

2024 Edition Vol. 13





## **DISCLAIMER**

The AUJUS journal was prepared by Auburn University Office of Undergraduate Research. Auburn University Office of Undergraduate Research does not make any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, or process disclosed. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not constitute or imply its endorsement, recommendation, or favoring by Auburn University Office of Undergraduate Research or Auburn University thereof. The views and opinions of the authors expressed herein are not intended to reflect those of the Auburn University Office of Undergraduate Research or Auburn University thereof.

## TABLE OF CONTENTS

8 AUJUS Editorial Team

#### **Research Highlights**

- A Flow Cytometric Approach to the Immunological Characterization of *Periplaneta americana* Tessa Allen, Faith Boyer-Millander, Arthur Appel, Zakee Sabree, and Elizabeth Hiltbold- Schwartz
- 19 Development and Optimization of Phase I and II Metabolites Identification Approach: Case of Bioactive Açaí Constituents

Meredith Almy, Zarna Raichura, and Angela I. Calderón

22 How Authorship Functions in TTRPG Actual Plays

R. Carson Barnes and Dr. Emily C. Friedman

- 24 Investigation Into the Optimization of Air-Cooled Battery Thermal Management Systems Nick A. Bensman and Mehmet Arik
- 28 Pitch Perception and Production in Music: A Study Determining the Benefits in Aided Versus Unaided Listening

Bailey Branham, Payton H. Lack, Au.D., Mary J. Sandage, SLP, Ph.D., Kathleen C. Lea, Au.D., Aurora J. Weaver, Au.D., Ph.D.

- 31 Development of a Pipeline for Low-thrust Trajectory Design and Validation in GMAT Barbara Brogan and Ehsan Taheri
- 35 Structured-Light 3D Imaging Using Off-the-Shelf Components
  Alex Brown and Zihe Gao
- 37 Dual-Task Balance in Healthy Adults

Connor Cantrell, Danielle M. Lang, and Kristina A. Neely

 $40 \quad \hbox{Engineering the Chicken Microbiome for the Prevention of Histomonias is}$ 

Jazmine D. Carroll and John F. Beckmann

43 Risk Perception Among Scavengers: How Does the Introduction of a Carcass into a Wildlife Landscape Affect the Presence and Overlap of Different Species?

Ally E. Cobern, Ansley L. Strength, Jean E. Fantle-Lepczyk, and Christopher A. Lepczyk

47 HYBRID-EXOSKELETON Design & Control

Joseph Crapet and Brendon Allen

51 Benzoic Acid Reduces Glycine Availability for Growth in Starter Pigs

M. Abigail Crosby, Paulo Henrique Amadeu de Azevedo, Alex C. Outlaw, and Marko Rudar

- Measuring Xylem Characteristics for Drought Tolerance in Peanuts Christopher Deveau, Sajid Hanif, Alvaro Sanz-Saez, Hasib Rahman, Tanzeel Rehman, Leah Hoffman, and Emily Graff
- Reading Recall Using Rapid Serial Visual Presentation (RSVP): The Impacts of Pauses on Reading Walton Ferguson, Alejandro Lazarte, and Joaquin S. Naraza
- 62 Students' Attitudes About Virtual Work PostPandemic: How Confidence Online and Virtual Communication Skills Drive Intentions for Future Remote Work
  Emily Findlay and Sara Driskell
- 65 Using Unoccupied Aerial Systems to Study the Effects of Wildflower Planting Adeline Flach, Zachary Beneduci, Anthony Abbate, Geoffrey Williams, and Stephanie Rogers
- 69 Evaluation of Agricultural and Clinical Antifungal Agents Against Members of the Fusarium solani Species Complex Amelia Flesner and Jeffrey Coleman
- 72 Microalgae Biomass Production Using Anaerobically Digested Solid Waste from Aquaponics Al Dean Francisco, Qichen Wang, and Brendan Higgins
- Mental Health Among Service Members Who Experienced Military Sexual Trauma: Exploring the Roles of Individual and Social Factors Melissa A. Garnes, Erin Cooper, and Mallory Lucier-Greer
- 80 Standardizing Success: Creating Gait and Behavioral Markers of Prognosis with Feline Models of Neurodevelopment and Neurodegenerative Diseases
  Olivia Grigsby, Jordan Towns, Dr. Emily Graff, and Dr. Douglas Martin
- Phuzzyphase: A Diploid and Polyploid Phasing Tool with Genotype Correction for HIFI and HIC Data

  Joshua Horton and Dr. Haynes Heaton
- The Changing Role of Occupational Characteristics in Socioeconomic Health Disparities Amy E. Hudson and Thomas Fuller-Rowell, Ph.D.
- 88 Researching Beneficial Bacteria That Help Plants Survive Cold Environments Sarah J. Hyde and Kathy S. Lawrence
- 91 **AAV-mediated Anti-hormone Antibody Therapy as a Treatment for Alzheimer's Disease** Emma E. Hruska, Arthur D. Zimmerman , and Douglas R. Martin
- 94 Modeling Ammonium To Prepare for a Nitrogen Mass Balance for an Algal-Bacterial System Ann E. Inskeep, Brendan T. Higgins, and Daniel E. Wells
- 98 Analyzing the Use of Historical Chairs in Modern Media Alissa L. Johnson, Darby Huber, and Anna Ruth Gatlin, Ph.D.

101	The Role of Anti-Müllerian Hormone Receptor Type 2 (AMHR2)-Expressing Cells in Adrenal Gland
	Development

Su Jeong Kim, Humphrey Yao, and Chen-Che Jeff Huang

#### A Centralized User Interface to Display Satellite Mega-Constellations in a Gamified System Emily Kimbrell and Daniel Tauritz

## 107 Internal Erosion in Unsaturated Slopes: Experimental Evaluation and Monitoring Anna Lancaster, Olaniyi Alfolayan, Dr. Jack Montgomery

## 114 High Dietary Salt Intake Induces Upregulation of Pro-Inflammatory Cytokine Involved with Cognitive Decline Within the Hippocampus

Abigail Law and Vinicia C. Biancardi

## 117 Teaching Away Anxiety: Psychoeducation Interventions for Subclinical Generalized Anxiety in Elementary-Aged Children

Katie E. Lawrence and Linda M. Gibson-Young

#### Improving the Accuracy of Atomic Fluorescence Efficiencies for Solar System Science Benjamin Lightfoot, John Noonan, and Dennis Bodewits

## Effects of Rear-Foot Center of Pressure on the Application of Forces During College Softball Hitting Aubrie K. Lisenby, Anthony W. Fava, Billy Lozowsk, and Gretchen D. Oliver

### 127 The Impact of Increased Financial Literacy Education and Awareness Through Marketing Approaches

Joseph M. Luria and Dr. Portia Johnson

## Justice System Contacts, Individual Differences, and Quality of Life Maddy J. McDaniel, Olivia H. Sawyer, Samantha J. Fede

## 133 **Investigation of Yellow-Pigmented Bacterial Catfish Pathogens**Benjamin A. Marshall, Courtney E. Harrison, and Timothy J. Bruce

## 138 **Hemocompatibility of Branched Amphiphilic Peptide Capsules** Emilee Middleton, Ethan Oesterle, and Adriana Avila-Flores

## 142 Classifying and Estimating Tremor Frequency via Inertial Measurement Unit Zachary R. Miller and Chad G. Rose

## 144 Understanding the Impacts of Genetic Counseling on Coping Styles of Families with Positive Cystic Fibrosis Carrier Screening

Hannah J. Montgomery, Joshua R. Novak, Kate M. Day, Kaleigh C. Miller, and Katy Marten

## 148 Environmental Initiatives in the Lodging Industry: Best Practices, Challenges and Efficacy in LEED Platinum Certified Properties

Shelby Morris and Dr. Imran Rahman

152	Analyzing Data-Driven CR3BP Orbit Representations for Immersive Astrodynamics Catalogs
	Eirik Mulder and Davide Guzzetti

#### 156 Captive Coral Breeding Methods Influence Restoration Success

Madeleine Park, Kelly Dunning, and Janna Willoughby

## 165 Investigation of the Tumor-Promoting Characteristics of the STK11 and MSK1 Genes in Canine Mammary Cancer

Caroline Parrish, Deepika Goyal, R.Curtis Bird

## 168 A Multimodal Study of Vocal Function and Upper Airway Temperature in Individuals with Asthma Versus Controls

Annie L. Pauley and Mary J. Sandage, PhD, CCC-SLP

## Distress Tolerance, Anxiety Sensitivity, and the Use of Cannabis as a Coping Mechanism Madeleine E. Rein, Mallory J. Cannon, B.A., Julia Y. Gorday, M.S., Richard Macatee Ph.D.

### 174 Localizing the Movement Protein (NSm) of Tomato Spotted Wilt Virus in Nicotiana Benthamiana Alexandra C. Rios, Abdelaal H.A. Shehata, Amanda Strayer-Scherer, Kathleen M. Martin

### 178 Isolating the Effects of Individual Nest Characteristics on Offspring Phenotypes of Brown Anole Lizards

John M. Rodgers, M. Christopher Norris, and Daniel A. Warner

## 183 Studying the Effect of Drought on Peanut's Stomatal Characteristics: Development of an Automatic Detection Method

Spencer Rubin, Sajid Hanif, Alvaro Sanz-Saez, Jónathan Heras, and Ángela Casado García

## 186 Maximum Thermal Tolerance of the Pinfish (*Lagodon rhomboides*) Across Different Populations of the Western Atlantic

Jacob E. Samenuk, Katie Eaton, and Moisés A. Bernal

## 190 Sexual Selection in the Wild Inferred using 3-D Printed Decoys and PIT Tags: Male Mate Choice in Yellow-Bellied Slider Turtles (*Trachemys scripta*)

McKae L. Sarkowski, Iwo Gross, and Matthew E. Wolak

#### 193 Representing the Spatial History of Alabama Authors: A Data Visualization Project

Kevien Shelton and Dr. Beverley Park Rilett

## 198 Characterization of Acoustic Pressure Waveforms in Rijke Tubes with Spatially Varying Temperature Distributions

Emma T. Signor, Cody M. Shelton, and Joseph Majdalani

## 202 Pecan Shells as an Alternative to Pine Bark as Substrate for Rabbiteye and Southern Highbush Blueberry Cultivation

Lucas K. Speer and James D. Spiers

#### 206 Ideal Gas Law Model for Presynapses and Presynaptic Vesicles

Henry Stephens, Noah Kim, Luca Cmelak, Mason Parkes, Paxton Wilson, and Michael Gramlich

- Nine-banded Armadillo (*Dasypus novemcinctus*) Burrow Use by Commensal Species Lauren R. Stevens, Wesley M. Anderson, and Olivia F. Sciandra
- Living in the Tensions: Investigations of Gender Identity and Performativity in STEM R. Smith Strain, Noah Leibnitz, Reagan Ruben, Doris Li, and Eric Burkholder
- 215 **Modelling Interorgan Transfer of Ionizing Radiation Amongst** *In Vivo* **Species** Jack Terry, Isaac Meyer, and Minseo Park
- A Landmark-Based Assessment of Humeri and Femora from Highly Fragmentary and Commingled Archaeological Skeletal Remains
  Cameron G. Troyer, Anna M. Buckingham, Toni M. Lee, and Kristrina A. Shuler
- Functional Analysis of Subpopulation-Specific DNA Ligase 1 Gene Polymorphisms in Human Cancers

Abigail Weir, Katherine Marlow, Razan Waliagha, and Amit Kumar Mitra

## **AUJUS EDITORIAL TEAM**



#### **Editor in Chief**

Lorenzo Cremaschi is a Henry M. Burt, Jr., Professor of Mechanical Engineering and Director of Auburn University's Undergraduate Research Programs (Auburn, AL, U.S.). He received his Ph.D. from the University of Maryland (College Park, MD, U.S.) in 2004. In 2001, he was awarded his M.S. and B.S. diploma degrees from the University of Modena (Italy). Before joining Auburn University, Dr. Cremaschi was a post-doctoral research associate at Purdue University's Herrick laboratories, followed by assistant and associate professor in the School of Aerospace and Mechanical Engineering at Oklahoma State University (Stillwater, OK).

Dr. Cremaschi focuses on energy efficiency, scalable thermal energy systems, water harvesting, desalination and purification, and the water-energy-food nexus. His current research includes climate-control transportation systems for biomedical applications, thermodynamics and heat transfer of low Global Warming Potential and natural refrigerants, air dehumidification processes, frost and defrost, and moisture transport in thermal insulation systems. He is the principal investigator of several research projects and the author of over 100 papers. He revised three book chapters and gave several presentations at conferences, meetings, and university seminars. Dr. Cremaschi is the inventor of three international patents.

Dr. Cremaschi developed and implemented strategic plans for increasing the visibility and involvement of undergraduates in research and was responsible for providing support and administrative leadership for undergraduate research programs, including developing new initiatives. He fostered several scholarships and awards for undergraduate students involved with research, including programs providing access to research fellowships to a broad range of students. He also serves on the organizing committees for the university's research symposia, specifically organizing the annual research symposium.

Dr. Cremaschi served as an associate editor for the Science and Technology for the Built Environment Journal. He has several honors and awards, including the 2024 Henry M. Burt, Jr. Endowed Professorship for the Samuel Ginn College of Engineering, the 2022 Research Award for Excellence in the College of Engineering, and the 2020 Exceptional Service Award from the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE).

Dr. Cremaschi teaches Thermodynamics, Refrigeration, Renewable Energy Systems, and Cryogenics courses. These courses are offered at the undergraduate mechanical engineering curriculum's second, third, and fourth years and the graduate level. Dr. Cremaschi advised 35 graduate students (14 Ph.D. and 21 M.S. students) and over 50 undergraduate students in academic curriculum and career choice. He mentored 23 undergraduate students and 10 international visiting scholars during research and scholarly activities. Dr. Cremaschi is the faculty advisor of the Auburn University ASHRAE student organization.

Dr. Cremaschi is the chair of the American Society of Thermal and Fluids Engineers

(ASTFE) Executive Committee and served on the ASHRAE Research and Administration Committee. He has served as the chair of the International Institute of Refrigeration (IIR) U.S. National Committee. He is a member of the International Energy Agency U.S. Heat Pump National team. He is currently the president of the IIR Commission B1, which focuses on thermodynamics and transfer processes fundamentals of refrigeration. He has served on several technical and standard committees for over 20 years. Dr. Cremaschi was the technical program chair for two ASTFE conferences in 2019 and 2021. He organized numerous technical sessions, panel forums, and seminars within ASHRAE, ASTFE, IIR, and the American Society of Mechanical Engineers (ASME).



#### **Production Editor**

Madison Russell is a second-year graduate student pursuing her Doctorate in Audiology. Madison graduated from The University of Alabama with a Bachelor of Arts degree in Communicative Disorders. She is a Graduate Assistant for the Office of Undergraduate Research. She is in charge of processing and editing AUJUS highlights and articles. Her research studies the overall well-being of those with hearing loss in rural areas. Her previous research included services in rural areas to improve the accessibility of hearing healthcare. Madison is currently involved with the Student Academy of Audiology as the Fundraising Chair. In her spare time, she enjoys reading, spending time with her family, and exploring new cooking and baking recipes.



#### **Production Editor**

Lexy Barraza is a third-year graduate student pursuing her Doctorate in Audiology. Lexy graduated from the University of South Florida with a Bachelor of Science degree in Health Science. She is a Graduate Assistant for the Office of Undergraduate Research. She is in charge of the mentor matching program, newsletter and editing for the AUJUS journals. Her research studies the effects of hearing loss on Hispanic cultures in Alabama. Lexy's other on-campus involvements include being a Student Academy of Audiology member and an Auburn University Rural Health Fellow. In her spare time, she volunteers at the Chambers County Community Health & Wellness Center and spends quality time with her family in Florida.



#### **Associate Editor**

**Esther Akinrinde** is a fourth-year PhD student in the Industrial and System Engineering department at Auburn University. She received her bachelor's degree in industrial and production engineering at the University of Ibadan, Nigeria and her MS in Industrial and systems engineering and supply chain management from auburn university in 2023 and 2024 respectively. Her research interest is in driving operational excellence by identifying, developing, and implementing methods to eliminate inefficiencies in processes. She is currently exploring the applications of overall equipment effectiveness approach to driving process improvement in poultry processing with the goal of enhancing operational excellence. In her spare time, she likes to cook and bake.



#### **Associate Editor**

Olivia Brahms is a Ph.D. candidate and graduate teaching assistant in the Department of Biological Sciences with a graduate certificate in Health Equity Science under the guidance of Dr. Jason Upton. She graduated from Auburn University in 2019 with a Bachelor of Science in Animal Sciences. Her current research focuses on how Cytomegalovirus modulates and manipulates host immune responses to aid in viral pathogenesis. In her spare time, Olivia enjoys walking her dog, Milo, playing with her guinea pig and cat, Winston and Nougat, and painting.



#### **Associate Editor**

Gabrielle Brown is a 4th year doctoral candidate in the Counseling Psychology PhD program and a graduate research assistant for the department of Special Education, Rehabilitation, and Counseling. She earned her Master of Public Health in Health Policy and Management from Emory University and Bachelor of Arts in Psychology from Vanderbilt University. Her current research focuses on exploring how the impact of oppressive social structures contribute to health disparities and the connection between applied psychology and health policy. In her spare time, she enjoys drinking tea, reading and spending time with friends and family.



**Associate Editor** 

**Olamide Isaac Durodola** is a graduate student of Biosystem Engineering. He had his undergraduate in Agricultural and Environmental Engineering at Obafemi Awolowo University in Nigeria. His research efforts have been dedicated to conducting spatial analysis within incubators, with the goal of achieving optimal performance and increased productivity by refining air velocity, temperature, and humidity conditions. In his spare time, he dedicates moments to staying updated on technological advancements, learning new skills, and enjoying valuable moments with his family and friends.



**Associate Editor** 

Olumide Falana is a Ph.D. student in Biosystems Engineering whose research focuses on optimizing the thermal environment in commercial poultry houses to improve animal welfare and production efficiency. With a B.Sc. and M.Sc. in Agricultural and Environmental Engineering from Obafemi Awolowo University in Nigeria, he applies his expertise in precision agriculture and environmental control systems to tackle challenges in food security and sustainability. His research interests span sustainable food systems, climate-resilient agriculture, environmental control, and energy efficiency. Outside academia, Olumide is actively involved in community outreach and educational initiatives.



**Associate Editor** 

**Temitope Ruth Folorunso** is a Ph.D. student in the Conservation and Genomics Laboratory at the College of Forestry, Wildlife, and Environment. Her academic background is rooted in molecular biology and genomics, with degrees from Obafemi Awolowo University, Nigeria. Her current research significantly contributes to the forest industry by focusing on mitigating the effects of Brown Spot Needle Blight, caused by the fungal pathogen *Lecanosticta acicola*, in pine species. She aim to compare patterns of genetic diversity with symptoms of Brown Spot Needle Blight to better understand how *L. acicola* strains are associated with symptom expression and severity.

Beyond her research endeavors, she has actively engaged in leadership roles across various student organizations, she previously served as the Secretary of the African Students Association and as the Graduate Senator for the College of Forestry, Wildlife, and Environment 2023/2024. Currently, she represent graduate students as a Senator in the Auburn University Student Government Association. She has communicated her research findings both through journal publications and conference presentations. At the core of her academic and professional pursuits is a commitment to fostering sustainability in agriculture and forestry through impactful research and leadership.



#### **Associate Editor**

**Pankaj Gaonkar** is a Ph.D. student and graduate research assistant in the Department of Pathobiology. He is a veterinarian with a Master's degree in Veterinary Pathology from Maharashtra Animal and Fishery Sciences University, India. His research focuses on the epidemiology of antimicrobial resistance in poultry production. Beyond academia, he's an avid traveler, constantly exploring new destinations, and actively practicing Karate.



#### **Associate Editor**

**Maliha Yel Mahi** is in her third year of pursuing a Ph.D. in Mechanical Engineering, specializing in Computational Fluid Dynamics (CFD) and Multiphase Fluid Flow. She has achieved her Master's degree in Mechanical engineering from the University of North Dakota. She enjoys reading books, spending time with family and friends, and traveling in her spare time.



#### **Associate Editor**

Onyedika Mbelu, is from Anambra state, Nigeria. He is a third-year PhD Student at the Department of Mechanical Engineering. Mr. Mbelu currently holds a master's degree in fluids engineering for Industrial processes from INSA Toulouse, France. He received his bachelor's degree in mechanical engineering from University of Nigeria. His research focuses on Direct-Contact Storage of Thermal Energy Utilizing Phase Change Materials under the supervisor of Dr. Jay Khodadadi. In his spare time, he likes to go to the gym, swim, and read.



#### **Associate Editor**

**Pritam Mitra** is a fourth year PhD student in the Department of Agricultural Economics and Rural Sociology. He has a bachelors (B.Sc) and master's degree (M.Sc) in Economics from the University of Calcutta, Kolkata, India (2018) and Auburn University (MS) (2023). His current research topics are food waste at the household level, the use and misuse of pesticides. he is also interested in behavioral economics and decision making. In his free time, he enjoys a cup of tea with music or an awesome book.



#### **Associate Editor**

Ifeoluwa Odeniyi is a PhD candidate and graduate research assistant in the Department of Nutritional Sciences. He obtained both his bachelor's and master's degrees from Nigeria in Biochemistry. He has rich work and research experience in patient tumor xenograft model and tumor dissociation, mouse breeding, genotyping and metabolic cage assessment and adipocyte differentiation. Currently, his PhD research in Dr. Greene's lab focuses on the link between obesity and its role in the development and progression of colorectal cancer through integrating molecular biology, genetics, and nutrition. Outside his academic undertakings, Ifeoluwa's leadership acumen shines through his role as the President of the African Students Association during the academic year of 2022/2023. His exceptional dedication was meritoriously acknowledged with the title of student leader of the year, accompanied by an array of additional awards. He has published 8 peer-reviewed journal articles and numerous conference presentations. During moments of respite, he finds solace and joy in strumming the guitar, a pursuit that harmoniously balances his multifaceted life.



#### **Associate Editor**

Abosede Onifade is a second-year graduate student in the Department of Sociology at Auburn University, where she focuses on the intersection of environmental justice, public health, and socio-economic equity. She earned her undergraduate degree in Sociology from Lagos State University, Nigeria, graduating as the best student in the Faculty of Social Sciences. Her current research explores the social impacts of wastewater infrastructure, addressing critical issues such as public health risks, environmental sustainability, and the challenges of equitable access in underserved communities. Outside of her academic pursuits, Abosede engages in professional development, participates in community service initiatives, and serves in leadership roles that advocate for diversity, inclusion, and sustainability. In her free time, she enjoys learning new skills, staying informed on global environmental challenges, and spending quality time with family and friends.



**Associate Editor** 

Mason Parkes is a PhD student in the Physics Department. He graduated from BYU in 2019 with a Bachelor of Science degree in Physics. His research focuses on the physics of resource sharing in hippocampal presynapses. He's particularly interested in why synaptic vesicles are shared between presynapses, what cytoskeletal elements support that sharing, and what that means for wider brain function. In his free time he enjoys spending time with his wife and two daughters, and playing Dungeons and Dragons with his friends.



**Associate Editor** 

Didimos Johnson Pulikkottil is a fourth-year PhD candidate in the Department of Chemistry and Biochemistry. He earned his Bachelor's (B.Sc.) and Master's (M.Sc.) degrees in Chemistry from Mahatma Gandhi University, Kottayam, India, in 2018. His research focuses on the synthesis of pyrene-extended molecules for applications in biology and material sciences. Beyond his academic pursuits, Didimos is passionate about science communication and creatively sharing scientific ideas through films. In his free time, he enjoys exploring photography through photo walks and creating short videos.



**Associate Editor** 

**Jeremiah Pfitzer** is a Ph.D. candidate committed to making a meaningful impact by developing therapeutics for hematological malignancies. Jeremiah's current research investigates novel therapeutics in drug-resistant multiple myeloma. His previous work involved deciphering the neural pathology in Alzheimer's disease and related dementias.



**Associate Editor** 

Claudia Ann Rutland Landrum is a PhD student and a graduate research assistant in the Department of Crop, Soil, and Environmental Sciences, studying weed science under Dr. Scott McElroy. She graduated from Auburn University in 2020 with a Bachelor of Science in Microbiology. Her current research focuses on target-site resistance in herbicide resistant weed species, more specifically understanding the mechanisms of resistance in auxin-mimic herbicides. In her free time, she enjoys reading, painting, and playing with her dog Obi.



**Associate Editor** 

**Pooja Zen Santhamoorthy** is a fourth-year Ph.D. student and a graduate research assistant in the Department of Chemical Engineering, working on developing optimization tools under Dr. Selen Cremaschi. She graduated from Anna University in 2019 with a Bachelor of Technology in Chemical Engineering. Her current research focuses on developing portfolio optimization models for planning carbon capture and utilization and solution approaches to solve the developed mathematical model. She enjoys reading fiction, playing DOTA, and caring for her sweet pets, Valerie and Rocket.



**Associate Editor** 

**Syed Hassan Raza Shah** is a PhD candidate, Course Instructor, and graduate teaching assistant in the Aerospace Engineering department. He possesses two master's degrees in aerospace, one from Auburn University and the other from Air University, Islamabad, Pakistan. His undergraduate degree also in Aerospace was acquired from the esteemed College of Aeronautical Engineering at NUST, Pakistan. His research is centered around unsteady and low Reynolds number aerodynamics, with a specific focus on investigating non-linear aerodynamic interactions. Beyond his academic endeavors, Syed finds gratification in cherishing moments with his family, traveling, and golfing.



**Associate Editor** 

Ying Yan is a Ph.D. candidate in the Consumer and Design Sciences. She has rich work and research experience in multiple fields, including industrial design, interior design, consumer behavior, and business. She previously graduated from Nanjing Agriculture University with a Bachelor of Industrial Design in Mechanical Engineering, as well as a Master of Industrial Design at Auburn University. Throughout her research, she has been continuously fascinated by the intersection of design and well-being. Recognizing the profound influence environments have on our mental and physical health, she was drawn to explore how design can positively impact human well-being. Currently, Ying's research focuses on increasing college students' learning motivation through game-based learning to enhance learning experiences in design education.

# A Flow Cytometric Approach to the Immunological Characterization of *Periplaneta* americana

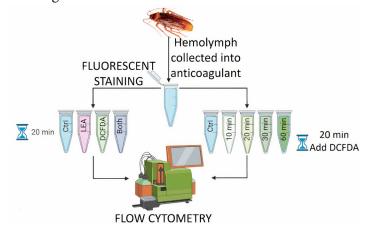
Tessa Allen<sup>1,\*</sup>, Faith Boyer-Millander<sup>2</sup>, Arthur Appel<sup>3</sup>, Zakee Sabree<sup>4</sup>, and Elizabeth Hiltbold-Schwartz<sup>5</sup>

<sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University
 <sup>2</sup> Graduate Student, Department of Biological Sciences, Auburn University
 <sup>3</sup> Associate Dean, Department of Entomology and Plant Pathology, Auburn University
 <sup>4</sup> Associate Professor, Department of Evolution, Ecology, and Organismal Biology, Ohio State University
 <sup>5</sup> Associate Professor and Assistant Dean, Department of Biological Sciences, Auburn University

The American cockroach (Periplaneta americana) is a globally distributed pest with a relatively complex gut microbiome, primarily composed of Bacteroides and Firmicutes. In mammalian systems, the impact of the gut microbiome on immunological composition and function has been well investigated; however, an insect system provides a unique platform for investigating this interaction with the innate immune system. Insect immune cells, called hemocytes, have been investigated primarily through microscopy, leaving a knowledge gap in relative abundance and specific functionality of these different cell types. To address this knowledge gap, we employ flow cytometry, cell sorting and functional assays to isolate and characterize hemocyte populations found in *P. americana*. Our flow cytometric data reveals distinct hemocyte populations based on lectin-binding capabilities (see Fig. 2). Based on these parameters, we can sort isolated cell populations and investigate individual population activity through functional assays (i.e., ROS production, antibacterial capabilities) (see Fig. 1). These data provide a foundation for developing a more robust characterization of hemocyte abundance and function. For future studies, we are annotating the hemocyte transcriptome and cultivating a colony of germ-free P. americana for investigation of the role of the gut microbiome in composition and function of the innate immune system.

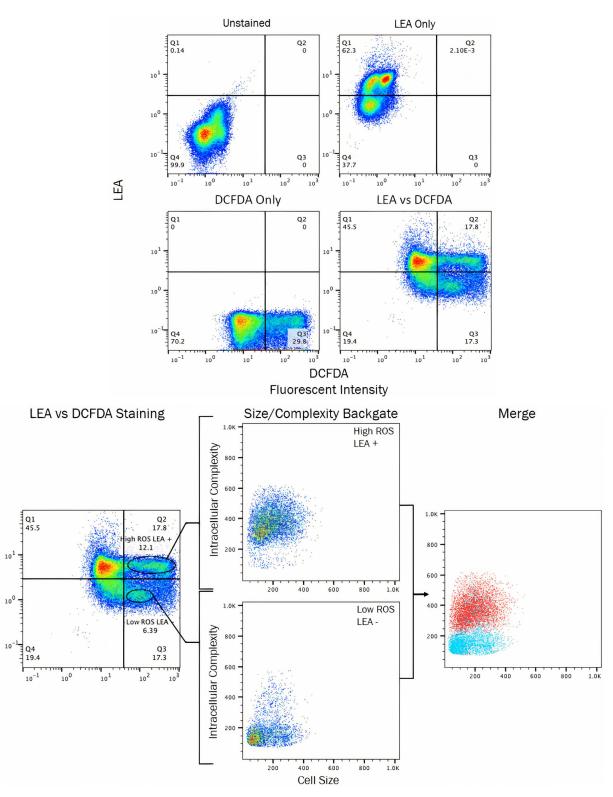
Lectins distinguish hemocyte populations in the absence of antibodies that can be used in other systems. Lectin staining (LEA) distinguished two populations of ROS producing hemocytes. Initial flow cytometric

assays with ROS indicator (DCFDA) show a complex hemocyte population produced ROS in response to a stimulus (see **Fig 3**). Repetition (N=7) of this assay shows inconsistent timing of this kinetic reaction (see **Fig 4**). These assays contribute to our baseline immunological characterization of *P. americana*, providing a necessary reference to understand contributions of the gut microbiota to the cellular diversity and functionality of hemocytes in immunological development in future germ-free.

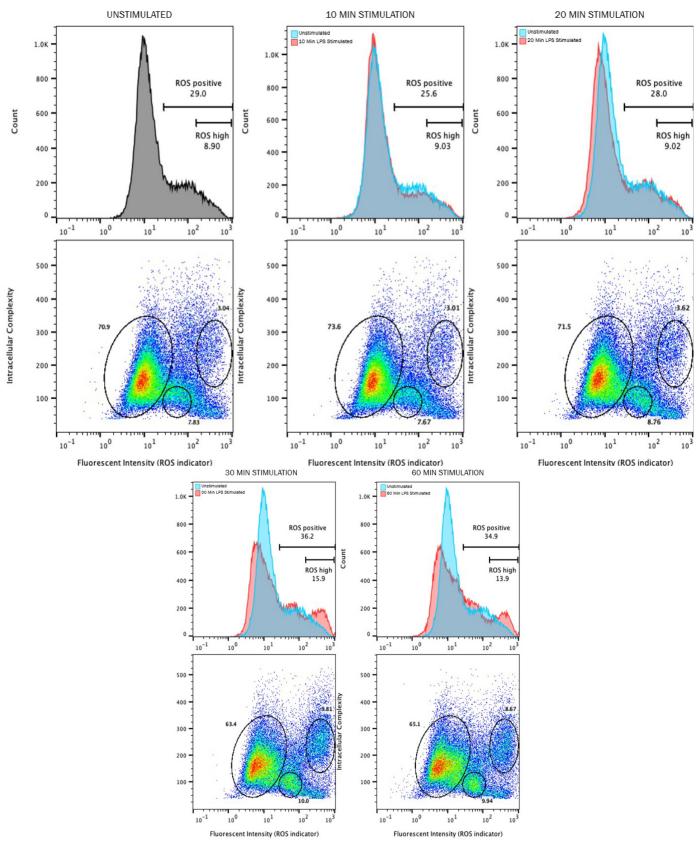


**Figure 1** Hemolymph is collected from adult male *P. americana* into anticoagulant. To determine which cells are producing ROS, cells were incubated with LEA (lectin) and DCFDA (ROS indicator) for 20 minutes at 30C. To determine when cells produce ROS in response to stimulus, hemocytes were exposed to 500ng of LPS at 10-, 20-, 30-, and 60-hour time points and incubated with DCFDA at 20 minutes. All samples were examined through flow cytometry. Figure generated in Biorender\*.

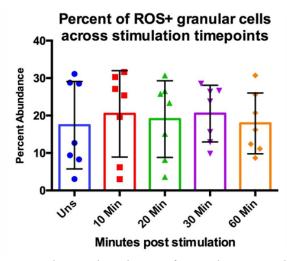
<sup>\*</sup> Corresponding author: tra0014@auburn.edu



**Figure 2** Lectin binding and DCFDA staining reveal two distinct ROS-producing hemocyte populations. Cell populations distinguished by LEA (lectin) binding and DCFDA staining (ROS indicator). 2A shows the unstained, LEA single stained, DCFDA single stained, and double stained samples. Two separate populations (LEA positive and LEA negative) produce reactive oxygen species. 2B shows the back gating of ROS-producing populations. LEA positive ROS producing cells are more complex than LEA negative ROS producing cells, revealing two distinct subsets of hemocytes.



**Figure 3** Time course of ROS production after stimulation with LPS at 10, 20, 30, and 60 minutes. The fluorescent overlays of the unstimulated and stimulated samples show a peak of ROS production indicated through fluorescent intensity at 30 minutes post-stimulation. Corresponding dot plots displaying ROS production vs intracellular complexity reveal that a population of granular ROS-producing cells increases in relative abundance, peaking at 30 minutes post-stimulation.



**Figure 4** Relative abundance of granular ROS + hemocytes over a time course of stimulation with LPS (N=7). Abundance at each time point is highly variable. Additional metrics should be used to understand the kinetics of ROS production in hemocytes responding to stimulation.

In the future, we will use additional metrics to evaluate and quantify ROS production from hemocyte subsets. Additionally, we will employ the use of plate reader assays to understand the kinetics of ROS production in insect immune cells responding to a stimulus. After generation of our germ-free *P. americana* stock, we will evaluate ROS production capabilities, among other immunological functions in conventional and germ-free cockroaches to better understand the contribution of a complex gut microbiome in development and functionality of the innate immune system.

#### **Statement of Research Advisor**

In the fall of 2023, Tessa began working with my PhD student, Faith Boyer-Millander. Faith taught her how to collect hemolymph from cockroaches, how to stain the cells to measure production of reactive oxygen radicals, and how to run and analyze this cellular function by flow cytometry. Tessa quickly became adept at these approaches and ran many of these experiments independently.

-Dr. Elizabeth Hiltbold-Schwartz, Biological Sciences, College of Science and Mathematics

#### References

[1] Eliachar S, Snyder GA, Barkan SK, Talice S, Otolenghi A, Jaimes-Becerra A, Sharoni T, Sultan E, Ha-

dad U, Levy O, Moran Y, Gershoni-Yahalom O, Traylor-Knowles N and Rosental B., Heat stress increases immune cell function in Hexacorallia. Front. Immuol. Retrieved April 19, 2024, from https://doi.org/10.3389/fimmu.2022.1016097 (2022)

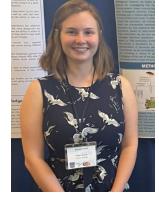
[2] Hillyer JF. Insect immunology and hematopoiesis. Dev Comp Immunol. Retrieved April 19, 2024, https://doi.org/10.1016/j.dci.2015.12.006, (2016)

[3] Liao W-Y and Fugmann SD. Lectins identify distinct populations of coelomocytes in Strongylocentrotus purpuratus. PLoS ONE. Retrieved April 19, 2024, from https://doi.org/10.1371/journal.pone.0187987, (2017)

#### **Authors Biography**



Tessa R. Allen is a senior-year student pursuing a B.S. degree in Biomedical Sciences and a B.A. degree in Spanish at Auburn University. She has been accepted and will be attending USA Frederick. P. Whiddon College of Medicine beginning this summer.



Faith Boyer-Millander is a graduate student pursuing a Ph.D. in the Department of Biological Sciences at Auburn University. She has been a vital contribution to the ongoing work of Schwartz Lab and is a valued undergraduate research mentor.



Dr. Arthur Appel is the Associate Dean for research in the Department of Entomology and Plant at Auburn University. He received his Ph.D. in Urban Entomology at the University of California in 1985 and conducts research in urban entomology, structural pests, insect physiology and behavior.



Dr. Zakee Sabree is an Associate Professor of Evolution, Ecology & Organismal Biology at Ohio State University. His areas of expertise include insect-microbe symbioses and microbial ecology and genome evolution.



Dr. Elizabeth Hilt-bold-Schwartz is an Associate Professor and Assistant Dean for Biomedical Sciences at Auburn University. She received her Ph.D. from Emory University in 1996 and conducts research in immunology and microbiology.

# Development and Optimization of Phase I and II Metabolites Identification Approach: Case of Bioactive Açaí Constituents

Meredith Almy<sup>1,\*</sup>, Zarna Raichura<sup>2</sup>, and Angela I. Calderón<sup>3</sup>

<sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University
 <sup>2</sup> Graduate Student, Department of Drug Discovery and Development, Auburn University
 <sup>3</sup> Gilliland Endowed Professor, Department of Drug Discovery and Development, Auburn University

Euterpe oleracea Mart. (açaí) is Arecaceae family (Figure 1). Botanical Drug Supplements (BDS) from açaí fruits are highly consumed worldwide, due to its antioxidant and anticarcinogenic effects. Recent statistics show that cancer patients use BDS at a much higher rate than non-cancer patients, in order to complement their conventional chemotherapeutic medications. Data analysis using the Food and Drug Administration Adverse Event Reporting System (FAERS) showed adverse events associated with concomitant use of BDS of açaí and anticancer drugs. For this reason, it has proven necessary to explore the mechanisms of action that cause these clinically relevant drug interactions. The main objective of our study is to develop a predictive preclinical approach to identify Phase I and II metabolites of the constituents of açaí extracts [4], and subsequently elucidate the corresponding metabolite structures using mass spectrometry and bioinformatic tools. The study focuses on predicting the metabolites of cyanidin 3-glucoside (C3G), as this compound is used for the standardization of açaí extracts and it is the only compound with reported pharmacokinetic studies.

The experiments involved conducting Phase I and II metabolic reactions of açaí constituent (C3G) using human liver microsomes (HLM). Phase I and II enzymes considered are cytochrome P450 (CYP) and uridine diphosphate glucuronosyltransferase (UGT). The resulting metabolites were monitored and assessed for their increase in polarity and decrease in toxicity as well as possible drug interactions. C3G was incubated for 10 minutes with HLM, phosphate buffer (pH 7.4), and nicotinamide adenine dinucleotide phosphate (NADPH) and/or uridine 5'-diphosphoglucuronic acid (UDPGA) to initiate Phase I and II reactions respectively. Fur-

thermore, for the Phase II experiment, alamethicin was added to the sample to activate UDPGA sequestered in the HLM, and saccharolactone was added to inhibit glucuronidase, which reverses the Phase II reactio [2]. The obtained metabolites of açaí were analyzed with liquid chromatography-mass spectrometry (LC-MS) and by elucidating the structure of a compound with software such as Xenosite and Biotransformer.



Figure 1. Açaí Fruit

The metabolism predicted by the biotransformation tools were oxygenation and quinonation reactions of phenolic and hydroxy groups present in C3G [1,5]. The potential enzymes responsible for such metabolites are CYP2A6, CYPC29, CYP2E1. Whereas Phase II enzymes resulted in glucuronidation, methylation, and sulfonation of the aromatic hydroxy groups [1,5]. On the other hand, LC-MS analysis was conducted using Poroshell 120 EC-C18 column and Q-TOF as a mass spectrometer. The resulting metabolites were protocatechuic acid, phloroglucinaldehyde, ferulic acid [3] as represented in figure 2. Potential enzymes for the

<sup>\*</sup>Corresponding author: mma0058@auburn.edu

formation of these metabolites are CYP1A2, CYP2C9, CYP2D6, CYP3A4. This result was supported by the information reported in literature

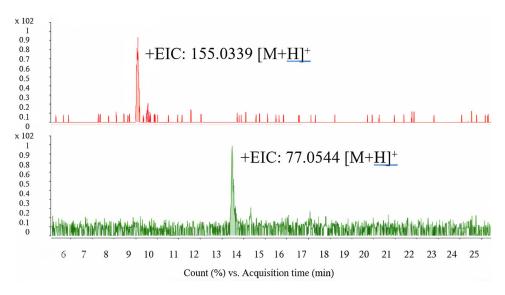
A future direction for this study would be to test other known açaí constituents and identify unknown metabolites. Furthermore, tandem mass spectrometry (MS/MS) would be considered to identify and confirm the existence of metabolites produced by these enzymes along with their fragmentation pattern. After finalizing the confirmation of produced metabolites, the samples will be tested for its possible inhibitory effect of CYP enzymes and will be incubated with and without an-

ticancer drugs to observe the inhibition and interaction of the metabolites. The study provides preliminary overview of açaí metabolites that may offer insight into bioactivity or potential drug interaction [5].

#### **Statement of Research Advisor**

Meredith is a brilliant student. Her scientific curiosity has helped her to participate and contribute to various small projects within my NIH R15 grant. Her work will help us to identify the important Phase I and Phase II açaí metabolites.

- Angela I. Calderón, Drug Discovery and Development, Harrison College of Pharmacy



**Figure 2.** Extracted ion chromatogram of protocate- chuic acid/ phloroglucinaldehyde ([M+H]+155.0339) and ferulic acid ([M-H2O+H]+177.0544).

#### References

- [1] Biotransformer- BioTransformer 3.0 (http://biotransformer.ca/new)Webpage: Nonperiodically Web Document
- [2] Fasinu et al. (2012). Liver-Based In Vitro Technologies for Drug Biotransformation Studies A Review. Current Drug Metabolism, 13(2), 215–224. Journal Paper
- [3] Fernandes et al. (2008). Enzymatic hemisynthesis of metabolites and conjugates of anthocyanins. Journal of Agricultural and Food Chemistry, 57(2), 735–745. Journal Paper

- [4] Wu et al. (2021). Metabolite Identification in the Preclinical and Clinical Phase of Drug Development. Current Drug Metabolism, 22(11), 838–857. Journal Paper
- [5] Xenosite- https://xenosite.org/ Webpage: Nonperiodically Web Document

#### **Authors Biography**



Meredith Almy is a junior-year student pursuing a B.S. degree in Integrative Biology at Auburn University, in order to pursue a career in veterinary medicine. She has played key research roles in Drug Discovery and Development.



Zarna Raichura is a graduate student pursuing her Ph.D. in Medicinal Chemistry at Auburn University's Harrison College of Pharmacy. She got her Bachelor's in Pharmacy from Bhanuben Nanavati College of Pharmacy in Mumbai, India. Her research focuses on the possible inhibition of ashwagandha extract on CYP enzymes.



Dr. Angela I. Calderón is a Gilliland Endowed Professor in the Department of Drug Discovery and Development at Auburn University Harrison College of Pharmacy. She received her Bachelor's in Pharmacy from the University of Panama, an M.S., in Pharmacognosy from the University of Illinois at Chicago, and a Ph.D. in Pharmacognosy from the University of Lausanne (Switzerland). Her research focuses on the mechanism of açaí BDS-anticancer drug interactions.

## How Authorship Functions in TTRPG Actual Plays

R. Carson Barnes<sup>1,\*</sup> and Dr. Emily C. Friedman<sup>2</sup>

Undergraduate Student, Department of Film & Media Studies, Auburn University
 Associate Professor, Department of English, Auburn University

This research project analyzed the emerging media space of online Tabletop Roleplaying Game (TTRPG) Actual Plays (AP) in order to discover how concepts such as authorship and intellectual property are understood in a field that blurs the lines between hobby and profession. Made attractive by the remote, isolated periods of the 2020 COVID-19 pandemic, there has been a proliferation of these new media online in recent years, as well as a growing audience for both new and old productions. Though such growth inspires questions pertaining to where and how Actual Play fits into a broader media culture, research on the topic is still relatively rare, as Actual Play's origins tend to denote it as 'niche'.

Due to the independent nature of AP productions and the different game systems that may be used in each, this research project's main objective was to compile data concerning how the producers and creators of Actual Play shows understand authorship and intellectual property in relation to their own work. The project was divided into two main parts.

First, an initial examination of the media landscape of TTRPG Actual Plays was conducted through engagement with an array of Actual Play texts produced by groups such as *Dimension 20 and 20 Sided Stories*. This period of primary research was bolstered by scholarship such as Stephanie Hedge and Jennifer Grouling's essay collection *Roleplaying Games in the Digital Age*, which provided a foundational comprehension of the many layers of authorship that exist within an Actual Play production.

The second part of this research project consisted of collecting data on how persons and creators within the AP field discuss and define their work; methods employed to collect such data included oral interviews with and

visits to a convention attended by participants in this field, which informed the design of a survey deployed in Spring 2024. This survey compiles data about participants' perceptions and lived experiences of monetization, intellectual property ownership, and other signs of professionalization in both TTRPGs and AP. This survey is also the first to collect demographic data about participants in the AP field. At the time of this report, over 1700 responses have been collected, with about 12 invalid entries (responses from underage participants).

Preliminary survey results have revealed information about how both authorship and creative ownership are understood and mediated within the AP field. Among self-identified AP creators, approximately 71% of respondents indicate that they consider themselves authors of their work in this space, and a majority of them own their work either as an individual or within a collective. The survey's results reinforce and put into words the reliance of the Actual Play medium on collaboration between creators. Nearly 68% of AP creator respondents to the survey who rejected the title of author in regard to their work in this field indicated that they did so because of the collaborative nature of their work.

Furthermore, the survey results reveal AP to be an aspirational field, with nearly 42% of respondents who do not own their work indicating that they would choose to own their work if they could. Nearly 75% of respondents volunteer their time in this field in some capacity, though only less than 1% have indicated that they do not believe in compensation for AP work. 43% indicate that Actual Play is connected to their career goals and 43% indicate they are working to monetize their work in Actual Play, but only around 15% of respondents indicate their work in Actual Play currently functions as an income stream for them.

<sup>\*</sup> Corresponding author: rcb0072@auburn.edu

More than anything, the survey's results reinforce and put into words the reliance of the Actual Play medium on collaboration between creators. Nearly 34% of AP creator respondents to the survey who rejected the title of author in regard to their work in this field indicated that they did so because of the collaborative nature of their work, viewing 'author' as an individual title not suited to this medium. As the 21st-century media landscape develops and boundaries between distinct types of media continue to dissolve and blur, we will most likely see the emergence and proliferation of more collaborative and transmodal media like TTRPG Actual Play. Data about creators in this field and how they comprehend their own work, such as those collected throughout this research project, are meant to provide information for other scholars interested in conducting research in the field.

**Statement of Research Advisor** 

Carson has worked hard to fully understand the TTRPG and AP space in order to co-author this survey, which has had a strong response rate. The sample she provides of some of the preliminary insights is just a small part of what we anticipate we will be able to draw from the collected data. We will continue to collaborate on this work, presenting and publishing fuller results. I anticipate that the insights gained from this research will be eye-opening to both new media and game studies scholars, as well as thought-provoking to the communities of practice we study.

- Dr. Emily C. Friedman, Department of English, College of Liberal Arts

#### References

Caldwell, John Thornton. *Production Culture: Industrial Reflexivity and Critical Practice in Film and Television*. Duke University Press, 2008.

Hedge, Stephanie, and Jennifer Grouling. *Roleplaying Games in the Digital Age: Essays on Transmedia Storytelling, Tabletop RPGs and Fandom.* McFarland & Company, Inc., Publishers, 2021.

Keogh, Brendan. The Videogame Industry Does Not Exist: Why We Should Think Beyond Commercial Game Production. The MIT Press, 2023.

Peterson, Jon. *Game Wizards*: The Epic Battle for Dungeons & Dragons. The MIT Press, 2021.

#### **Authors Biography**



R. Carson Barnes is a senior-year student pursuing a B.A. degree in Media Studies – Film Option at Auburn University. As a student, she has a particular interest in new media and transmodal stories that defy traditional genre and industry categorizations. Her research on authorship in these areas will continue beyond her undergraduate career.



Emily C. Friedman is an Associate Professor of English at Auburn University. An eighteenth-centuryist and book historian by training, her current work focuses on the long history of creativity outside commercial mass media, from never-published manuscript fiction of the 18th and 19th centuries to creator cultures of the 21st. Her current book project is *Improvised Worlds: Digital Storytelling through Play*.

## **Investigation Into the Optimization of Air-Cooled Battery Thermal Management Systems**

Nick A. Bensman<sup>1,\*</sup> and Mehmet Arik<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Mechanical Engineering, Auburn University

In recent years, the amount of electric vehicles produced has increased tremendously, most automotive manufacturers now have production lines for electric vehicles. Electric vehicles use large battery packs to power the electric motors. These battery packs are comprised of thousands of small batteries, ranging from 5,000 to 10,000 cells, depending on the manufacturer. The common types of battery cells used in electric vehicles are Lithium-Ion cells (Li-Ion). Lithium-Ion cells offer superior energy density, low-memory loss effects, and are easy to work with, due to recent advancements in cell production. In electric vehicles, Li-Ion battery cells are cylindrical, due to the strong energy density per volume that the shape allows. The three common sizes of the cells are 18650, 217000, and 4860, where the first two numbers are the cell diameter in millimeters, and the second two numbers are the height of the cell in millimeters. The downside of Lithium-Ion battery cells is that they are extremely prone to temperature variations. Li-Ion battery cells' effective temperature range is 15 – 40 (°C) (1). Battery cells exposed to temperatures outside of this range are prone to losing their ability to hold a charge. When battery cells encounter temperatures higher than 80 (°C), thermal runaway becomes a concern for the cells (2). Thermal runaway is when a battery cell reaches a temperature that the cell will continue to increase in temperature until failure. Due to these reasons, battery thermal management systems are included in electric vehicles to prevent the cells from exceeding this temperature range.

The types of battery thermal management systems used are air-cooled, liquid-cooled, and thermos-electric coolers. Most manufacturers use air-cooling systems for their EV battery packs because they offer great cooling capabilities without worrying about the cooling liquids. In air-cooling there are two types: forced

and natural. In forced air-cooling, the air is accelerated over the cells using a fan or an air intake from the outside air. This increases the cooling effectiveness of the air but at the cost of requiring external power. The second form of air-cooling is natural cooling. In natural cooling, the cells are exposed to the air outside, but this is much more susceptible to overheating due to the decrease in cooling effectiveness. Due to the effectiveness of forced air-cooling, most electric vehicles rely on forced air-cooling to maintain battery cells within their operating temperatures (3).

The goal of this study is to optimize the arrangement of 12, 21700 lithium-ion battery cells in an air-cooled battery thermal management system. To do this, six varying layout arrangements were created, Fig. 1. The six layout variations will then be simulated withing ANSYS Icepak, a numerical thermal and CFD solution software, to simulate a battery thermal management system.

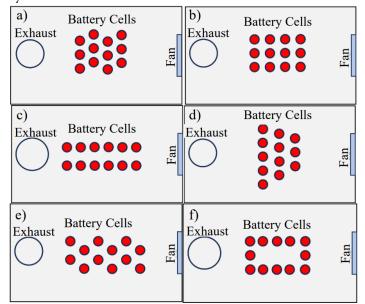


Fig. 1 Layout Variations: a) 3 x 4 staggered, b) 3 x 4 in-

<sup>&</sup>lt;sup>2</sup> Associate Professor, Department of Mechanical Engineering, Auburn University

<sup>\*</sup> Corresponding author: nzb0051@auburn.edu

line, c) 2 x 6 in-line, d) triangular, e) 2 x 6 staggered, f) rectangular

The layout variations were tested with varying heat generation wattages for each cell, 3 W, 15 W, 30 W, 50 W, and 120 W. Typically, lithium-ion battery cells of this size generate about 3 W of heat during discharge and recharge periods. Due to the limited number of cells in the study, increased wattages were used to simulate larger numbers of cells in the pack, thus allowing for larger gradients in temperature variation.

ANSYS Icepak is a computational fluid dynamics simulation software that uses three fundamental conservation equations to effectively model fluid dynamics and thermal problems. The fundamental equations used to solve the problem are the conservation of mass (1), conservation of momentum (2), and the conservation of energy (3).

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho V) = 0 \tag{1}$$

$$\rho\left(\frac{\partial V}{\partial t} + V \cdot \nabla V\right) = -\nabla p + \nabla \cdot \tau + \rho g \tag{2}$$

$$\frac{\partial}{\partial t}(\rho e) + \nabla(\rho eV) = \nabla \cdot (q + \tau V) - p\nabla \cdot V \tag{3}$$

The simulation solver was verified by using a mesh sensitivity analysis. In this, the number of elements within the solution is adjusted, a temperature value is recorded, and the element size is increased until the temperature value plateaus. This is the point at which a increase in the number of elements does not affect the solution, Fig. 2. The final number of selected elements was 365,428.

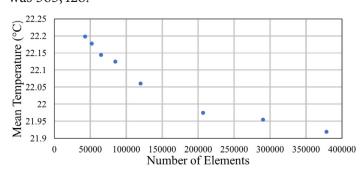


Fig. 2 Mesh Sensitivity Analysis

The simulations were run in ANSYS Icepak, the six variations were tested within each heat generation value. The battery cell temperatures for the 3 W, 15 W, 30

W, 50 W and 120 W amounts are located in Tables 1, 2, 3, 4, and 5, respectively.

**Table 1** 3 W Cell Heat Generation Cell Temperature Values

Layout	Minimum (°C)	Maximum (°C)	Mean (°C)
3x4 Organized Rows	21.54	22.20	21.96
3x4 Staggered Rows	21.55	22.21	21.97
2x6 Organized Rows	21.45	22.27	21.99
2x6 Staggered Rows	21.45	22.29	21.99
Triangular	21.60	22.14	21.93
Rectangular	21.47	22.19	21.91

**Table 2** 15 W Cell Heat Generation Cell Temperature Values

Layout	Minimum (°C)	Maximum (°C)	Mean (°C)
3x4 Organized Rows	42.74	46.01	44.84
3x4 Staggered Rows	42.77	46.05	44.86
2x6 Organized Rows	42.46	46.55	45.13
2x6 Staggered Rows	42.20	46.34	44.88
Triangular	43.0	45.70	44.67
Rectangular	42.47	46.07	44.69

**Table 3** 30 W Cell Heat Generation Cell Temperature Values

Layout	Minimum (°C)	Maximum (°C)	Mean (°C)
3x4 Organized Rows	50.48	57.02	54.68
3x4 Staggered Rows	50.54	57.11	54.73
2x6 Organized Rows	49.93	58.10	55.26
2x6 Staggered Rows	49.40	57.69	54.76
Triangular	51.03	56.40	54.34
Rectangular	49.94	57.14	54.38

**Table 4** 50 W Cell Heat Generation Cell Temperature Values

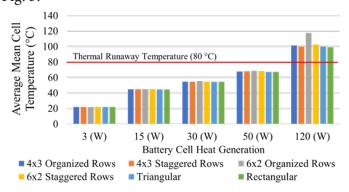
Layout	Minimum (°C)	`Maximum (°C)	Mean (°C)
3x4 Organized Rows	60.81	71.70	67.80
3x4 Staggered Rows	60.90	71.85	67.88
2x6 Organized Rows	53.89	73.51	68.78
2x6 Staggered Rows	59.00	72.83	67.94
Triangular	61.72	70.66	67.24
Rectangular	59.90	71.90	67.30

**Table 5** 120 W Cell Heat Generation Cell Temperature Values

Layout	Minimum (°C)	Maximum (°C)	Mean (°C)
3x4 Organized Rows	83.84	110.84	101.27
3x4 Staggered Rows	83.11	109.79	100.20
2x6 Organized Rows	95.86	127.97	117.59
2x6 Staggered Rows	80.04	114.75	102.56
Triangular	86.89	108.31	100.14
Rectangular	80.14	110.37	98.971

The cell temperature values are the minimum, maximum, and mean temperatures of all of the cells within the model.

The values calculated in the simulation were plotted against each other to examine the most effective layout, Fig. 3.



**Fig. 3** Comparison plot of the average cell temperature for each layout at each heat generation value

The following figures show the temperature gradients of the individual cells and of the air in the simulation results, Fig. 4, Fig. 5, and Fig. 6.

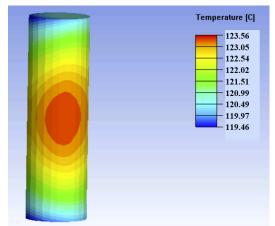
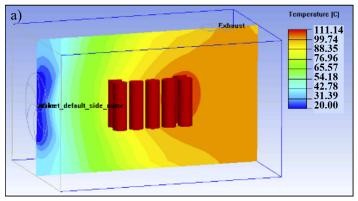
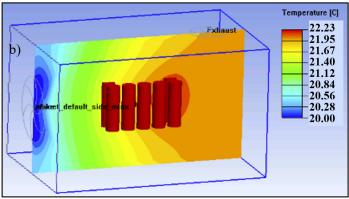
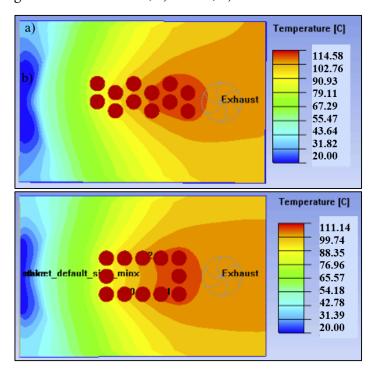


Fig. 4 Individual Cell from 120 W Rectangular Layout





**Fig. 5** Rectangular layout variations due to cell heat generation variations, a) 120 W, b) 3 W



**Fig. 6** Variations in temperature due to layout variations at 120 W cell heat generation, a) 2 x 6 staggered, b) rectangular

From the previous results in the numerical simulation, in ANSYS Icepak, conclusions about the optimal layout arrangement as well as the maximum wattage that can be produced from the cells in these arrangements.

- 1. The rectangular layout proved to have the lowest mean temperature in all of the cell heat generation amounts. The difference between the rectangular and 2 x 6 organized rows at 120 W is 18.619 (°C)
- 2. To stay within the optimal cell temperature range, less than 15 W must be generated in the cell, in any arrangement. To stay within the thermal runaway temperature region, less than 50 W must be generated for any arrangement

#### Nomenclature

P = density (kg/m3)

t = time(s)

V =velocity (m/s)

g = gravity (m/s2)

 $\tau$  = shear stress (N/m2)

p = pressure (Pa)

e = energy(J)

#### **Statement of Research Advisor**

This research done by Nick Bensman on battery thermal management systems is important as the demand for more efficient electric vehicles is growing.

- Dr. Mehmet Arik, Department of Mechanical Engineering, Samuel Ginn College of Engineering

#### References

- (1) Waldmann, T., Scurtu, R. G., Richter, K., & Wohlfahrt-Mehrens, M. (2020). 18650 vs. 21700 Li-ion cells—A direct comparison of electrochemical, thermal, and geometrical properties. Journal of Power Sources, 472, 228614.
- (2) Tete, P. R., Gupta, M. M., & Joshi, S. S. (2021). Developments in battery thermal management systems for electric vehicles: A technical review. Journal of Energy Storage, 35, 102255.
- (3) Vendra, C. M., Shelke, A. V., Buston, J. E., Gill, J. Howard, D., Read, E., ... & Wen, J. X. (2022). Numerical and experimental characterisation of high energy density 21700 lithium-ion battery fires. Process Safety and

Environmental Protection, 160, 153-165.

#### **Authors Biography**



Nick A. Bensman is a senior-year student pursuing a B.S. degree in Mechanical Engineering at Auburn University. He has focused on thermodynamic systems and electronics thermal management while at Auburn. He is graduating in May, 2024 with his bachelor's degree in Mechanical engineering, and will be working for Bollinger Shipyard as a thermal design engineer for naval vessels.



Dr. Mehmet Arik is an associate professor in the Mechanical Engineering Department. His research focuses on electronics thermal management, energy systems and photonics systems.

# Pitch Perception and Production in Music: A Study Determining the Benefits in Aided Versus Unaided Listening

Bailey Branham<sup>1,\*</sup>, Payton H. Lack, Au.D.<sup>2</sup>, Mary J. Sandage, SLP, Ph.D.<sup>3</sup>, Kathleen C. Lea, Au.D.<sup>3</sup>, Aurora J. Weaver, Au.D., Ph.D.<sup>3</sup>

Undergraduate Student, Department of Speech, Language, and Hearing Sciences, Auburn University
 Auburn Alumnus, Department of Speech, Language, and Hearing Sciences, Auburn University
 Faculty, Department of Speech, Language, and Hearing Sciences, Auburn University

To date, there is limited insight into how hearing aid technology influences musicians' singing accuracy. This case study investigated the benefits of properly fit amplification for a musically inclined individual, specifically, music perception, pitch perception, and singing accuracy (i.e., pitch production) were measured with and without amplification. Outcomes with properly fit (verified) amplification resulted in enhanced pitch discrimination and music perception scores; however, the Seattle Singing Accuracy Protocol resulted in a robust (34%) improvement in singing imitation accuracy. The case study suggests that the SSAP would be a promising tool to validate (measure benefit outcomes with) amplification fitting for singers.

Pitch discrimination is a fundamental part of everyday listening and is the defining aspect of melody in music (Oxenham, 2012). Hearing loss distorts an auditory signal in many ways, including diminished pitch perception and discrimination. Today's hearing aids use rapid digital signal processing to alleviate some of the burden that individuals with hearing loss experience in difficult listening situations. While there is ongoing research aimed to improve both the perception of speech and music (Groth, 2018; Coburn & Seitz, 2017; Frohlich & Littmann, 2017), tools to validate such benefits are lacking. This case study aimed to identify tools in agreement with the self-reported perceived benefit of hearing aids in pitch perception and production of a hearing aid user. Two tools were investigated: AngelSound and the Seattle Singing Accuracy Protocol (SSAP). AngelSound has a music module used for assessment, and auditory training for those with hearing loss (AngelSound, 2006). SSAP is a newer tool designed to provide a basis for the study of singing with populations of different ages and levels of training to compare performance accuracy (Demorest, 2015). This case study investigated the benefits of amplification in relation to music listening and singing production abilities, as well as patient-reported outcomes under two conditions: aided (with hearing aids) versus unaided (without hearing aids).

Data was collected on a case series manner with two participants, one male participant, age 23 years, and one female, age 21 years, presenting with a bilateral, sensorineural hearing loss sloping from within normal limits to a moderately-severe high-frequency loss.

Data collection included comprehensive hearing evaluation, real ear measures for verification of prescribed gain, Melodic contour recognition and segregation (AngelSound), SSAP (Seattle singing accuracy protocol) and a validation questionnaire (SASS- Self-assessment of Amplification with Singing Scale), which was created for the purpose of this study. The results with amplification included subjective measurement of participants' self-perceived reactions of confidence, perceived accuracy, engagement, and expectations. With properly fit amplification, we would assume scores would decrease showing higher (lower scored) confidence, perceived accuracy, engagement, and expectations.

Data collection is ongoing, trying to rectify software server limitations. Efforts are being made to revive the SSAP program in a standalone using OpenScience-Framework; ideally, the SSAP will be running again to finish data collection on participants in the up-and-

<sup>\*</sup> Corresponding author: bmb0109@auburn.edu

coming months.

As of now, there are positive trends that show an increase in pitch perception and production with amplification versus without amplification. From gathered data, singing accuracy and pitch perception (16% average across listening conditions) and production abilities are improved (up to 34% increase in singing accuracy) with the use of properly fit amplification. There is room to say that proper fitting amplification can elicit positive outcomes in pitch perception and production in hearing impaired individuals who are musically inclined.

#### Statement of Research Advisor

Bailey was an integral part of the progression of this project during her Undergraduate Research Fellowship. She revised the literature and manuscript, collected additional data, and participated in the dissemination of the findings to both hearing aid manufactures and audiologist working with patients that use Hearing aids. She presented the work at a national conference; AAA (American Academy of Audiology) 2024+HearTECH. - Aurora J. Weaver, Speech, Language, and Hearing Sciences, College of Liberal Arts

#### References

Coburn, C., & Seitz, K. (2017). Music Streaming with Halo 2: Fitting Tips for Patient Success. https://star-keypro.com/pdfs/technical-papers/Music\_Streaming\_with\_Halo\_2.pdf

Demorest, S. M., et al. (2015). "Methodological perspectives on singing accuracy: An introduction to the special issue on singing accuracy (Part 2)." Music Perception: An Interdisciplinary Journal 32(3): 266-271.).

Fröhlich, M., & Littmann, V. (2017). Comparison of Music Sound Quality Between Hearing Aids and Music Programs. https://www.audiologyonline.com/audiology-ceus/course/comparison-music-sound-quality-between-29850

Groth, J. (2018). The technical proof for clearer, fuller, and richer sound with ReSound LiNX Quattro. https://pro.resound.com/en-sg/research

Oxenham, A. J. (2012). Pitch perception. Journal of Neuroscience, 32(39), 13335-13338.

#### **Authors Biography**



Bailey Branham is a junior-year student pursuing a B.S. degree in Speech, Language, and Hearing Sciences at Auburn University. Bailey has been a member of the Auditory Music Perception lab at Auburn for three years and has plans to pursue her Doctorate in Audiology post-graduation



Payton Lack, Au.D., received his Doctorate of Audiology, in 2023 at Auburn University. Dr. Lack contributed to the development of the protocol for and data collection protocol for this study. He currently practices at Atlanta Hearing Associates in Greensboro, Georgia.



Mary Sandage, Ph.D., is a Professor in the Department of Speech, Language, and Hearing Sciences. Her research covers Hormonal influences on voice function, muscle bioenergetics, performance, & fatigue aspects of voice physiology. Dr. Sandage contributed to the planning and design of the study.



Kathleen Lea, Au.D., is an Assistant Clinical Professor in the Department of Speech, Language, and Hearing Sciences at Auburn University. Dr. Lea is a practicing clinical audiologist in the Auburn University Speech and Hearing Clinic, and she oversaw all clinical and diagnostic real ear measurements for the study.



Aurora J. Weaver, Au.D., Ph.D., is an Associate Professor in the Department of Speech, Language, and Hearing Sciences at Auburn University. Dr. Weaver is the lead researcher in the Auditory Music Perception Lab, and directly oversaw the planning, design, and execution of the study.

## Development of a Pipeline for Low-thrust Trajectory Design and Validation in GMAT

Barbara Brogan<sup>1,\*</sup> and Ehsan Taheri<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Aerospace Engineering, Auburn University <sup>2</sup> Assistant Professor, Department of Aerospace Engineering, Auburn University

#### **Abstract**

The main objectives of the proposed research project were to develop a pipeline for validating and verifying low-thrust trajectories generated using optimal control theory in one of NASA's trajectory optimization tools - the General Mission Analysis Toolkit (GMAT), and to enable low-thrust trajectory design in GMAT by providing externally-generated, high-quality, initial guesses to GMAT. The proposed research consists of tasks related to generating initial guesses for low-thrust trajectory optimization problems. Electric low-thrust propulsion systems have garnered significant attention due to their potential for reducing mission costs and enabling extended mission durations. However, their continuous operation and low-thrust nature of the trajectories presents unique challenges to the trajectory optimization task compared to when traditional impulsive propulsion systems are used. These optimization challenges necessitate the development of sophisticated trajectory design methodologies that can accommodate various mission constraints and objectives. To address these challenges, the project aims at leveraging optimal control theory to accurately model and optimize lowthrust trajectories within GMAT. The project seeks to integrate advanced numerical algorithms to generate high-quality initial guesses, improving the convergence and efficiency of the optimization process. This approach aims to produce more accurate and reliable trajectory solutions, thereby advancing the capabilities of GMAT and contributing to space exploration and technology. By combining these approaches, the research aims to improve the convergence and efficiency of the optimization process within GMAT, ultimately leading to more accurate and reliable trajectory solutions. By integrating optimal control theory techniques with GMAT's capabilities, the research project aims to facilitate the design of efficient and reliable low-thrust trajectories for a wide range of space missions, ultimately contributing to the advancement of space exploration and technology.

#### Introduction

Low-thrust electric propulsion systems have emerged and been used for several decades as a promising propulsion system for space exploration, offering the potential for reduced mission costs and extended mission durations. However, their continuous operation and low-thrust nature poses unique challenges to trajectory optimization problems compared to when traditional impulsive propulsion systems are used. Addressing these challenges requires sophisticated trajectory design methodologies capable of accommodating diverse mission constraints and objectives.

One of the key aspects of creating these optimization problems began with MATLAB's capabilities for optimization of trajectories using various numerical methods. MATLAB's capabilities consist of an optimization toolbox that is useful for solving many trajectory optimization problems. MATLAB also allows researchers to use CasADi [2], which is an efficient optimization package. The indirect and direct methods for solving optimization problem were initially studied and thoroughly examined for their potential in creating better optimization techniques using higher-fidelity dynamical models [1]. The optimization of low-thrust trajectories demands a departure from conventional methodologies, necessitating the development of sophisticated techniques capable of navigating the intricate landscape of space motion under the continuous operation of thrusters. Factors such as the varying thrust magnitude, directionality, and the nuanced interplay with orbital dynamics introduce layers of complexity that is beyond the capability of traditional optimization techniques.

<sup>\*</sup> Corresponding author: bdb0067@auburn.edu

In response to these challenges, the proposed research project embarks on a journey at the intersection of optimal control theory and trajectory optimization within the General Mission Analysis Toolkit (GMAT) framework. By harnessing the principles of optimal control theory, which provide a rigorous mathematical framework for optimizing the performance of various dynamical systems subject to operational constraints, this research endeavors to unlock verification in lowthrust trajectory design. The crux of this endeavor lies in the development of a streamlined pipeline for validating and verifying low-thrust trajectories that are generated using optimal control theory within GMAT. Central to this pipeline is the provision of high-quality initial guesses, meticulously crafted to guide the optimization process towards convergence and accuracy. Through the seamless integration of advanced numerical algorithms and methodologies, the research aims to surmount the computational hurdles inherent in lowthrust trajectory optimization, empowering GMAT to produce trajectory solutions that are not only efficient, but also robust and reliable. Moreover, this research is not merely confined to the realm of theoretical exploration; it is driven by a pragmatic vision of enabling real-world applications in space mission planning and execution. By bridging the gap between theoretical advancements and practical implementation, this research seeks to democratize access to cutting-edge trajectory design capabilities, fostering innovation and efficiency across a diverse spectrum of space missions.

#### **Methods**

The verification step begins with the necessary assumptions made in each of the orbit and trajectory sequences. The simplest case of orbit propagation must be examined to ensure the consistency of the fidelity between the MATLAB model and GMAT's software. To achieve it, a spacecraft was selected to fly a low-earth orbit (LEO) over a span of one month using a MATLAB script under the two-body dynamics only. GMAT's script was then researched and modified to recreate the same two-body dynamical model. The state vector of the spacecraft (i.e. both position and velocity vectors) was then compared between the two simulations to verify their consistency and accuracy.

Once the simplest orbit was verified between the two interfaces, a constant-thrust model was considered to verify that the dynamic models were identical between MATLAB and GMAT. This was achieved by propagating a constant thrust along the velocity vector of the spacecraft over a ten-day time span. Thrust can be modeled using the following equation of motion:

$$\ddot{r} = -\frac{\mu}{r^3}r + \frac{T_{\text{max}}}{m}\frac{r}{|r|},$$

where r denotes the position vector from the Earth to the center of mass of the spacecraft,  $\mu$  denotes the gravitational parameter of the Earth,  $T_{\text{max}}$  denotes the constant maximum thrust magnitude produced by the propulsion system, and m denotes the mass of the spacecraft. The spacecraft mass was assumed to be constant. The thrust time history from MATLAB was then converted into a compatible version for GMAT and a simulation was run and the state vector, consisting of position and velocity vectors, was compared again to verify the fidelity of the dynamics and the results were consistent.

After confirming that the dynamics of both models are consistent, more complex trajectories can be considered and compared. Different dynamics were analyzed including a transfer orbit within the Earth's atmosphere and an interplanetary trajectory on the heliocentric phase of an Earth-to-Mars transfer orbit. Next, an Earth-to-Mars trajectory optimization problem was analyzed under the assumptions of two-body dynamics. Considering the simplest version of this problem as a Hohmann Transfer during the heliocentric phase, we can assume that the first phase is a geocentric hyperbola to escape Earth's sphere of influence (SOI) and a final phase that is a hyperbolic capture trajectory. Using CasADi [2] optimization in MATLAB, the following equations of motion are considered for generating fuel-optimal trajectories:

$$\dot{\mathbf{r}} = \mathbf{v}$$

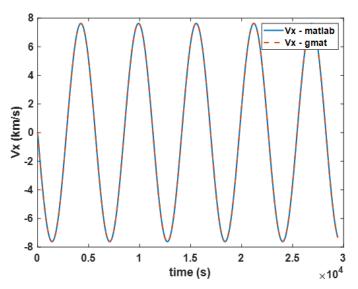
$$\dot{\mathbf{v}} = -\frac{\mu}{r^3}\mathbf{r} + \delta \frac{T_{\text{max}}}{m}\mathbf{u}$$

$$\dot{\mathbf{m}} = -\frac{T_{\text{max}}}{c}\delta$$

where r is the position vector, v is the velocity vector, and u is the thrust steering unit vector. Here,  $0 \le \delta \le 1$  is the throttle setting. When  $\delta = 1$ , the engine is operating and when  $\delta = 0$ , the engine is shuts down. In the equations, c denotes a (constant) effective engine exhaust velocity.

#### **Conclusions**

Confirming the dynamics between the two models proved to be a larger challenge as the GMAT is a relatively complex software. Once dynamic documentation was studied for GMAT, the fidelity of GMAT was able to be reduced to the assumptions of the two-body motion in MATLAB.



**Fig 1.** Portion of velocity vector in GMAT and MAT-LAB

Dynamics are confirmed through the constant thrust model between MATLAB and GMAT through the comparison of state vectors. Figure 1 shows the time history of the x coordinate of the velocity vector. Different line styles are used to show the solutions obtained using MATLAB and GMAT confirming that the solutions are identical.

The optimization model for the fuel-optimal trajectory corresponds to the heliocentric phase of an Earth-to-Mars rendezvous maneuver. The optimization problem was originally solved using CasADi. The thrust vector with respect to a Sun inertial coordinate system can be visualized through the simulation (Figure 3). The spacecraft departs from the Earth with an initial mass of 1000 kg. The trajectory shows that the fuel-optimal solution consists of three segments during which the thruster is operating (i.e.,  $\delta$ =1) and two segments during which the thrust is shut down ( $\delta$ =0). During the thrusting arcs, the magnitude of the thrust is T\_max=0.5 N, but the orientation of the thrust vector changes. The spacecraft reaches Mars with a final mass of 603.83 kg, which is the known optimal solution for

this benchmark problem [3]. Figure 2 depicts the minimum-fuel trajectory.

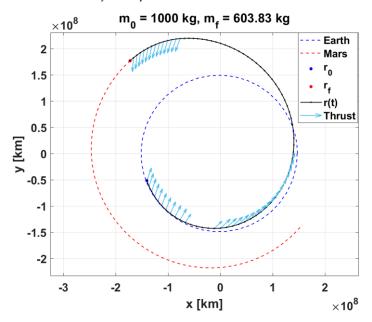


Fig 2. Fuel-optimal trajectory with thrust vectors

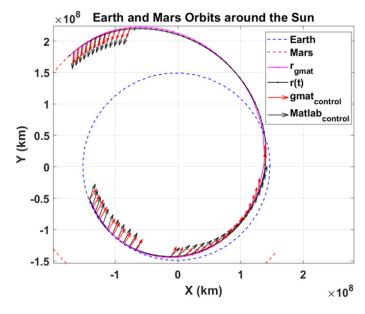


Fig 3. MATLAB and GMAT trajectory comparison

The solution that was generated in MATLAB is implemented in GMAT to compare the two models with an interplanetary transfer orbit. However, we used the thrust history of the unit vector as an initial guess in GMAT. In GMAT, a trajectory targeting sequence has to be defined and this targeting scheme can be initialized. We used the same time discretization between CasADi and GMAT. The comparison between these two models validate the ability to utilize GMAT's fidelity for more

complex problems of interplanetary trajectories in the future. Providing the solution obtained in MATLAB as an initial guess to GMAT improved convergence performance of the optimizer in GMAT (Figure 3). The difference between the trajectories is small with GMAT final mass being 598 kg.

Finalizing this comparison, is the first step, in developing a stream-lined pipeline between MATLAB and GMAT for future obtaining solutions to more complex problems. GMAT's high-fidelity, flight-proven models provide the opportunity to validate future low-thrust trajectories generated in MATLAB with assumptions that can be tailored to the problem at hand as this study provides the framework for modifying a higher-fidelity software for a simplistic problem.

#### **Statement of Research Advisor**

Barbara has taken my graduate-level course titled optimal control of aerospace vehicles. She has worked with MATLAB scripts and GMAT to complete the objectives outlined in this research. She has contributed substantially to the development of the numerical examples.

- Ehsan Taheri, Department of Aerospace Engineering, Samuel Ginn College of Engineering

#### References

- [1] Betts, John T. Survey of numerical methods for trajectory optimization. 21.2 (1998): 193-207, **Journal of guidance, control, and dynamics.**
- [2] Andersson, Joel AE, et al. CasADi: a software framework for nonlinear optimization and optimal control. 11 (2019): 1-36 **Mathematical Programming Computation.**
- [3] Taheri, Ehsan, Ilya Kolmanovsky, and Ella Atkins. Enhanced smoothing technique for indirect optimization of minimum-fuel low-thrust trajectories. 39.11 (2016): 2500-2511, **Journal of Guidance, Control, and Dynamics.**

#### **Authors Biography**



Barbara Brogan is a senior-year student pursuing a B.S. degree in Aerospace Engineering at Auburn University. She has played key research roles in verification of trajectory models between interfaces for MATLAB and GMAT.



Ehsan Taheri is an assistant professor at Auburn University where he leads the Aero-Astro Computational and Experimental (ACE) Lab. Dr. Taheri and his students attempt to solve optimal control problems associated with nonlinear systems and apply numerical methods to astrodynamics problems for deep-space trajectory design.

## **Structured-Light 3D Imaging Using Off-the-Shelf Components**

Alex Brown<sup>1,\*</sup> and Zihe Gao<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Electrical and Computer Engineering, Auburn University <sup>2</sup> Assistant Professor, Department of Electrical and Computer Engineering, Auburn University

Structured-light 3-dimensional (3D) imaging, a method of capturing three dimensional images using a light projector and camera, is a powerful technique widely used in 3D scanning, machine vision, and surface inspection. Structured light allows for highly precise 3D scanning, meaning it is highly useful for taking measurements and constructing accurate 3D models. This study focuses on the design and implementation of a structured-light imaging system, alongside a comprehensive evaluation of various structured light patterns and their effectiveness on diverse surfaces.

Structured-light projection works by projecting multiple light patterns onto a surface. One or multiple cameras capture images of the projections. The projected pattern appears distorted on the surface, and the images of multiple distortions can be used to find the corresponding points from the projector's perspective and construct an image from the perspective of the projector. This new image can be utilized with the existing camera image to compute the depth of the surface. The computed depth is used to generate a three-dimensional image.

The two patterns selected were gray coded [1] and sinusoidal [2] Gray code patterns use vertical stripes of light where the stripe is either completely on or off, while sinusoidal patterns project a light pattern where the intensity of the light is represented by a sinusoidal function. The patterns were projected onto surfaces with varying textures and reflectivity. The captured data was analyzed to determine the robustness and accuracy of 3D reconstructions derived from each pattern type.

The resolution of our 3D scanning was limited by both the camera resolution and the projector fringe quality. To increase the resolution, we moved the camera to zoom in onto the object (Figure 1). To improve the projector fringe, we used an external lens to shrink the image size and achieved better spatial resolution in the projected image.



Fig. 1. Projector and camera setup using external lens for increased special resolution.

The projector and camera were calibrated using a standard calibration procedure to ensure accurate correspondence between the projected patterns and captured images. Both Gray coded and sinusoidal patterns were projected onto the object sequentially. For Gray coded structured light, 10 binary patterns were used, while for sinusoidal structured light, 3 sinusoidal patterns with varying phases were projected. The captured images were processed to decode the Gray codes and sinusoidal phases [3]. Correspondence maps were generated for both methods (Figure 2). The 3D shape of the object was reconstructed using the correspondence maps obtained from both methods.

The experiment showed that both Gray coded and sinusoidal structured light methods produced accurate 3D reconstructions. The sinusoidal structured light

<sup>\*</sup> Corresponding author: kab0182@auburn.edu

method demonstrated higher accuracy, especially for smooth surfaces, due to its continuous nature and finer phase resolution. There were noticeable gaps in the reconstructions generated by the gray coded methods. Both methods of structured light were unable to generate accurate reconstructions of reflective surfaces.

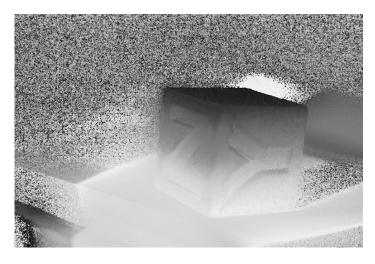


Fig. 2. Projector Correspondence Image using Sinusoidal Structured Light.

This experiment provides valuable insights into the performance of Gray coded and sinusoidal structured light methods. Both methods have their strengths and can be selected based on the specific needs of the application. Future research could explore methods for effectively utilizing structured light on reflective surfaces and achieving better resolution with differing projector and camera setups and different equipment.

#### Statement of Research Advisor

Alex, together with his teammates, constructed a structured-light 3D imaging system using off-the-shelf components. The 3D imaging system has demonstrated enough resolution to view ~mm scale features on 3D-printed components. Alex's direct contributions in both the construction of the hardware and the fringe-based projection code in the software made this demonstration possible. This system will serve as the platform and performance baseline to guide future miniaturized and polarization-based 3D imaging systems.

- Zihe Gao, Department of Electrical and Computer Engineering, Samuel Ginn College of Engineering

#### References

[1] Zhang, S., High-speed 3D shape measurement with structured light methods: A review, Optics and Lasers in Engineering, Volume 106, 2018, Pages 119-131, https://www.sciencedirect.com/science/article/pii/S0143816617313246 [accessed May 14, 2024]

[2] Geng, J., "Structured-light 3D surface imaging: a tutorial," Adv. Opt. Photon. 3, 128-160 (2011).

[3] Xu, Yi, and Daniel G. Aliaga. "Robust pixel classification for 3D modeling with structured light." In Proceedings of Graphics Interface 2007, pp. 233-240. 2007.

#### **Authors Biography**



Alex Brown is a senior-year student pursuing a B.S. degree in Electrical Engineering at Auburn University. He assisted with the creation of the structured light scanner and the software for the different structured light methods.



Dr. Zihe Gao is an Assistant Professor in the Department of Electrical and Computer Engineering at Auburn University. Previously, he was a postdoctoral researcher at the University of Pennsylvania, and prior to that, he served as a postdoctoral research scientist at Meta. Dr. Gao received his PhD in electrical and computer engineering from the University of Illinois at Urbana-Champaign and MS and BS degrees in physics from the University of Illinois at Urbana-Champaign and Nanjing University, respectively. His research interest is in photonic devices and imaging systems.

### **Dual-Task Balance in Healthy Adults**

Connor Cantrell<sup>1,\*</sup>, Danielle M. Lang<sup>2</sup>, Kristina A. Neely<sup>3</sup>

<sup>1</sup> Undergraduate Student, Department of Biomedical Sciences, Auburn University
 <sup>2</sup> Post-Doctoral Fellow, Center for Military Neuroscience and Psychiatry, Walter Reed Army Institute of Research
 <sup>3</sup> Associate Professor, School of Kinesiology, Auburn University

Postural control is the ability to regulate balance and stability and is affected by increases in cognitive load (i.e., a dual-task situation) [1]. In a dual-task paradigm, participants perform a cognitive and motor task simultaneously. Also, dual-task balance is associated with specific brain wave functions [2]. This study aims to determine whether we can replicate and extend prior work using a new, affordable, and portable mobile electroencephalography (mEEG) device, the Muse headband.

The purpose of this study was to determine the relationship between postural control and changes in cognitive load: surface, vision, and task. The feasibility of the mEEG device, Muse Headband, was tested. The Muse mEEG device has not been reported in a standing balance study.

Twenty-five young adults (19-25 years, 13 women) who were able to walk and stand without assistance completed this study. Before data collection, participants completed written informed consent. Participants completed a demographic questionnaire and the Patient Health Questionaire-9 (PHQ-9) to assess the degree of depression severity over the past two weeks. Participants completed eight conditions on the Biodex Balance System: surface (firm/foam), vision (full vision/ no vision), and task (single-task/dual-task). Two 30-second trials were completed for each condition. Postural sway and mEEG data were recorded for the 30-second trials. The order of the conditions was pseudorandomized to prevent any bias.

For all trials, participants were instructed to rest their arms by their sides and look straight ahead. For each dual-task trial, participants were given a 3-digit starting number between 200-999. Participants were instructed

to subtract by three from the given three-digit number. Verbal responses in the dual-task trials were recorded. During no-vision trials, participants were instructed to close their eyes at the beginning of the trial and instructed when to open their eyes.

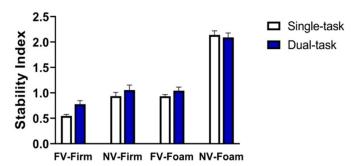
Postural control was measured by the Stability Index (SI) on the Biodex Balance System. SI is the angular movement of the center of gravity [3]. A larger SI is associated with more movement off the center of gravity. For all conditions, participants' feet were positioned at the same place on the Biodex Balance System by lab assistants across both the firm and foam surfaces. The Muse mEEG device was placed around the participant's head at the beginning of the study. The mEEG device enabled real-time recordings of electrical activity in the brain.

There was no observed correlation between SI and age, body mass index, or depression. Therefore, none of these variables were used as a covariate in the analysis. We conducted a 2 (surface: firm/foam) by 2 (vision: full vision/ no vision) by 2 (task: single-task/dual-task) repeated measures analysis of variance. We observed the main effects of vision, surface, and task (ps < .05) for the SI. As expected, the SI increased as a function of cognitive load. The largest SI occurred during no vision and foam surface trials independently of the task [Figure 1].

There was an interaction between vision and surface (ps < .001). SI was greater when standing on the foam surface compared to the firm surface; however, as shown in Figure 2, the magnitude of the difference was greater in the no vision condition. There was an interaction between vision and task (ps < .001), such that SI was greater in the dual-task compared to the single-task

<sup>\*</sup> Corresponding Author: clc0166@auburn.edu

condition, but only in the full vision condition [Figure 3].



**Fig. 1** Stability Index for all Trials

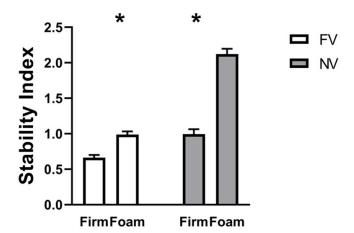


Fig. 2 Vision by Surface

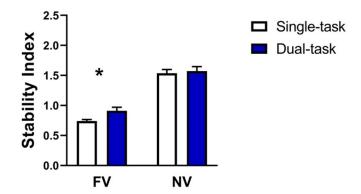


Fig. 3 Vision by Task

The SI results are consistent with previous literature in that increased cognitive load and increased difficulty in motor tasks influence the SI.

Currently, we are using spectral analysis to determine if changes in cognitive load are associated with changes in EEG activity and power. Alpha waves are associated with relaxed and/or meditative states, while beta waves are associated with alertness and increased concentration. Based on previous studies, it is predicted that the absolute power of brain waves will be correlated with task performance and cognitive load. Specifically, it is predicted that the absolute power of alpha waves will decrease in dual-task conditions due to increased concentration and stimulation [4]. The absolute power of beta waves is predicted to increase during dual-task trials since beta waves are associated with alertness.

#### **Statement of Research Advisor**

Connor's research represents an important step in determining the feasibility of using a mobile electroencephalography (mEEG) device during whole-body postural control. He replicated previous work demonstrating that cognitive load impacts sensorimotor control and indeed demonstrated that mEEG is a viable option for data collection. Ultimately, his work has a pivotal role in shaping the future work in our lab. Connor assisted in the design of the research, planned and executed data collection, and assisted with the signal processing and statistical analysis. He presented his project locally at the AU student symposium and will present nationally at the annual meeting of the North American Society for the Psychology of Sport and Physical Activity in June.

- Kristina A. Neely, School of Kinesiology, College of Education

#### References

[1] Ghai, S., Ghai, I., & Effenberg, A. O. (2017) Effects of dual tasks and dual-task training on postural stability: a systematic review and meta-analysis. Clinical Interventions in Aging, 12, 557-577.

[2] Kahya, M., Gouskova, N. A., Lo, O. Y., Zhou, J., Cappon, D., Finnerty, E., Pascual-Leone, A., Lipsitz, L. A., Hausdorff, J. M., & Manor, B. (2022). Brain activity during dual-task standing in older adults. Journal of Neuroengineering and Rehabilitation, 19(1), 123.

[3] Oh, Ki & Kim, Soo & Lee, Seung Yeol & Lee, Young. (2011) Comparison of manual balance and balance board test in healthy adults. Annals of Rehabilitation Medicine. 35, 873-9.

[4] Xavier, Gregory et al. (2020). "Exploratory study of brain waves and corresponding brain regions of fatigue

on-call doctors using quantitative electroencephalogram." Journal of Occupational Health, 62(1), 243.

#### **Authors Biography**



Connor Cantrell is a third-year student pursuing a B.S. in Biomedical Sciences with a minor in Business at Auburn University. He has been a research assistant for the past 2 years and is currently a research fellow in the Brain and Behavior Lab leading the Dual-Task Balance in Healthy Adults study.



Dr. Danielle Lang is a recent Auburn graduate currently working as a post-doctoral researcher at Walter Reed Army Institute of Research. Her research focuses on the use of wearables in analyzing physiological parameters of health across various populations.



Kristina A. Neely, Ph.D. is an Associate Professor in the School of Kinesiology and director of the Brain and Behavior Lab. Her research focuses on the influence of movement on cognitive functions, such as inhibitory control and working memory.

# **Engineering the Chicken Microbiome for the Prevention of Histomoniasis**

Jazmine D. Carroll<sup>1,\*</sup> and John F. Beckmann<sup>2</sup>

Undergraduate Student, Department of Entomology and Plant Pathology, Auburn University
 Associate Professor, Department of Entomology and Plant Pathology, Auburn University

Histomoniasis is a parasitic disease seen in poultry that is known to cause severe lesions in the liver. The disease, once contracted by a single bird, can spread rapidly across the population, causing up to 100% flock mortality in turkeys and 10-20% flock mortality in chickens (McDougald, 2005). Treatments for histomoniasis include chemical compounds such as nitroimidazoles. However, there are no treatments or preventative drugs that are currently approved by the FDA for use in food producing birds, leading to large economic losses (Regmi, Hungerford, Messenheimer, Zhou, Pillai & Gilbert, 2016). The causal parasite of this disease, Histomonas meleagridis, is a protozoan that is transmitted primarily in the eggs of the cecal worm Heterakis gallinarum. The goal of this research is to identify culturable microorganisms within the cecum that can be genetically engineered to eradicate H. meleagridis within the cecum, developing an effective probiotic preventative of histomoniasis.

Our intention was to isolate fast-growing culturable microbes as subsets of the chicken ceca microbiome for downstream engineering. Five chickens were chosen at random to be euthanized by CO2 inhalation and cervical separation. Each of these chickens were dissected with sterile technique. The contents of each cecum were removed and mixed with phosphate buffered saline (PBS) at a ratio of 9 mL per gram of body fluid sample. Each ceca sample was then serially diluted in PBS with 10-fold dilutions down to concentrations of 10-4, 10-5, 10-6, 10-7, and 10-8. The three lowest concentrations (10-6, 10-7, and 10-8) were plated on three different media including Luria Broth (LB), tryptic soy agar (TSA) + 5% sheep blood, and plate count agar (PCA) plates, each with 100 µL of dilution sample. Additionally, concentrations of 10-4, 10-5, and 10-6 were plated on yeast extract peptone dextrose (YPD) plates, also with 100 μL of the given sample dilution. The intention of plating on various media was to acquire a diverse array of culturable microbes. In general, each ceca sample was plated on a total of 12 plates. All plates were then incubated for 24 hours at 37°C. After incubation, the plates were imaged and isolated colonies from the most concentrated dilutions were used to start liquid LB cultures. From these liquid cultures, 25% glycerol stocks were created and stored at -80°C.

After culturing the microbes, the next step was to attempt strain identification using molecular biology methods. The primary method of species identification in the 8 unique samples was polymerase chain reaction (PCR) and Sanger sequencing of the 16S and 16S-23S rDNA region. The primers JFB1058 and JFB1060 were used to target the 16S region, while primers JFB1058 and JFB1063 were used to target the 16S-23S region as shown in Table 1. Samples were amplified using the Bio Rad T100 Thermo Cycler for a total of 35 cycles. PCR products were then confirmed using agarose gel electrophoresis as shown in Fig. 1.

To attempt to confirm sequencing identifications via a secondary method, a Gram-stain procedure was performed on samples of each strain to compare the morphologies to those of known species. Gram stain results for each sample were found to match the known morphology of the species as shown on Table 2.

To be able to measure if these bacteria would localize to the chicken cecum when administered orally, we needed to develop trackable fluorescent transformants. Thus, the next step was to transform them with a fluorescent protein marker plasmid. Transformation attempts proceeded using various techniques. These methods included the CaCl2 chemical transformations, electroporations, and conjugations. When each of these methods were attempted, a few colonies grew. However,

<sup>\*</sup> Corresponding author: jdc0120@auburn.edu

when DNA was isolated from these colonies and the same rDNA regions were amplified and sequenced, all samples were confirmed to be Escherichia coli strains different from those identified in the cecum study. These results suggested that the isolated bacteria might be recalcitrant to transformations and that contamination was a concern. While no strain has shown initial success in expressing the fluorescent protein, additional trials will be performed to eliminate E. coli contamination and increase transformation efficiency.

**Table 1** Database of primers used for rDNA sequencing.

Primer Name	Sequence	Description	Annealing Temperature
JFB 1058	5'-AGAGTTTGATCCTGGCTCAG-3'	Forward primer for 16S and 16S-23S regions	58°C
JFB 1060	5'-CGGTTACCTTGTTACGACTT-3'	Reverse primer for 16S region	56°C
JFB 1063	5'-GTACTTAGATGTTTCAGTTCCC-3'	Reverse primer for 16S-23S region	58°C

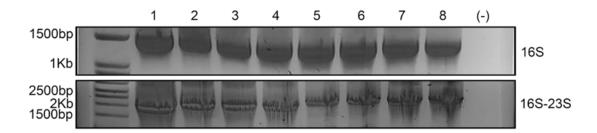


Fig. 1 Agarose gel electrophoresis of rDNA PCR amplicons.

**Table 2** Gram-stain results on bacterial strains.

Sample	Gram Stain Result	Support of Identification	
1. Bacillus amyloliquefaciens	Gram positive rods	✓	
2. Escherichia coli	Gram negative rods	✓	
3. Escherichia coli	Gram negative rods	<b>√</b>	
4. Enterococcus faecium	Gram positive cocci	<b>√</b>	
5. Proteus mirabilis	Gram negative rods	✓	
6. Proteus mirabilis	Gram negative rods	✓	
7. Escherichia coli	Gram negative rods	<b>√</b>	
8. Escherichia coli	Gram negative rods	√	

#### **Statement of Research Advisor**

Jazmine initiated the study with chicken cecum dissections in collaboration with faculty from the Department of Poultry Science. Thereafter, she cultured and isolated colonies, extracted DNA, designed PCR primers, and amplified confirmatory PCR amplicons. Jazmine then spearheaded all transformation attempts using three distinct methodologies which remain to be optimized.

- John Beckmann, Entomology and Plant Pathology, College of Agriculture



John F. Beckmann is an Associate Professor of Biotechnology in the Department of Entomology and Plant Pathology. Beckmann is pursuing a B.S. degree in Computer Science at Auburn University in addition to his other degrees. His research focuses on techniques to limit the transmission of diseases in agricultural contexts.

#### References

McDougald, L. R. (2005). Invited Minireview: Blackhead Disease (Histomoniasis) in Poultry: A Critical Review. Avian Diseases, 49(4), 462–476. http://www.jstor.org/stable/4099188

Regmi, P. R., Shaw, A. L., Hungerford, L. L., Messenheimer, J. R., Zhou, T., Pillai, P., Omer, A., & Gilbert, J. M. (2016). Regulatory Considerations for the Approval of Drugs Against Histomoniasis (Blackhead Disease) in Turkeys, Chickens, and Game Birds in the United States. Avian Diseases, 60(4), 725–730. https://www.jstor.org/stable/26432161

#### **Authors Biography**



Jazmine D. Carroll is a senior-year student pursuing a B.S. degree in Applied Biotechnology at Auburn University. As an undergraduate research assistant, she has contributed work to multiple research projects including microbiome engineering and screening PG-PRs for insecticidal properties.

# Risk Perception Among Scavengers: How Does the Introduction of a Carcass into a Wildlife Landscape Affect the Presence and Overlap of Different Species?

Ally E. Cobern<sup>1,\*</sup>, Ansley L. Strength<sup>1</sup>, Jean E. Fantle-Lepczyk<sup>2</sup>, and Christopher A. Lepczyk<sup>3</sup>

Undergraduate Student, Department of Wildlife Ecology and Management Auburn University
 Assistant Research Professor, Department of Wildlife Ecology, Auburn University
 <sup>3</sup>Professor, Department of Wildlife Sciences, Auburn University

Animals that feed on dead plant and animal matter, called scavengers, play a vital role in maintaining ecosystem health. They perform valuable ecosystem functions, such as recycling nutrients into usable forms, shaping the structure of food webs, and reducing the risk of disease spread from carcasses [3,5]. Despite their established importance as environmental regulators, scavenging dynamics and trophic interactions have been understudied in the southeastern USA. Understanding these dynamics is particularly important in the Southeast, as the region is suffering from biodiversity loss, habitat degradation, and disease [6]. One disease that has already infiltrated the southeast is Avian Influenza A. This disease poses a serious threat to the economy and human and animal health. It can potentially decimate wild and domestic bird populations and infect mammals, including humans [1]. Further-more, It can be spread through interactions at carcasses between scavengers, and because many scavengers are also prey animals, it can eventually be spread to predators as well [4]. Disease transmission through carcass interactions can jeopardize vulnerable southeastern ecosystems, which is why these encounters must be documented.

The objective of our research was to document and understand scavenger interactions and dynamics at carcasses in the Southeast. To study these interactions, we decided to replicate the occurrence of a carcass in an area actively managed for wildlife and habitat management in the southeast. We chose the Wehle tract of the Forever Wild Land Trust in Bullock County, Alabama, which is actively removing wild pigs (Sus scrofa) from its boundaries. Wild pigs are invasive and cause significant property damage and degrade wildlife habitat. We

decided to use the carcasses of these wild pigs to attract the scavengers because they were being dispatched on the property, which means there is a significantly reduced risk of introducing any new pathogens or diseases from the introduction of the carcass. Lastly, we felt that the scavenger population at Wehle was representative of typical southeastern species and that the results would apply to many other wildlife management areas. icant property damage and degrade wildlife habitat. We decided to use the carcasses of these feral hogs to attract the scavengers because they were being dispatched on the property, which means there is a significantly reduced risk of introducing any new pathogens or diseases from the introduction of the carcass. Lastly, we felt that the scavenger population at Wehle was representative of typical southeastern species and that the results would apply to many other wildlife management areas.



**Fig.1.** Turkey vultures (*Cathartes aura*, left two individuals) and American black vultures (*Coragyps atratus*, right two individuals scavenging.

\*Corresponding author: aec0115@auburn.edu

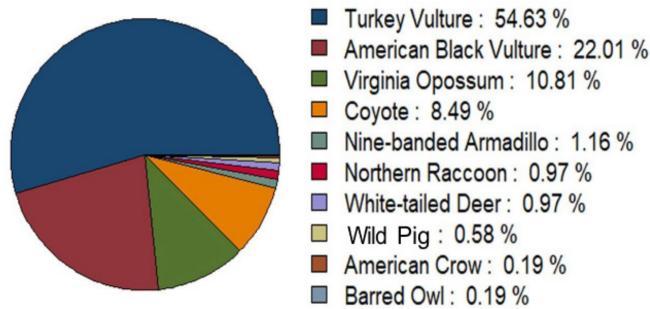
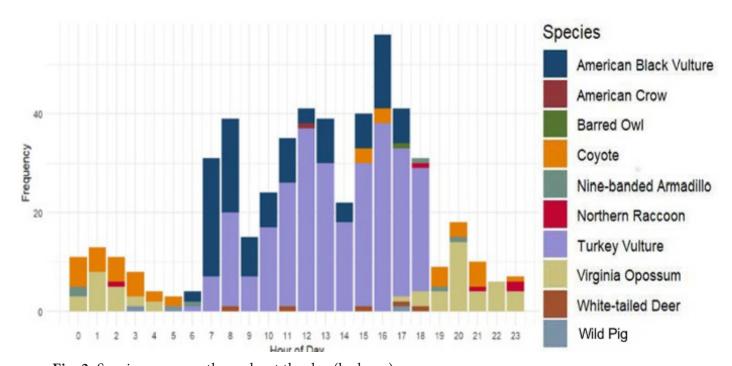


Fig. 2. Proportions (%) of Species in Camera Sequences.



**Fig. 3.** Species presence throughout the day (by hour).

To fully capture the extent of scavenger interactions, we intended to manipulate factors such as cover and perceived risk as they relate to distance from humans and predators. To test these factors, we planned to place multiple carcasses at locations with varying levels of vegetative cover and with varying levels of closeness to human structures and water sources (which were inhabited by alligators). We felt that these considerations

would accurately capture each species' general behaviors as they pertain to scavenging in different types of places. However, we faced several obstacles in implementing these plans and were forced to pivot. The primary challenge was a delay in obtaining the necessary permits to follow through with this experiment. State and Forever Wild permits were not granted challenge was a delay in obtaining the necessary permits to follow

through with this experiment. State and Forever Wild permits were not granted until August, which delayed our intended start in June. The Scientific Collection Permit was granted in August only after being altered to enforce stricter regulations regarding avian flu. While there have been no reported cases of avian flu on the Wehle tract, there have been in other nearby counties [2].

Many of the infected birds were black vultures, (Fig. 1) which are obligate scavengers that have been documented to exist in large numbers at the Wehle tract. This situation limited the collection of carcasses to Bullock County only and banned us from placing carcasses during the peak of avian flu (October 15th, 2023 - February 15th, 2024). It also confined us to one location, meaning we could not compare cover or risk variables. Additionally, because of the reduced timeline, we were only able to place one carcass throughout the duration of the study. Despite these major delays, we were still successful in placing one carcass and have analyzed our findings, which will still be valuable in understanding scavenger dynamics and forming disease models.

We placed one wild pig carcass on the Wehle land tract on October 10th, 2023, and deployed a motion-triggered camera to photograph every species that interacted with the carcass until December 13th. We used Wildlife Insights to identify and catalog every individual and then analyzed using R Studio and Excel. A total of 12,772 photographs and 469 sequences were taken, and we identified ten unique species (Fig. 2). The most prevalent species was the Turkey Vulture (Cathartes aura, n=283), followed by the American Black Vulture (Coragyps atratus, n=114), the Virginia Opossum (Didelphis virginiana, n=56), and the coyote (Canis latrans, n=18). We observed the highest volume of vulture sequences (Turkey and American Black) during the day (6:00 AM-6:00 PM) and it was not uncommon for the two species to appear together (Fig. 3). We did notice an upward trend in black vulture numbers over time, and they were photographed more in the second half of the study. Mammalian scavengers were much more likely to appear at night, and the only other in-ter-species overlap photographed was between rac- coons (Procyon lotor) and opossums (Didelphis virginiana). Three sequences included another wild pig; one was the second day after deployment, and the others were

after the carcass had been entirely scavenged and only bones were left. Despite the lack of variables we were able to test, these results are vital to our understanding of scavenger interaction and can be important tools in understanding disease movement. While not all of the species included in this study have been reported to have avian flu, we can infer that they could all potentially serve as hosts since the disease infects both birds and mammals. Further, each of these species can potentially interact with more species not included in this study based on overlaps in home range and resource use.

#### **Statement of Research Advisor**

Scavengers are an integral part of food webs, though their importance is often neglected and understudied. Ally's research exploring when and how Southeastern wildlife species frequent carcasses provides important information about which species are scavenging carcasses and how these species partition their time to avoid stress or danger from other species.

- Jean Fantle-Lepczyk, College of Forestry, Wildlife and Environment

#### References

[1] Centers for Disease Control and Prevention, Bird Flu Detections Reporting in Wild Birds. Retrieved April 15, 2024, from https://www.cdc.gov/flu/avianflu/data-map-wild-birds.html (2024).

[2] Centers for Disease Control and Prevention, Spread of Bird Flu Viruses Between Animals and People. Retrieved April 15, 2024, https://www.cdc.gov/flu/avianflu/virus-transmission.htm (2024).

[3] DeVault, T.L., Rhodes, Jr., O.E. and Shivik, J.A., Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems. Retrieved April 15, 2024, from https://nsojournals.onlinelibrary.wiley.com/doi/10.1034/j.1600-0706.2003.12378.x, (2023).

[4]OGADA, D.L., TORCHIN, M.E., KINNAIRD, M.F. and EZENWA, V.O., Effects of Vulture Declines on Facultative Scavengers and Potential Implications for Mammalian Disease Transmission. Retrieved April 15, 2024, from https://conbio.onlinelibrary.wiley.com/

doi/10.1111/j.1523-1739.2012.01827.x (2012).

[5] Patterson, J. R., DeVault, T. L., & Beasley, J. C., Integrating terrestrial scavenging ecology into contemporary wildlife conservation and management. Retrieved April 15, 2024, from https://onlinelibrary.wiley.com/doi/10.1002/ece3.9122, (2022).

[6] USGS, Ecosystems in the Southeastern U.S. are Vulnerable to Climate Change. Retrieved April 15, 2024, https://www.usgs.gov/news/national-news-re-lease/ecosystems-southeastern-us-are-vulnerable-climate-change (2024).

#### **Authors Biography**



Ally Cobern is a senior pursuing a B.S. degree in Wildlife Ecology and Management at Auburn University. As an undergraduate research fellow, she played key research roles in study design and implementation, as well as data collection, communication, and presentation.



Ansley Strength is a 2023 graduate from Auburn University with a B.S. degree in Wildlife Ecology and Management. She has played a key role in implementing the experimental design and collecting data at the Wehle Tract. She has worked for the last two years under Dr. Jean Fantle-Lepczyk and Dr. Chris Lepczyk.



Dr. Jean Fantle-Lepczyk is an Assistant Research Professor in the College of Forestry, Wildlife and Environment at Auburn University. She received her B.S. in Wildlife Ecology from the University of Wisconsin-Madison and her M.Sc. and Ph.D. in Zoology from the University of Hawai'i at Mānoa. Her research involves the conservation of wildlife, especially species that are understudied and of conservation concern, using a variety of non-invasive



Dr. Christopher Lepczyk is an Alumni Professor in the College of Forestry, Wildlife and Environment at Auburn University. He received his B.S. in Biology and Geology from Hope College, M.S. in Wildlife Ecology from the University of Wisconsin-Madison, and dual Ph.D. in Fisheries and Wildlife; and Ecology, Evolutionary Biology, and Behavior from Michigan State University. He is an ecologist and conservation biologist who focuses on wildlife, landscapes, citizen science, biodiversity, urban ecosystems, and both endangered and invasive species

## **HYBRID-EXOSKELETON Design & Control**

Joseph Crapet<sup>1,\*</sup>, and Brendon Allen<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Mechanical Engineering, Auburn University <sup>2</sup>Assistant Professor, Department of Mechanical Engineering, Auburn University

Neurological conditions (NC) affect millions of Americans and often reduce the quality of life for those afflicted by decreasing muscular strength and limb control. These can then lead to secondary health conditions such as obesity, muscle atrophy, and other chronic conditions [1]. Although common recreational activities could be beneficial, these individuals may lack the strength or limb coordination required to perform these activities. However, performing these actions with neuromuscular electrical stimulation (NES) can provide sensory afferent feedback for those with NCs. This approach promotes enhanced bone density, muscle development, and motor control, although the individual's endurance limits the benefits. A limitation that rehabilitation robots can and have overcome [2]

Hybrid exoskeletons combine the advantages of NES with rehabilitation robots to effectively help those with NC. The project at hand is to design and construct a hybrid exoskeleton device that will act as a test bed for future studies. The design has two degrees of freedom which allows movement about the patient's elbow and shoulder joints. The NES of the device will eventually be controlled using simple control principles [2]. Participants can utilize the constructed device to perform various actions such as arm flexion and extension.

The project aimed to develop a device allowing motion parallel to the transverse plane and rotation at the elbow and shoulder joints, leading to the creation of a prototype. The first prototype is shown in Fig. 1, and it was meant to help get a simple idea of the device.

Once a basic design was modeled, numerous prototypes were designed and constructed to improve the design. Different methods of approach were considered as well, such as a choice between gears or capstans as a means of torque amplification. This amplification was needed for the system because motors are generally designed

for high speed and low torque applications. Torque amplification solves this problem by taking advantage of basic mechanical advantages principles. Ultimately, capstans were chosen for the setup as Fig. 2 shows.

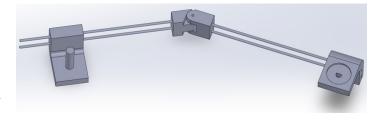


Fig. 1. Basic Design

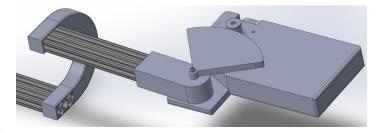


Fig. 2. Initial Capstan Shoulder design

Capstans were chosen as the amplification method because of easy customizability. Moreover, the tension system utilized results in minimal losses due to friction compared to gears. Lastly, the wire tension acts as a hard stop when reaching the end of the capstan's range of motion to provide safety for participants.



Fig. 3. Physical Prototype

With this initial concept designed for the elbow and

<sup>\*</sup> Corresponding author: jlc0130@auburn.edu

shoulder, the parts were 3D printed for evaluation and testing. This physical prototype is shown in Fig. 3.

The physical prototype parts successfully fit together in such a way that allows smooth movement of joints. However, there were noticeable areas for improvement. Firstly, the capstans did not allow the wires to be secured to the design. Secondly, the shoulder capstan moves too closely to the head. Lastly, there were some general areas of improvement needed for the design.

With these areas for improvement being realized, work for improving the design began. These improvements resulted in the shoulder design shown in Fig. 4.

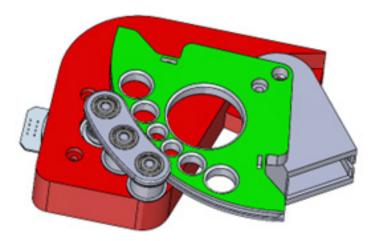


Fig. 4. Current Shoulder Design

This current shoulder design has a few improvements from the initial design. First, the design was updated to allow for a reasonable range of motion for a human with hard stops preventing over extension. Secondly, idler gears with ball bearings were added to offset the capstan's rotation such that it is away from the user's head. Lastly, holes were added to the sides of the capstan that can house a nut and bolt, and other holes on the top and bottom to cut back on material use. Wire that has been crimped together can be looped around the screw to for a strong connection. From here, the screw's depth can be adjusted to loosen or tighten the wire.

With these concepts applied to the design, the current prototype was modeled and can be seen in Fig. 5.

As Fig. 5 shows, the prototype can fit well on a modeled person. Although the idler offset rotated the capstan

away from the person's head, it also added a limit to the shortness of the user's arm. A problem with the blue part in the image was alleviated.

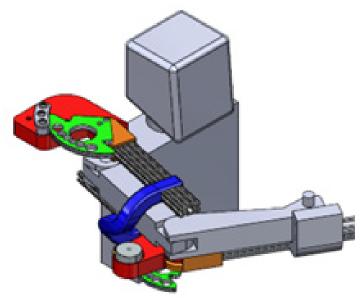


Fig. 5. Complete SolidWorks Design with Person

Once the CAD prototype was completed, the parts were 3D printed and assembled for usage. Leaving motor power and support construction as the main tasks remaining for the physical design.

With most of the physical design constructed, a basic control law was created for the system's motors. To begin with, a parametric equation of a desired path of the hand was derived in terms of the x and y coordinates. The coordinates were then converted into two vectors (representing the desired locations at each time instant), and the associated joint angles of the links were found. This resulted in desired angles of the system that changed as a function of time.

With the desired path determined, an equation of motion for only the exoskeleton was derived using the Lagrange approach. Lastly, PD gains were chosen for each motor and a simulation was performed. This then resulted in Fig. 6 and Fig. 7.

In Fig. 6,  $q_1$  is the angle the upper arm makes with the torso (i.e., the shoulder angle) and  $q_2$  is the angle the lower arm makes with the upper arm (i.e., the elbow angle). Additionally, in Fig. 6 and 7, the solid blue line is the desired path while the dashed orange line is the simulated path.

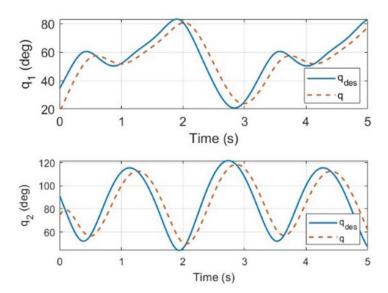


Fig. 6. Angles for Desired and Simulated Paths

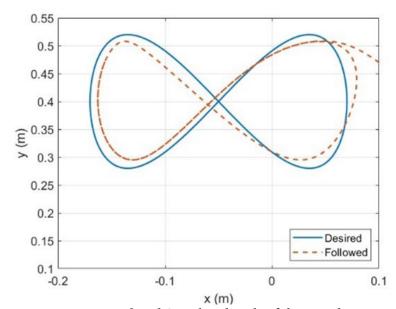


Fig. 7. Desired and Simulated Path of the Hand

As Fig. 6 and 7 show, the basic controller can track closely with the desired path. There is a slight delay though, which causes errors in the path. This delay can be elevated through the development of an estimator or utilizing a square wave method.

With regards to the control law, there are a few simulated iterations that can be developed. Firstly, a constant force can be added that acts perpendicular to the desired path to simulate an ideal scenario of someone wearing the exoskeleton. From there noise can be added to the applied force's amplitude and direction to simulate a person with tremors. With these features added to the system, control laws can be developed to handle

the different scenarios.

Once the exoskeleton is completed and paired with FES, it will act as a test bed to gather data on how effective hybrid exoskeletons can be in rehabilitating people who suffer from NCs [2].

#### **Statement of Research Advisor**

The Controls, Autonomy, and rehabilitative Engineering (CARE) lab in the Auburn University Department of Mechanical Engineering is focused on the design of controllers for nonlinear dynamical systems. In this work, Joseph led the design and construction of a hybrid exoskeleton to assist in the rehabilitation of individuals with movement disorders. The exoskeleton incorporates capstans to reduce friction in the joints and is capable of being adjusted to properly fit individuals with ranging arm sizes. Furthermore, Joseph developed a controller to control the developed exoskeleton. The exoskeleton will be used during experiments in the CARE lab for years to come.

- Brendon Allen, Department of Mechanical Engineering, Samuel Ginn College of Engineering

#### References

[1] Bellman, M. J., Cheng, T. H., Downey, R. J., and Dixon, W. E., "Stationary cycling induced by switched functional electrical stimulation control," 2014 American Control Conference, pp. 4802-4809, (2014).

[2] Anaya, F., Thangavel, P., and Yu, H., "Hybrid FES-robotic gait rehabilitation technologies: a review on mechanical design, actuation, and control strategies," Int. J. Intell. Robot. Appl. 2, pp. 1–28, (2018).

#### **Authors Biography**



Joseph Crapet is a junior-year student pursuing a B.S. degree in Mechanical Engineering at Auburn University. He has primarily worked on the elbow joint design for the hybrid-exoskeleton and control development.

#### Aubufft University Journal of Undergraduate Scholarship



Brendon Allen received his Ph.D. in 2021 from the Department of Mechanical and Aerospace Engineering from the University of Florida. He joined the Department of Mechanical Engineering at Auburn University in 2021. His main research interests include the development of robust, adaptive, or learning Lyapunov-based control techniques for uncertain nonlinear systems such as: rehabilitation robotics, and autonomous systems.

# Benzoic Acid Reduces Glycine Availability for Growth in Starter Pigs

M. Abigail Crosby<sup>1,\*</sup>, Paulo Henrique Amadeu de Azevedo<sup>2</sup>, Alex C. Outlaw<sup>2</sup>, and Marko Rudar<sup>3</sup>

<sup>1</sup> Undergraduate Student, Department of Animal Sciences, Auburn University

The swine industry is not only an economically important industry but an important source of animal protein to the U.S. Maximizing the efficient use of dietary protein for growth while minimizing nitrogen excretion into the environment is important for profitable and sustainable pork production. Diets fed to pigs normally supply protein above their estimated requirements for growth. Although this appears to be wasteful, pig growth performance is sometimes reduced when protein supply is lowered, suggesting that estimated protein or amino acid requirements are not completely understood (Limbach et al., 2021; Rocha et al., 2022). In other words, the amino acid composition of protein, above the requirement for dietary essential amino acids, becomes increasingly important as total protein in the diet is reduced. Moreover, newly weaned pigs can benefit from reductions in dietary protein because flow of undigested protein into the large intestine can have negative effects on pig health (Diether and Willing, 2019; Nyachoti et al., 2006). Before dietary protein can be reliably decreased in swine diets without affecting growth, especially in the nursery, the amino acid composition of protein above the requirement for dietary essential amino acids must be better understood.

Benzoic acid is an organic acid and common feed additive in starter pig diets as an antimicrobial, diet acidifier, and feed preservative. A unique characteristic of benzoic acid metabolism is that it is excreted in urine as its glycine conjugate, hippuric acid, in pigs (Kristensen et al., 2009). Glycine is a dietary non-essential amino acid that, while not usually considered when formulating swine diets, appears to increase growth performance in pigs under specific conditions (He et al., 2023). This study was designed as a proof-of-concept study to demonstrate that the addition of benzoic acid to low protein diets will reduce growth performance in

starter pigs. Further understanding of dietary non-essential amino acid metabolism in pigs will help optimize dietary amino acid compositions to refine swine rations, increase feed efficiency, and decrease environmental nitrogen excretion.

All animal procedures were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2023-5306). A total of 180 pigs were weaned at 28 days (d) age and divided into mixed-sex pens according to body weight (five pigs per pen) across three experimental blocks. Pigs were fed a commercial starter diet for 4 d after weaning before introducing one of three treatment diets: 1) high crude protein (HCP; 19.75% crude protein; n = 12); 2) low crude protein (LCP; 15.75% crude protein; n = 11); and 3) low crude protein + benzoic acid (LCP+BA; 15.75% crude protein and 0.9% benzoic acid; n = 12). Treatment diets were formulated mainly with corn, soybean meal, and dried whey. The HCP treatment diet met the dietary essential amino acid and total protein requirements of starter pigs; the LCP and LCP+BA treatment diets met the dietary essential amino acid requirement but supplied protein at 85% of the total protein requirement of starter pigs. The LCP+BA treatment diet also included benzoic acid at an amount equivalent to the amount of glycine in the LCP diet. Treatment diets were fed to pigs for 28 d.

Average daily gain (ADG) was determined by weighing pigs at the start of the study and every 7 d thereafter for 28 d. Average daily feed intake (ADFI) was determined by calculating the difference between the amount of feed added to pens and the amount of feed leftover in pens at the end of each week. Feed efficiency (FE) was calculated as the ratio between ADG and ADFI (i.e., gain-to-feed ratio). Blood was collected by jugular veni-

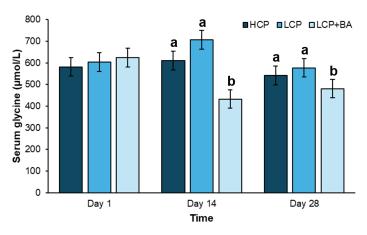
<sup>&</sup>lt;sup>2</sup> Graduate Student, Department of Animal Sciences, Auburn University

<sup>&</sup>lt;sup>3</sup>Assistant Professor, Department of Animal Sciences, Auburn University

<sup>\*</sup> Corresponding author: mac0209@auburn.edu

puncture at the start of the study and every 14 d thereafter for 28 d. Blood was centrifuged at  $2500 \times g$  for 20 min. After centrifugation, serum was collected and stored at -20°C until glycine analysis by HPLC.

Data were analyzed with Statistical Analysis Software (SAS; version 9.4) using the generalized linear mixed model procedure. For growth performance data, treatment was considered a fixed effect and pen and block were considered random effects. For serum glycine concentration, treatment and time were considered fixed effects, pen and block were considered random effects. Differences among treatment diets were determined by the Tukey honest significant difference (HSD) test. Results are reported as least-squares means ± standard error of the mean (SEM). Differences among treatments were considered significant when P-values were less than 0.05.



**Fig. 1.** Serum glycine concentrations on day 1, day 14, and day 28 of the study. Means without a common letter differ (P < 0.05).

Growth performance results are reported in Table 1. Average daily gain was greatest in the HCP group, intermediate in the LCP group, and lowest in the LCP+BA group. Although ADFI was not different among groups, FE was approximately 7% lower in the LCP+BA group compared to the HCP and LCP groups. Serum glycine concentration is reported in Fig 1. Serum glycine concentration was not different among groups on day 1 but was lower in the LCP+BA group compared to either the HCP and LCP groups on day 14 and day 28 of the study.

**Table 1.** Growth performance of starter pigs fed HCP, LCP, and LCP+BA diets for 28 d.

	Treatment <sup>1</sup>			SEM
	НСР	LCP	LCP+BA	SEM
ADG, g/d	580ª	544 <sup>b</sup>	503°	11
ADFI, g/d	1001	944	942	19
FE, g/g	0.58 <sup>a</sup>	$0.58^{a}$	0.54 <sup>b</sup>	0.01

<sup>&</sup>lt;sup>1</sup> Means without a common superscript letter differ (P < 0.05).

These results indicate that adding benzoic acid to diets at or below the total protein requirement of starter pigs reduces body weight gain and feed efficiency. Lower growth performance is likely related to lower serum glycine concentrations. The reduction in serum glycine concentration indicates that glycine was needed to excrete benzoic acid in urine as hippuric acid, lowering glycine available to the pig for growth. This work recommends against using benzoic acid as a feed additive in low protein diets without adding more protein. This work also offers a new prospect of using benzoic acid as a tool to reduce glycine availability for studying dietary non-essential amino acid metabolism in pigs. Overall, maximizing growth performance in pigs fed low protein diets will help reduce both feed costs and environmental nitrogen pollution in pork production systems.

#### **Statement of Research Advisor**

Abigail's work evaluating starter pig responses to benzoic acid will provide the basis for future work in our laboratory studying the metabolism of specific dietary non-essential amino acids, including glycine, in pigs. Abigail was responsible for daily animal care, collecting and analyzing pig growth performance data, and assisting with blood collections and serum glycine analysis.

- Dr. Marko Rudar, Department of Animal Sciences, College of Agriculture

#### References

[1] Diether, N.E. and B.P. Willing. 2019. Microbial fermentation of dietary protein: an important factor in diet-host-microbe interaction. Microorganisms 7: 19. doi: 10.3390/microorganisms7010019

[2] He, W., E.A. Posey, C.C. Steele, J.W. Savell, F.W. Bazer, G. Wu. 2023. Dietary glycine supplementation enhances postweaning growth and meat quality of pigs with intrauterine growth restriction. J. Anim. Sci. 101: 1-11. doi: 10.1093/jas/skad354

[3] Kristensen, N.B., J.V. Norgaard, S. Wamberg, M. Engbaek, J.A. Fernandez, H.D. Zacho, H.D. Poulsen. 2009. Absorption and metabolism of benzoic acid in growing pigs. J. Anim. Sci. 97: 2815-2822. doi: 10.2527/jas.2009-2003

[4] Limbach, J.R., C.D. Espinosa, E. Perez-Calvo, H.H. Stein. 2021. Effect of dietary crude protein level on growth performance, blood characteristics, and indicators of intestinal health in weanling pigs. J. Anim. Sci. 99: 1-14. doi: 10.1093/jas/skab166

[5] Nyachoti, C.M., F.O. Omogbenigun, M. Rademacher, G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 84: 125-134. doi: 10.2527/2006/841125x

[6] Rocha, G.C., M.E. Duarte, S.W. Kim. 2022. Advances, implications, and limitations of low-crude-protein diets in pig production. Animals 12: 3478. doi: 10.3390/ani12243478

#### **Authors Biography**



M. Abigail Crosby graduated in December 2023 with a B.S. degree in Animal Sciences (Animal & Allied Industries option). She has recently completed an internship at American Paint Horse Association.



Paulo Henrique Amadeu de Azevedo is pursuing an M.S. degree in Nonruminant Nutrition in the Department of Animal Sciences, Auburn University. His research focuses on challenges and opportunities related to feeding pigs low protein diets.



Alex C. Outlaw is pursuing an M.S. degree in Nonruminant Nutrition in the Department of Animal Sciences, Auburn University. His research focuses on how variable feed intake affects sulfur amino acid metabolism in newly weaned pigs.



Marko Rudar is an Assistant Professor of Protein Nutrition in the Department of Animal Sciences, Auburn University. His research focuses on protein and amino acid nutrition and metabolism and aims to reduce the inefficient utilization of amino acids for lean protein accretion in pork production systems.

# **Measuring Xylem Characteristics for Drought Tolerance in Peanuts**

Christopher Deveau<sup>1,\*</sup>, Sajid Hanif<sup>2</sup>, Alvaro Sanz-Saez<sup>3</sup>, Hasib Rahman<sup>4</sup>, Tanzeel Rehman<sup>5</sup>, Leah Hoffman<sup>6</sup>, Emily Graff<sup>7</sup>

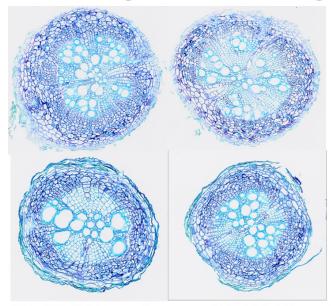
<sup>1</sup>Undergraduate Researcher, Department of Crop, Soil, and Environmental Sciences, Auburn University

#### **Abstract**

Peanuts are commonly cultivated across the world in semi-arid climates and regularly susceptible to droughts and heat stress, causing drastic losses in the production of peanuts. This environment accounts for the exposure of about 65% of peanuts in the United States to extreme abiotic stress, resulting in a significant decrease in yield. Improvements to resistance against abiotic stresses such as droughts is vital for the long-term viability of U.S. peanut production. Xylem characteristics of different xylem sizes can affect the overall plant transpiration efficiency, this has been identified as one of the possible drought tolerant characteristics in plants. We expect to find smaller xylem on more drought tolerant cultivars, allowing the plant to preserve more water during a drought and provide more stability for the plant during drought conditions (Fig. 1.). The process of measuring each individual xylem is a vastly slow and inefficient method to collect data on the characteristics of each root sample. In this project we are adapting an existing AI software, Segment Anything, which is a Meta AI program that can be used in scientific and normal images to be able to segment whatever needs to be analyzed within the image. The segment anything project is to reduce the need for task-specific modeling expertise, so that way anyone can use the AI software to analyze their images, for this project we are using the AI software to measure xylem characteristics in peanuts (Fig. 2.). With this proposal, we grew 5 peanut cultivars in the greenhouse under irrigated and drought conditions and took pictures of the roots. Once the pictures were taken, images were acquired with an Olympus Slide View VS200 microscope. Those images were run through CVAT utilizing Segment Anything for semi-automatic measuring of xylems in each picture, allowing a much more efficient and accurate measurement of the root's xylem characteristics to identify optimum drought tolerant characteristics in peanuts.

PI-502120 Irrigated

**AU-16-28 Irrigated** 



PI- 502120 Drought

AU-16-28 Drought

**Fig. 1.** A Cross section comparison of two cultivars PI-521200 and AU-16-28 grown under irrigated and drought conditions.

#### Introduction

Peanuts are commonly cultivated across the world and regularly susceptible to droughts and heat stress,

<sup>&</sup>lt;sup>2</sup> Graduate Researcher, Department of Crop, Soil, and Environmental Sciences, Auburn University

<sup>&</sup>lt;sup>3</sup> Assistant Professor, Department of Crop, Soil, and Environmental Sciences, Auburn University

<sup>&</sup>lt;sup>4</sup> Graduate Researcher, Department of Biosystems Engineering, Auburn University

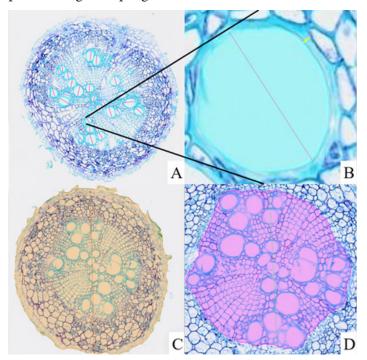
<sup>&</sup>lt;sup>5</sup> Assistant Professor, Department of Biosystems Engineering, Auburn University

<sup>&</sup>lt;sup>6</sup> Research Assistant, Department of Pathobiology, Auburn University

<sup>&</sup>lt;sup>7</sup> Associate Professor, Department of Pathobiology, Auburn University

<sup>\*</sup> Corresponding author: cbd0038@auburn.edu

causing drastic losses in production. In the US, 65% of peanuts grow without irrigation and therefore are susceptible to drought stress. This costs 50 million annually to the peanut industry. Improvements to resistance against abiotic stresses such as droughts are vital for the long-term viability of US peanut production. In other crops it has been demonstrated that having smaller and more abundant root xylem vessels may be a drought tolerant characteristic. The process of measuring each individual xylem is a vastly slow and inefficient method to collect data on the characteristics of each root sample, causing slow progression of data collection.



**Fig. 2.** (A) A root image with xylem annotations done to it. (B) Enlarged picture of the xylem showing xylem area, length, and thickness. (C) Root image showing root area and length. (D) An enlarged picture of the cortex area and length.

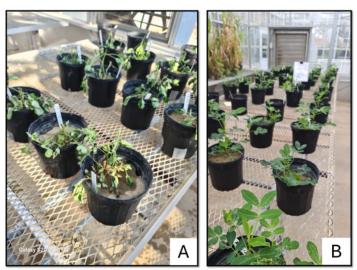
#### Materials and Methods

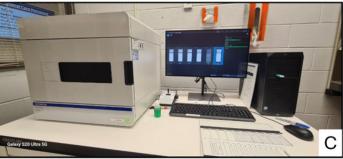
Four peanut lines (PI-502120, AU-NPL-17, AU-16-28, Line 8) with known drought tolerance and one drought sensitive line (AP-3) were grown in the Plant Science Research Center at Auburn, AL (Fig. 3.). Plants were grown under controlled water conditions for 40 days during which the drought treatment started by reducing the soil water capacity to 20% (Drought) while maintaining half of the plants at 100 % of soil water content (Well-water control). Twenty days after drought establishment, the plants were harvested, the

roots cleaned, and secondary roots samples were taken for cross section root analysis. Root xylem histology samples were prepared by the histology service at the Department of Pathobiology and images were captured using an Olympus Slide view VS200 at 20 X of magnification (Fig. 3.).

#### **Results and Future Directions**

A protocol on how to harvest samples, take histologic samples and take images has been created. A step-by-step image acquisition and analysis protocol has been created using the AI software "Segment anything" which will facilitate future experiments in the lab. It can be observed that the cultivars tested here (Figure 1) seem to show differences in number and size of xy-lem vessels and that these characteristics are affected by drought. In future research, all samples from this experiment will be analyzed, and the effect of drought and cultivars will be studied. In addition, an AI model to automatically measure xylem characteristics will be developed.





**Fig. 3.** (A) Peanut plants going through drought phase. (B) The peanut plants being grown under well water conditions. (C) The microscope, Olympus Slide View. V200.

#### **Statement of Research Advisor**

Chris studied how plants modify the xylem vessels, like the blood vessels in animals, when they are under drought stress. To do this Chris teamed up with a graduate student, Sajid Hanif, to take histological images in collaboration with the College of Veterinary Medicine. After that he collaborated with another graduate student in Biosystems engineering (Hasibur Rahman) to use Artificial Intelligence models to automatize the process of image analysis. Although there is still a lot that needs to be done to produce software that is able to recognize and count xylem vessels in plants automatically, Chris took the first steps in this direction. His work has led my lab to new directions in research that look very promising in the future.

- Alvaro Sanz Saez de Jauregui, Crop, Soil, and Environmental Sciences, College of Agriculture

#### References

Thangthong, N.; Jogloy, S.; Punjansing, T.; Kvien, C.K.; Kesmala, T.; Vorasoot, N. Changes in Root Anatomy of Peanut (Arachis hypogaea L.) under Different Durations of Early Season Drought. Agronomy 2019, 9, 215. https://doi.org/10.3390/agronomy9050215

Prince, S. J., Murphy, M., Mutava, R. N., Durnell, L. A., Valliyodan, B., Shannon, J. G., & Nguyen, H. T. (2017). Root xylem plasticity to improve water use and yield in water-stressed soybean. Journal of experimental botany, 68(8), 2027–2036. https://doi.org/10.1093/jxb/erw472

#### **Authors Biography**



Christopher Deveau is a senior-year student pursuing a B.S. degree in Applied Biotechnology. He has currently been involved in Environmental Research for the past three years as a student worker and research fellow, in Dr. Alvaro's Lab. He has contributed to research in the development of A.I. system for measuring xylem and other root characteristics.



Sajid Hanif is a current Ph.D. student at Auburn University, in the department of Crop, Soil, and Environmental Sciences. His research interests are in improving crop growth and performance.



Dr. Alvaro Sanz Saez de Jauregui is an Assistant Professor at Auburn University, in the Crop, Soil, and Environment Science Department. Dr. Alvaro's research is focused on detecting genotypic variation to abiotic stress, like high temperatures, elevated carbon dioxide (CO<sub>2</sub>), and drought. His studies are also looking for new and improved methods of measuring plant responses to environmental stress, as it is a difficult process to do in the field. His research is mostly focused on peanut plants.

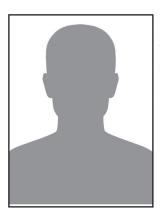


Hasibur Rahman is a current Master's Student at Auburn University and is in the Department of Biosystems Engineering. His main area of research interests is in Artificial Intelligence, Computer Vision, Machine Learning, and Sensors.

#### Aubusa University Journal of Undergraduate Scholarship



Dr. Tanzeel Rehman is an assistant professor at Auburn University, in the Department of Biosystems Engineering and Horticulture. His main area of research is in Artificial Intelligence, Computer Vision, Automation, Robotics, and Precision Horticulture.



Leah Hoffman is a Research Assistant II, with the Ritchey Endowment Income, at the Scott Ritchey Research Center, at Auburn University.



Dr. Emily C. Graff is an Associate Professor at Auburn University, in the Department of Pathobiology. Her main research interest is the investigation of the relationship between adipose tissue and the immune system, with cats as the focus for the intermediate model of human obesity and disease. Her additional research is in the investigation of the physiologic role of the HCA2 receptor in both adipose tissue and immune cells.

# Reading Recall Using Rapid Serial Visual Presentation (RSVP): The Impacts of Pauses on Reading

Walton Ferguson<sup>1,\*</sup>, Alejandro Lazarte<sup>2</sup>, and Joaquin S. Naraza<sup>3</sup>

- <sup>1</sup> Undergraduate Student, Department of Psychological Sciences, Auburn University
  - <sup>2</sup> Associate Professor, Department of Psychological Sciences, Auburn University
  - <sup>3</sup> Undergraduate Student, Department of Computer Science, Auburn University

The Internal Prosody Hypothesis asserts that effective readers will silently mimic regular speech while reading a text (Fodor, 2002). Languages tend to have their own prosodic structure that is most conducive to reading comprehension, and evidence suggests that readers may learn different prosodic pauses and tempos (Wade-Woolley et al., 2022; Speer & Ito, 2009). Further evidence suggests that various aspects of reader prosody can be altered by computer-assisted training (Hardison, 2004). However, most research has focused on prosody as stresses within words (i.e., emphasis given to certain syllables; Nayak et al., 2022) and no research has dealt with prosodic pauses.

Efficient readers will instate short pauses at the end of phrases (Andrews & Veldre, 2021). This phenomenon, labeled by Justen and Carpenter (1980) as the "wrapup effect," occurs when individuals take a short pause at boundary words (e.g., words immediately followed by punctuation marks such as periods or comas). This effect allows readers more time to "dwell" on the information in one clause before moving on to the next one (Breen, 2014; Fodor, 2002). Disrupting this natural pause at the end of clauses has been shown to negatively impact one's ability to recall information from the text (Busler & Lazarte, 2017). Busler and Lazarte demonstrated this effect by using Rapid Serial Visual Presentation (RSVP), a text-presentation technique that allows the manipulation of a reader's tempo by presenting one word at a time. Their experiment included a condition in which participants read the text at their own pace (Self-paced presentation) and two RSVP conditions: one in which each word receives the same amount of time (a "flat" condition) and another in which additional time is provided at boundary words (mimicking the

wrap-up effect). Reading difficulty served as another controlling variable, with each participant being presented with readings of "easy" and "hard" difficulties.

The current project is a pilot study intending to extend Busler and Lazarte's results by (1) replicating the main findings in a new sample, (2) adding an additional experimental condition, and (3) testing a new analysis plan for reading recall. The additional experimental condition provided at random additional time for words in the middle of phrases, i.e. not at end of clauses or end of sentence words, where the wrap-up effect occurs. Extending these results will further validate the benefits of the wrap-up effect by engaging in pausing at the right positions in a sentence and inform future use of computer-assisted prosody training.

We hypothesized that reading recall would be higher for "easy" readings and that recall scores as function of presentation condition would display the following pattern: additional time for random words in the middle of phrases < a "flat" condition where no word received additional time < additional time at the end of phrases, imitating the wrap-up effect < Self-Paced reading. We included general measures of vocabulary, reading comprehension, and working memory to aid in interpretation (Brown, 1981; Oswald et al., 2015).

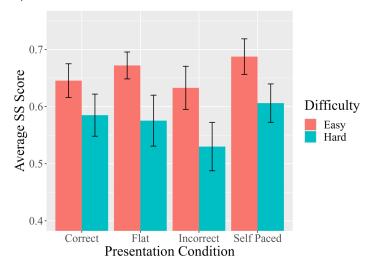
Using reading extracts from early English novels, participants (N=18) were presented 8 readings of "easy" difficulty and 8 of "hard" difficulty and prompted to recall what they read. Readings were randomly presented using one self-paced (SP) and three RSVP conditions: a "flat" condition with no pauses (FL), a "correct" condition with pauses imitating the wrap-up effect (CP), and

<sup>\*</sup>Corresponding author: wfergus2@nd.edu

an "incorrect" condition with random pauses within phrases (IP). Further, participants completed the Nelson-Denny reading assessment as well as a short working memory task as additional auxiliary variables.

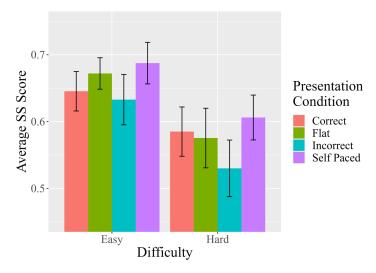
We utilized the Sentence Transformers Python module to calculate a semantic similarity (SS) score for each response, indicating the similarity between the response and the reading (Reimers & Gurevych, 2019). SS scores were averaged by difficulty level at each presentation condition. We then ran a repeated measures ANOVA to compare the impact of presentation condition, reading difficulty, and their interaction on SS scores. Finally, correlation analyses were conducted between main study variables and auxiliary variables.

Results indicated that SS scores were significantly related to reading difficulty (p = 0.02, see Fig. 1) but NOT to presentation condition (p = 0.16, see Fig. 2). Though not significant, the average SS scores for each reading condition trend in the hypothesized direction. (see Fig. 2).



**Fig. 1** Impact of Reading Difficulty on Semantic Similarity Scores

Correlation analyses were conducted with a smaller sample (N = 13) due to missing data. Analyses only revealed two significant correlations, though there were notable trends. Reading comprehension was poorly correlated with all experimental variables while working memory and vocabulary displayed consistently larger correlations for hard readings at each presentation condition (see Tables 1 and 2).



**Fig. 2** Impact of Presentation Condition on Semantic Similarity Scores

**Table 1** Correlation Matrix between auxiliary variables and Self-paced, Flat RSVP conditions. \* = statistically significant correlation at p < 0.05

	SP,	SP,	FL,	FL,
	Easy	Hard	Easy	Hard
Working Memory	0.29	0.43	-0.13	0.22
Vocabulary	0.30	0.66*	0.08	0.21
Comprehension	0.09	-0.01	0.34	-0.33

**Table 2** Correlation Matrix between auxiliary variables and Correct, Incorrect Pause RSVP conditions. \* = statistically significant correlation at p < 0.05

	CP,	CP,	IP,	IP,
	Easy	Hard	Easy	Hard
Working Memory	0.14	0.66*	0.02	0.31
Vocabulary	0.52	0.49	0.32	0.53
Comprehension	0.02	-0.32	-0.18	-0.12

With this small pilot sample, our hypotheses were partially supported. Participants were able to recall more from texts that were less complex, though presentation condition seemed to have no effect on text recall. The SS scores for presentation condition trended in the hypothesized direction (IP < FL < CP < SP) for the "hard" reading condition. This trend is promising for future work even though it did not reach significance.

Though there were few significant results (again, likely due to a small sample size), there were notable trends in the correlation analyses. SS scores tended to be more correlated with hard readings, suggesting that high levels of any auxiliary variable did not affect performance on text recall. Further, reading comprehension displayed poor correlations with SS scores. Assuming that an individual who is better able to comprehend a text will display better recall, these poor results may be highlighting a validity concern with the SS score itself.

The small sample size likely contributed to the lack of significant findings. Further, the SS scores may not measure appropriately the actual recall and therefore may contribute to a lack of significance. While the SentenceTransformers package for testing semantic similarity has been shown to perform well in other contexts, this is the first study in which it has been used to infer recall ability (Reimers & Gurevych, 2019). Future work should consider replicating this study using larger sample sizes and alternative measures of assessing text recall.

#### Statement of Research Advisor

Walton Ferguson has done a superb job in re-starting and updating a line of research that promises exciting developments in training for reading. During this researching endeavor, Walton exceled in training himself in the required software for designing, collecting, and analyzing the data (E-prime, R, Qualtrics) and to contribute with new insights about the use of AI procedures for analyzing the text recalls. Walton designed and presented the poster summarizing our pilot data and received an award for his effort during the AU Undergraduate Research Symposium in 2023.

- Alejandro A. Lazarte, Psychological Sciences, College of Liberal Arts

#### References

Andrews, S., & Veld re, A., "Wrapping up sentence comprehension: The role of task demands and individual differences," Scientific Studies of reading, 25(2), pp. 123-140, (2021).

Breen, M., "Empirical Investigations of the Role of Implicit Prosody in Sentence Processing, "Language and Linguistics Compass, 8(2), pp. 37-50, (2014).

Brown, James I., "The Nelson-Denny reading test: vocabulary, comprehension, reading rate: Forms G and H," Riverside, (1981).

Busler, J. N., & Lazarte, A. A., "Reading time allocation strategies and working memory using rapid serial visual presentation," Journal of Experimental Psychology: Learning, Memory and" Cognition, 43(9), pp. 1375-1386, (2017).

Fodor, J. D., "Prosodic disambiguation in silent reading," Proceedings of the Northeast Linguistics Society, vol. 32, pp. 113-132, (2002).

Justen, M.A., & Carpenter, P.A., "A theory of reading; From eye fixations to comprehension," Psychological Review, 87(4), pp. 329-354, (1980).

Oswald, F. L., McAbee, S. T., Redick, T. S., & Hambrick, D. Z., "The development of a short domain-general measure of working memory capacity," Behavior research methods, 47(4), pp. 1343–1355, (2015).

Reimers, N. & Gurevych, I., Sentence-BERT: Sentence Embeddings using Siamese BERT-Networks. Retrieved March 25, 2024, from https://doi.org/10.48550/arXiv.1908.10084, (August 27, 2019).

Speer, S. R., & Ito, K., "Prosody in first language acquisition: Acquiring intonation as a tool to organize information in conversation," Language and Linguistics Compass, 3(1), pp. 90–110, (2009).

Wade-Woolley, L., Wood, C., Chan, J., and Weidman, S., "Prosodic competence as the missing component of reading processes across languages: theory, evidence and future research," Sci. Stud. Read. 26, pp. 165–181, (2022).

#### **Authors Biography**



Walton Ferguson is a senior undergraduate pursuing a B.A. degree in Psychology at Auburn University. He has spent time working with several faculty at Auburn and has produced research in the areas of interoception and text recall. He has experience in the foundations of psychology and data analytics and will pursue a PhD in Quantitative Psychology at the University of Notre Dame. On this project, Walton served as the lead author and designed and implemented data collection programs.



Dr. Alejandro Lazarte is an Associate Professor in the Department of Psychological Sciences at Auburn University. Originally from Peru, Dr. Lazarte obtained his PhD from Purdue University and has since spent time researching the effects of reading presentation on text recall as well as other topics in cognitive and industrial psychology. Dr. Lazarte produced the foundational ideas for this project and served as the lead faculty mentor.



Joaquin S. Naraza is an undergraduate computer science student at Auburn University. He is an international student originally from Peru and hoped to work in the fields of Natural Language Processing and Machine Learning. Joaquin completed large portions of the analysis work for the current project.

## Students' Attitudes About Virtual Work Post-Pandemic: How Confidence Online and Virtual Communication Skills Drive Intentions for Future Remote Work

Emily Findlay<sup>1</sup> and Sara Driskell<sup>2,\*</sup>

<sup>1</sup> Undergraduate Student, Department of Psychological Sciences, Auburn University <sup>2</sup> Lecturer, Department of Psychological Sciences, Auburn University

Due to the COVID-19 pandemic, a significant portion of the population has experience with online work. While younger generations have spent extended portions of their lives interacting with technology and are known as digital natives, missed socially significant milestones and time spent away from friends and loved ones could also contribute towards negative attitudes about the remote workplace.

The purpose of this study is to examine how students, who will enter the workforce in a few short years, perceive remote work. This work is important so that we can inform policies for re-integrating people into workspaces post- pandemic and improve how organizations and academic institutions utilize remote work moving forward. This study investigates students' self-efficacy (or confidence) in using online tools, their perceived online communication skills, their motivation for completing remote coursework, how much they are satisfied with remote work, and how much they value remote work.

695 Undergraduate students (74.5% women, 82.7% white American) were surveyed through the Auburn University Psychology Department's participant pool. Participants were asked to rate the extent to which they agreed with statements on a 1-5 Likert scale (5 being strongly agree). Sample items from the survey include "Remote work experiences (ex: internships) develop my career just as much as in-person work experiences," to measure students' value of remote work and "I can communicate effectively in one-on-one Zoom or video conferences," to measure students' self-rated online communication skills.

Results provided interesting insights into students' online self-efficacy and virtual communication abilities. Students felt positive about their online self-efficacy (M=4.29, SD=.73), as seen in Figure 1, but they struggled with online communication and participation using Zoom (M=2.86, SD=.55), even after experience during the pandemic. Many students reported being on the receiving end of virtual communications (e.g., listening passively during an online lecture) but rarely presented on Zoom themselves (M=1.85, SD=.98). Additionally, students rated themselves higher for their abilities in email communication but not as highly as they could have for such a common form of communication (M=3.56, SD=.82).

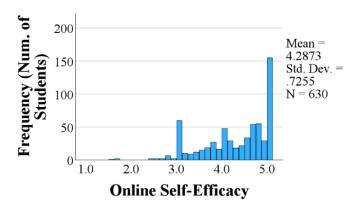
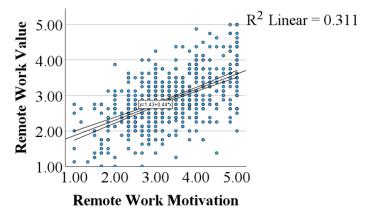


Fig. 1. Students' ratings of their online self-efficacy

Students had lower ratings for remote work satisfaction (M=2.94, SD=.64) and remote work value (M=2.88, SD=.71) but higher ratings for remote work motivation (M=3.34, SD=.91). Online self-efficacy correlated positively with students' remote work satisfaction (r=.227 p<.001), students' remote work value (r=.227 p<.001), and remote work motivation (r=.372 p<.001). Online self-efficacy also positively correlated with stu-

<sup>\*</sup> Corresponding author: driskell@auburn.edu

dents' Zoom and email communication skills (r=.158, p<.001 and r=.394, p<.001, respectively). Furthermore, both Zoom and email communication skills correlated positively with students' Auburn University Journal of Undergraduate Scholarship remote work satisfaction (r=.234, p<.001 and r=.131, p<.001, respectively), remote work value (r=.209, p<.001 and r=.126, p<.001, respectively), and remote work motivation (r=.334, p<.001 and r=.248, p<.001, respectively). Remote work value (r=.557, p<.001) also correlated positively with students' remote work motivation, as seen in Figure 2.

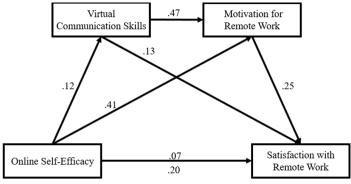


**Fig. 2.** The correlation between students' ratings of their motivation toward remote work and how much they value remote work.

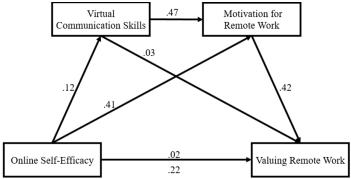
Serial mediation analyses indicate that increased self-efficacy is linked to higher virtual communication skills, which in turn increases motivation for remote work and then predicts students' satisfaction for remote work, as seen in Figure 3 (b=.01, t=3.51, 95% CI [0.0074, 0.02333]), and students' value of remote work, as seen in Figure 4 (b=.02, t=3.70, 95% CI [0.0126, 0.0385]). Because students' self-efficacy ratings were already high, this suggests that increasing students' online communication skills could improve their online motivation, which is linked to higher satisfaction and valuing of remote work.

Future research with Auburn students should develop interventions to improve comfort in online communication and examine their impact on students' attitudes towards and valuing of remote work. However, we may need to examine these patterns at other universities to better understand how universal these findings are and how to best intervene with other populations.

This research shows that even digital natives experience challenges in online work. Organizations and academic institutions can utilize this research to improve how remote work is implemented moving forward.



**Fig. 3.** The serial mediation analyses for online self-efficacy, virtual communication skills, motivation for remote work, and students' satisfaction for remote work.



**Fig. 4.** The serial mediation analyses for online self-efficacy, virtual communication skills, motivation for remote work, and students' valuing of remote work.

#### Statement of Research Advisor

Emily conducted a scoping correlational study to examine undergraduate students' perceptions of virtual work and what factors contribute to these views. Learning more about these factors can help more young people thrive in online work environments. It can also be used by businesses and colleges and universities to better help their students prepare for the future and to improve their online work skills through interventions. - Sara Driskell, Department of Psychological Sciences, College of Liberal Arts

#### References

Adamovic, M., Gahan, P., Olsen, J., Gulyas, A., Shall-cross, D., & Mendoza, A., "Exploring the adoption of virtual work: The role of virtual work self-efficacy

and virtual work climate," The International Journal of Human Resource Management, 33(17), pp. 3492–3525,(2022). Journal Paper

Akos, P., Leonard, A. J., & Hutson, B., "Virtual federal work-study and student career development," The Career Development Quarterly, 70(1), pp. 16–26,(2022). Journal Paper.

AlGhamdi, R. A., "Virtual internship during the COVID-19 pandemic: Exploring IT students satisfaction," Education & Training, 64(3), pp. 329–346,(2022). Journal Paper.

Chatterjee, S., Chaudhuri, R., & Vrontis, D., "Does remote work flexibility enhance organization performance? Moderating role of organization policy and top management support," Journal of Business Research, 139, pp. 1501–1512,(2022). Journal Paper.

Khorakian, A., Jahangir, M., Rahi, S., Eslami, G., & Muterera, J., "Remote working and work performance during the covid-19 pandemic: The role of remote work satisfaction, digital literacy, and cyberslacking," Behaviour & Information Technology, (2023). Journal Paper.

Shen, D., Cho, M.-H., Tsai, C.-L., & Marra, R., "Unpacking online learning experiences: Online learning self-efficacy and learning satisfaction," The Internet and Higher Education, 19, pp. 10–17,(2013). Journal Paper.

Yarberry, S., & Sims, C., "The impact of COVID-19-prompted virtual/remote work environments on employees' career development: Social learning theory, belongingness, and self-empowerment," Advances in Developing Human Resources, 23(3), pp. 237–252,(2021). Journal Paper.

#### **Authors Biography**



Emily Findlay is a senior-year student pursuing a B.A. degree in Psychology at Auburn University. She is in Auburn's I/O Psychology Advanced Bachelors-Master's Program and will return Fall 2024 to complete her M.A. in I/O Psychology. Her research interests include remote work and organizational culture.

Dr. Sara Driskell received her B.A. in Psychology and Studies of Women and Gender from the University of Virginia in 2005. She received her Ph. D. in Social Psychology from Indiana University, Bloomington in 2018, after which she joined Auburn University's Department of Psychological Sciences. Dr. Driskell's research involves looking at intergroup processes in applied social psychology, including how group members perceive and interact with each other and the consequences of group membership in a variety of modern contexts, including the classroom, the workplace, and the courtroom.

# Using Unoccupied Aerial Systems to Study the Effects of Wildflower Planting

Adeline Flach<sup>1,\*</sup>, Zachary Beneduci<sup>2</sup>, Anthony Abbate<sup>3</sup>, Geoffrey Williams<sup>4</sup>, and Stephanie Rogers<sup>5</sup>

Undergraduate Student, Department of Crop, Soils, and Environmental Sciences, Auburn University
 Graduate Student, Department of Entomology and Plant Pathology, Auburn University
 Assistant Research Professor, Department of Entomology and Plant Pathology, Auburn University
 Associate Professor, Department of Entomology and Plant Pathology, Auburn University
 Assistant Professor, Department of Geoscience, Auburn University

Pollinators are essential in natural and managed ecosystems (Baden-Bohm, Thiele, Dauber 2022). However, the loss of floral resources, primarily due to land use changes, has contributed to widespread pollinator decline (Steffan-Dewenter, et al 2006). Therefore, planting abundant and diverse forage for pollinators is essential in promoting pollinator health (Klein, et al 2006). The positive effects of biochar, a carbon-based soil amendment, on the growth of agricultural crops suggest it may benefit the growth and health of pollinator-friendly wildflowers. However, this has been sparingly tested in field settings.

The Normalized Difference Vegetation Index (NDVI) – a proxy for plant health – is a standard index used in remote sensing to measure the health and density of vegetation (Huang, et al 2021). NDVI has commonly been used to assess crop vegetation health in field settings for agricultural studies (Huang, et al 2021). However, recent studies have shown the potential of NDVI in pollination studies. For example, John et al. (2020) utilized NDVI to identify peak flowering in a field study and linked average NDVI values with high flowering periods and Anderson et al. (2023) applied the metric to quantify floral species and floral color diversity as potential correlations of wild bee population abundance. This study will help close the gap of knowledge with biochar application as biochar-treated wildflowers have not yet been assessed with Unoccupied Aerial Systems (UASs) in a field setting. Thus, we have used imagery collected from a multispectral UAS along with NDVI calculated from respective raster bands as the metric to quantify and compare the wildflower's planting health between biochar and non-biochar plots.

This study was completed at three sites across Auburn, Alabama (Figure 1). Each site was ~10,000 ft2 in size and subdivided into eight subplots: four experimental (containing biochar) and four control (not containing biochar). To distinguish plot variability, a DJI Mavic 3 Multispectral UAS was used to collect high resolution (0.55 cm/pixel) aerial (Red, Green, Blue [RGB] bands) and multispectral imagery (Green [G], Red [R], Near-Infrared [NIR], and Red Edge [RE] bands). The drone was flown at a 12m altitude, five times at each site between May to September 2023. The flights from May to August were flown with a front and side overlap of 80% and 70% respectively. Due to the increase in the density of vegetation, the overlap for the flights in September were flown at 85% and 85% respectively.

With recent improvements and increased usage of UASs, there is an opportunity to further investigate their potential in differing environments. Traditional techniques of measuring wildflower planting health are costly in time and labor, so UAS technology could be a non-invasive, cost and time-effective substitution (Tabor, 2022). When compared with satellite imagery, typically used to study vegetation health, UASs are an ideal tool as they provide higher spatial and temporal resolutions (Zhang, et al 2019). Using a multispectral UAS to analyze wildflowers is a new field within pollination research and there is a need for monitoring the spatial and temporal distribution of wildflowers (Gonzales, et al 2022). Thus, the goal of this study was to investigate whether a multispectral UAS would be a useful tool for determining wildflower plant health differences in NDVI between wildflower plots amended with biochar versus those that were not.

<sup>\*</sup> Corresponding author: arf0046@auburn.edu

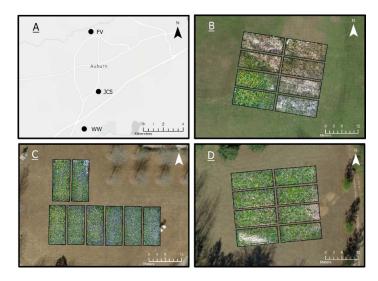


Fig. 1 The three sites for this experiment were located in Auburn, AL [A], over existing wildflower plots at the H.C. Morgan Water Pollution Control Facility (WW) [B], The Jule Collins Smith Museum of Fine Arts (JCS) [C], and the Auburn Bee Lab Apiary at the Farmville Bee Yard (FV) [D]. Eight subplots for each site have been depicted on each map as well as drone aerial imagery collected for this study overlaid onto a satellite imagery base layer provided by Esri.

Once the UAS imagery was collected, it was processed using Pix4Dmapper to produce the raster bands required to run NDVI analysis: R and NIR. NDVI values were calculated for each subplot using Raster Calculator in ArcGIS Pro using the NDVI equation (Equation 1). Next, the Zonal Statistics as Table tool was used to calculate the mean, maximum, minimum, and standard deviation of NDVI values for each subplot (Table 1).

#### **Equation 1**

Normalized Difference Vegetation Index (NDVI)
$$= \frac{(NIR - R)}{(NIR + R)}$$

All the statistics gathered from the five flights at the three sites were then compared between experimental (biochar) and control (non-biochar) subplots using general linear mixed effect models from the package 'lme4' (Bates, et al 2015) in the R statistical computing environment (R Core Team, 2023).

**Table 1** Depicts a sample portion of the output from the Zonal Statistic Tool in ArcGIS Pro. This is an example from one flight at the Jule Collins Smith Museum Site. STD refers to standard deviations away from the mean value.

Date	Subplot	Treatment	Mean	Min	Max	STD
6/9/23	1	Control	0.1986	-0.2916	0.6949	0.1391
6/9/23	2	Biochar	0.2188	-0.2068	0.7051	0.1368
6/9/23	3	Control	0.2044	-0.2693	0.6854	0.1333
6/9/23	4	Biochar	0.2242	-0.2125	0.7019	0.1456
6/9/23	5	Control	0.2161	-0.2442	0.6804	0.1491
6/9/23	6	Biochar	0.2248	-0.1954	0.6876	0.1637
6/9/23	7	Biochar	0.201	-0.3029	0.6881	0.1556
6/9/23	8	Control	0.2348	-0.3063	0.7054	0.1657

The hypothesis at the beginning of the project assumed that subplots treated with biochar would have a higher average NDVI value compared to the subplots not treated with biochar. The mean was chosen to be the representative variable of the NDVI value of the subplots (Zhang, et al 2019). The results showed that there was no statistically significant difference between the experimental and control plots [mean NDVI score difference of 0.009 (+/- 0.021 C.I., p=0.37)]. These results indicate no effect of biochar on the NDVI values within the experimental time period. Future work can investigate time lag effects by expanding the experimental period.

#### **Statement of Research Advisor**

Adeline Flach has been involved in this research for three semesters and was responsible for all aspects of the project. She obtained her FAA Part 107 Remote Pilot's license and became proficient with drone data collection. Adeline completed most analyses herself with statistical guidance from co-author Zach Beneduci. She has made immense progress in research throughout this endeavor! We appreciate the Bee Lab's collaboration in this study.

- Stephanie Rogers, Department of Geosciences, College of Science and Mathematics

#### References

Anderson, M., Crubaugh, F., Greenslit, C., Hill, E., Kroth, H., Stanislawski, E., Ribbons, R., & Toro, I. D. (2023). B.Y.O. Bees: Managing wild bee biodiversity in urban greenspaces. PLOS ONE, 18(4), e0281468. https://doi.org/10.1371/journal.pone.0281468

Baden-Böhm, F., Thiele, J., & Dauber, J. (2022). Response of honeybee colony size to flower strips in agricultural landscapes depends on areal proportion, spatial distribution and plant composition. Basic and Applied Ecology, 60, 123–138. https://doi.org/10.1016/j.baae.2022.02.005

Bates D, Mächler M, Bolker B, Walker S (2015). "Fitting Linear Mixed-Effects Models Using lme4." Journal of Statistical Software, 67(1), 1–48. doi:10.18637/jss.v067. i01.

Gonzales, D., Hempel de Ibarra, N., & Anderson, K. (2022). Remote Sensing of Floral Resources for Pollinators – New Horizons From Satellites to Drones. Frontiers in Ecology and Evolution, 10. https://www.frontiersin.org/articles/10.3389/fevo.2022.869751

Huang, S., Tang, L., Hupy, J. P., Wang, Y., & Shao, G. (2021). A commentary review on the use of normalized difference vegetation index (NDVI) in the era of popular remote sensing. Journal of Forestry Research, 32(1), 1–6. https://doi.org/10.1007/s11676-020-01155-1

John, A., Ong, J., Theobald, E. J., Olden, J. D., Tan, A., & Hille Ris Lambers, J. (2020). Detecting Montane Flowering Phenology with CubeSat Imagery. Remote Sensing, 12(18), Article 18. https://doi.org/10.3390/rs12182894

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2006). Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences, 274(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Steffan-Dewenter, I., Potts, S., & Packer, L. (2006). Pollinator diversity and crop pollination services are at risk. Trends in Ecology & Evolution, 20, 651–652; author reply 652. https://doi.org/10.1016/j.tree.2005.09.004

Tabor, J. (2022). Quantifying Floral Resource Avail-

ability Using Unmanned Aerial Systems and Machine Learning Classifications to Predict Bee Community Structure. All Graduate Theses and Dissertations. https://doi.org/10.26076/5052-f814

Zhang, J., Virk, S., Porter, W., Kenworthy, K., Sullivan, D., & Schwartz, B. (2019). Applications of Unmanned Aerial Vehicle Based Imagery in Turfgrass Field Trials. Frontiers in Plant Science, 10. https://doi.org/10.3389/fpls.2019.00279

#### **Authors Biography**



Adeline Flach is a senior-year student pursuing a BS degree in Environmental Sciences and a GIS Certificate. She is an Undergraduate Research Fellow in the GeoIDEA Lab, developing research skills and learning about geospatial applications in science. She is also on the Auburn Women's Tennis Team.



Zachary Beneduci is a graduate student in the Department of Entomology and Plant Pathology at Auburn University. His research interests lie in native bee community ecology, with particular focus on managing landscapes for their conservation.



Anthony Abbate is an Assistant Research Professor in the Department of Entomology and Plant Pathology at Auburn University. His research interests focus on investigating how anthropogenic disturbances affect pollinator abundance and diversity, determining the pollination requirements of native wildflowers and specialty crops grown in the southeastern US, and how tillage practices affect ground-nesting bees.

#### Auburg University Journal of Undergraduate Scholarship



Geoffrey Williams is an Associate Professor in the Department of Entomology and Plant Pathology at Auburn University. He holds a Ph.D. from Dalhousie University, Halifax, Canada, and has research, instruction and Extension responsibilities concerning insect pollinators, with a focus on honey bees, at Auburn University.



Stephanie Rogers is an Assistant Professor in Geographic Information Science (GIScience) in the Department of Geosciences at Auburn University. She runs the GeoIDEA (Geospatial Innovation, Development, and Environmental Applications) Lab on campus and integrates geospatial concepts and methods into a variety of applications to improve understanding about the environment.

## Evaluation of Agricultural and Clinical Antifungal Agents Against Members of the Fusarium solani Species Complex

Amelia Flesner<sup>1,\*</sup> and Jeffrey Coleman<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Entomology and Plant Pathology, Auburn University
<sup>2</sup> Associate Professor, Department of Entomology and Plant Pathology, Auburn University

Members of the Fusarium solani species complex (FSSC) are important pathogens in both agricultural and clinical settings. The Coleman lab has successfully demonstrated that isolates from a clinical setting are capable of infecting and causing disease on produce as well as proving that some agricultural isolates can cause human infection [1]. This pathogenicity has caused the need for further antifungal testing in both clinical and agricultural realms. Treating fungal infections is difficult because fungal cells and human cells share many basic structures as well as possessing the ability to easily develop resistance to drugs. The objective of this research is to investigate how the FSSC isolates from varying environments react to different antifungal agents, determining potential treatment options and exposing possible resistance. The minimum inhibitory concentration of various antifungal agents will be analyzed and compared to determine effectiveness. Ten-fold antifungal dilutions are added to a set number of spores and incubated for three days at thirty-five degrees Celsius. Fungal growth within each dilution is examined macroscopically to determine the minimum amount of antifungal needed to inhibit the growth of that particular infection (Fig. 2). Agricultural fungicides such as prochloraz and clinical antifungals such as amphotericin B show inhibition of both clinical and antifungal isolates. The concentration needed of each can vary by isolates, indicating possible resistance. The FSSC has been denoted as a fungal species complex of particular concern by the WHO in 2022, and this research will contribute significant information surrounding treatment in both clinical and agricultural environments.

This research aims to investigate the response of FSSC isolates from different environments to various anti-

fungal agents, providing valuable insight for treating infections in both clinical and agricultural settings, especially considering the WHO's designation of FSSC as a fungal pathogen of particular concern.

The *Fusarium solani* species complex comprises filamentous fungi that are globally distributed and capable of causing disease in both crops and humans. This species complex is notably recognized for its pathogenicity in economically significant crops such as cotton and soybean. The spores of the FSSC can also lead to human mycoses either through direct inoculation or inhalation, posing a significant risk, particularly to immunocompromised individuals. The risk for immunocompromised farmers is even higher as engaging in agricultural activities involving *Fusarium*-infected crops elevates the risk of spore exposure. Consequently, the identification of optimal treatment methods for these diseases has become crucial over recent years.

This study involved the evaluation of both clinical and agricultural antifungal agents due to the ability of these fungal isolates to infect both humans and crops. The clinical antifungal agents investigated are: amphotericin B, fluconazole, voriconazole, triamcinolone, caspofungin, ketoconazole, miconazole, clotrimazole, and posaconazole. The agricultural antifungals examined are: tebuconazole and prochloraz.

In clinical practice, amphotericin B and voriconazole are the preferred drugs for combating *Fusarium solani* species complex infections. This study revealed that both antifungals demonstrated inhibitory effects at lower concentrations compared to other options such as fluconazole, which is widely used for other medical-

<sup>\*</sup> Corresponding author: ajf0058@auburn.edu

ly important fungi. Amphotericin B exhibited a lower inhibitory concentration than voriconazole against the same isolates but is often associated with more severe side effects. Because of this, voriconazole has become the more frequently prescribed treatment due to its relatively lower adverse effects. However, this research indicates potential alternative options like clotrimazole, which exhibited the same minimum inhibitory concentration (MIC) as voriconazole (Fig. 1).

In the agricultural domain, prochloraz is extensively used to manage FSSC infection of crops in other countries. This study has shown that even a minute concentration of this fungicide, approximately  $0.06~\mu g/m L$ , can impede the growth of *Fusarium* isolates. However, prochloraz is considered a hazardous substance with significant long-term environmental repercussions and is banned within the United States. Tebuconazole also inhibits isolate growth but at a higher concentration. It is deemed less hazardous and has additional benefits such as controlling bacterial and viral pathogens affecting plants. Assessing the efficacy of higher verses lower concentrations requires a comprehensive consideration of the associated benefits and consequences, as well as the specific species complex causing the infection.

The minimum inhibitory concentrations were determined according to a protocol for filamentous fungi from the Clinical and Laboratory Standards Institute [2].

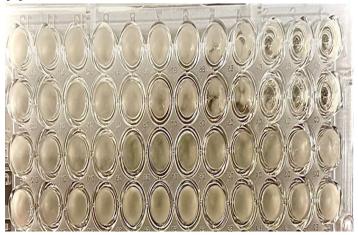


Fig. 2 involves a study involving the isolate Cherokee 2-1 and four distinct antifungal agents. The first row denotes a minimum inhibitory concentration of 4  $\mu g/mL$  with voriconazole. The second row signifies an MIC of 2  $\mu g/mL$  with tebuconazole. Conversely, rows three and four indicate MIC values exceeding 32  $\mu g/mL$  with caspofungin and triamcinolone respectively. Demonstrating the inability of these antifungals to impede the growth of the isolate at the highest concentration tested.

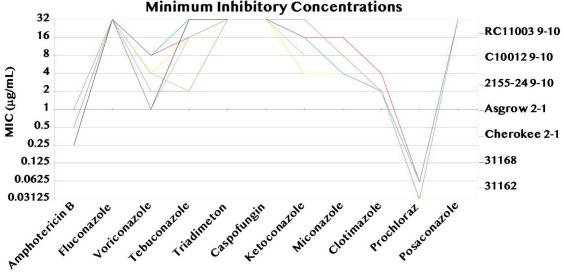


Fig. 1 demonstrates the minimum inhibitory concentrations (MICs) of all tested Fusarium solani species complex isolates and antifungal agents investigated in this study. The scale ranges from 0.03  $\mu$ g/mL, denoting the lowest concentration tested and the minimum amount of antifungal required to inhibit the isolate, to >32  $\mu$ g/mL, indicating a concentration beyond visual determination in this assay. An MIC of 32  $\mu$ g/mL signifies observable growth of fungal spores in all wells, while an MIC of 0.03 $\mu$ g/mL indicates the absence of fungal growth across all wells. The distinct lines in the figure correspond to different isolates tested. Variability is observed among isolates and antifungals, suggesting potential resistance, while no variability is observed among others indicating uniform effectiveness and lack of variation in pathogenicity.

The examination of agricultural and clinical isolates against various Fusarium solani species complex strains revealed varying effectiveness in inhibiting fungal growth among different antifungal agents. Prochloraz demonstrated exceptional efficacy even at low concentrations, indicating minimal antifungal resistance and a potential for treating Fusarium infections. In comparison, several antifungal agents assessed in this study exhibited an inability to hinder fungal growth even at the highest concentration tested, 32 µg/mL. Fluconazole, triamcinolone, caspofungin, and posaconazole fell into this category, suggesting limited effectiveness in treating FSSC infections. Higher concentrations of antifungals can lead to more severe side effects. Therefore, prescribing the lowest effective concentration capable of eliminating the infection is crucial. Among the clinical antifungals evaluated, amphotericin B, voriconazole, and clotrimazole emerged with the lowest MICs, indicating their potential as preferred options for treating human mycoses involving Fusarium.

#### **Statement of Research Advisor**

Members of the FSSC have a broad host range and are encountered under field conditions and in hospitals. Management of FSSC infections may involve the use of antifungal compounds in a class termed the triazoles. As *Fusarium* persists in such broad environments, their exposer to antifungal agents used in one setting can potentially confer cross resistance to treatment options in the other environment. Amelia's research has indicated FSSC isolates exposed to fungicides used in agriculture, may potentially be selecting for resistance to antifungals used in the medical setting for people infected with isolates of the FSSC.

- Jeff Coleman, Entomology and Plant Pathology, College of Agriculture

#### References

[1] Coleman, J.J., Muhammed, M., Kasperkovitz, P.V., Vyas, J.M., Mylonakis, E., "Fusarium pathogenesis investigated using Galleria mellonella as a heterologous host," https://www.sciencedirect.com/science/article/abs/pii/S1878614611001759, (4 October 2011). Journal Paper

[2] Rex, J.H., Alexander, B.D, Andes, D., Arthington-Skaggs, B., Brown, S.D., Chaturveli, V., Espinel-In-

groff, A., Ghannoum, M.A., Knapp, C.C., Motyl, M.R., Ostrosky-Zeichner, L., Pfaller, M., Sheehan, D.J, Walsh, T.J., "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved Standard-Second Addition," chromeextension://efaidnbmnnnibpcajpcglclefindmkaj/https://clsi.org/media/1455/m38a2\_sample.pdf, (April 2008) Journal Paper

#### **Authors Biography**



Amelia Flesner is a senior-year student pursuing a B.S. degree in Clinical Laboratory Science within the Biochemistry Department at Auburn University. She has played a key role in the research of clinical and agricultural antifungal agents against a fungal species complex of particular concern.



Jeffrey J. Coleman is an Associate Professor in the Department of Entomology and Plant Pathology at Auburn University. After obtaining his Ph.D. in Plant Pathology at the University of Arizona, Dr. Coleman conducted postdoctoral research in medical mycology at Harvard Medical School. Since joining the faculty at Auburn University in 2014, he has developed a laboratory focused on investigating fungal pathogenesis using advanced molecular techniques.

# Microalgae Biomass Production Using Anaerobically Digested Solid Waste from Aquaponics

Al Dean Francisco<sup>1,\*</sup>, Qichen Wang<sup>2</sup>, and Brendan Higgins<sup>3</sup>

Undergraduate Student, Department of Biosystems Engineering, Auburn University
 Post-Doc, Department of Biosystems Engineering, Auburn University
 Associate Professor, Department of Biosystems Engineering, Auburn University

#### **Abstract**

Soluble nutrients from fish waste are reutilized for plant growth within an aquaponics system. However, solid waste remnants persist and could build up within the otherwise sustainable aquaponics system. These solid wastes are typically removed from the system. But these solid wastes still contain valuable nutrients that could be extracted using microbial processes and recycled into algal production systems. However, if not managed appropriately, these same plant nutrients could cause environmental problems such as toxic algal bloom growth. To repurpose these nutrients, these solid wastes can undergo an anaerobic digestion process which results in solubilized organic nutrients. These nutrients are readily available for re-use by algae.

Our study observes the use of anaerobic digestate from aquaponics waste to grow algae while also assessing the risk of potential toxic cyanobacteria (blue green algae) to grow within the algal production system. The long-term goal is aims to use the algae grown in our bio-processing system to feed the fish and create a closed, cyclical aquaponics system.

Sustainable agricultural practices such as aquaponics have increased the efficiency of nutrient utilization by integrating two processes into one cycle (Forchino et al., 2018). However, with a focus on soluble nutrients in water, the nutrient discharge in the solid waste from aquaponics, which consists of organic carbon, nitrogen, and phosphorus, is still a critical environmental issue. Anaerobic digestion is an effective method for converting these organic rich compounds into biogas and solubilize organic nitrogen and phosphorus back into a more bioavailable form. Past work in our

team have developed an effective biological pretreatment which could achieve identical algal productivity in full strength municipal sludge anaerobic digestate when compared to the same strain of algae in synthetic media (Wang et al., 2019). Most algal-wastewater treatment processes are carried out in non-axenic conditions which could be a concern as contamination of cyanobacteria can happen in the algal production system. Studies have shown cyanobacteria's toxin-producing ability (Hitzfeld et al., 2000). There have also been many health concerns within seafood algal toxins that are ingested from fish that cause food poisoning (Liang et al., 2015). Previous work has observed cyanobacteria presence in municipal wastewater.

The objectives of this study are to 1. test the growth performance of green algae in anaerobically digested aquaponics solid waste; and 2. Test the potential risk of potential cyanobacteria contamination when using anaerobic digestate as a non-sterile nutrient media for algal biomass production. We hypothesized that 1. digestate from aquaponics solid waste could support the growth of green algae; and 2. The growth of cyanobacteria is strongly suppressed in the condition of anaerobic digestate while the growth of green algae is not impacted after bacterial pretreatment.

#### **Experiment 1**

Municipal anaerobic digestate (MAD) was collected from the Colombus Wastewater Treatment Plant in Colombus, Georgia. This digestate was used in the first trial because it has already been proven for algal growth in past work. Anaerobic digestate underwent bacterial pre-treatment (Wang et al., 2019). Briefly, 1% v/v activated sludge collected from the activated sludge ba-

<sup>\*</sup> Corresponding author: adf0038@auburn.edu

sin in the same plant is inoculated in the digestate. The composition underwent constant stirring and aeration (0.1vvm) for five days to allow the inoculated bacteria break down potential inhibitors. The effluent was collected from this process and filtered down. For a sterile media, it was filtered down to 0.22mm pore size; for the non-sterile condition, it was filtered down to 0.7 mm. Synthetic media BG-11 and modified BG-11 with ammonium as the nitrogen source were controls for the growth of cyanobacteria. With these varying media, cyanobacteria were inoculated in a bioreactor - 5 groups of triplicates - and observed with bidaily sampling for two weeks (Fig. 1). One group also had a coculture of both cyanobacteria and green algae. In this group, equal parts of cyanobacteria and green algae, on dry-mass basis, were inoculated in the municipal aerated digestate.



**Fig. 1** Experimental Setup of Bubble Column Tubes to Grow Cyanobacteria in Different Media

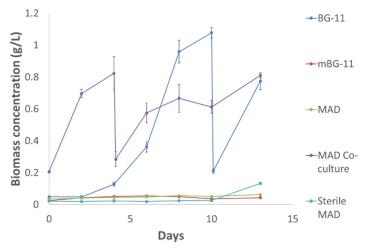
#### **Experiment 2**

Fish solid waste was collected in settling tanks from the Aquaponics stations at the E.W. Shell Fisheries at Auburn University. The fish solid waste was combined with food waste which was collected from Auburn University Student Dinning Hall. The mix of fish sludge and food waste was anaerobically digested with a hydraulic retention time of 20 days. Afterwards, the digestate underwent aerobic bacterial pre-treatment identically to the process described in the methods of the cyanobacteria experiment: bacteria from activated sludge were inoculated in the fish digestate, mixed and aerated up to 4 days, then filtered down to 0.7um. The filtered digestate media was used to grow C. sorokiniana. 4 groups of triplicates were tested: an N8-NH4 control group, an untreated fish digestate, a digestate pretreated and aerated for 1 day, and another group pretreated for 4 days. Here, this experiment lasted for 7 days and was sampled daily.

#### **Experiment 1 Results**

For the first experiment (Method 2.1), there was no

growth in cyanobacteria in non-sterile MAD, mBG-11, and Sterile filtered MAD. For the MAD Co-culture and BG-11, results show an initial growth within a week, but then sudden drop and regrowth (Fig. 2).



**Fig. 2** Concentration of UTEX 2385 in municipal digestate. Error bars represented standard deviations (n= 3).

Ammonia was the dominant nitrogen in non-sterile MAD, Sterile MAD, and mBG-11. However, this experiment revealed that ammonia showed a strong inhibition to the growth of cyanobacteria which resulted in their near complete suppression. This is also why we saw prolific growth in the standard BG-11 group: that media contains nitrates instead of ammonia.

#### **Experiment 2 Results**

In the second experiment (Method 2.2), growth of microorganisms was detected using optical density measurements (Fig. 3). However, upon closer inspection of the groups, it appears that the readings were not detecting algal growth, but instead general aerobic bacteria growing in the media. A possible reason for the lack of algal growth is that the fish digestate was too strong. An analysis of Volatile Fatty Acids (VFAs) of the fish digestate was measured to explain this inhibition of growth (Fig. 4). When observing concentrations of acetic acid, propionic acid, and butyric acid, results showed that the media had averages of 11.3 g/L, 3 g/L, and 20.1 g/L respectively. These amounts are too high to sustain algal growth. Past work has shown that the green algae C. sorokiniana is inhibited at ~450 mg/L of both propionic and butyric acids (Wang et al., 2018).

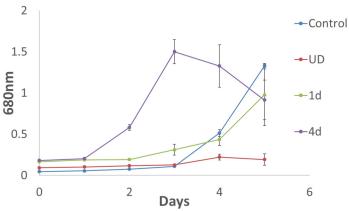
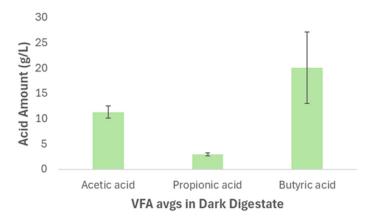


Fig. 3 680nm optical density of C.sorokiniana in Fish Digestate. Error bars represented standard deviations (n=3).



**Fig. 4** VFA concentrations in raw fish sludge digestate. Error bars represented standard deviations (n=3).

#### **Conclusions**

Overall, these experiments have allowed more understanding to the algal-bacteria processes of fish digestate as a tangible idea to create fish feed. From the cyanobacteria experiment, it has been observed that waste digestate inhibits cell growth, and thus, one does not have to worry about the presence of cyanotoxins. From this, algal was cleared to be grown in fish digestate to become fish feed. However, more work needs to be done and extra steps within the treatment process needs to be added to reduce VFAs in the fish digestate for green algae to sustain growth.

#### **Statement of Research Advisor**

Al Dean's project advances our understanding of how harmful cyanobacteria might behave in a system designed to grow algae for fish feed. This is an important step for concept viability and feed safety. He also showed that systems that work for one digestate may

not necessarily work for another and that additional process development is needed. Pending better control of volatile fatty acids, this process could advance the commercial potential for using waste-grown algae as a fish feed.

- Brendan Higgins, Department of Biosystems Engineering, Samuel Ginn College of Engineering

#### References

Forchino, A. A., Gennotte, V., Maiolo, S., Brigolin, D., Mélard, C., & Pastres, R. (2018). Eco-designing Aquaponics: A Case Study of an Experimental Production System in Belgium. Procedia CIRP, 69, 546-550. https://doi.org/10.1016/j.procir.2017.11.064

Hitzfeld, B. C., Höger, S. J., & Dietrich, D. R. (2000). Cyanobacterial toxins: Removal during drinking water treatment, and human risk assessment. Environmental Health Perspectives, 108(suppl 1), 113–122. https://doi. org/10.1289/ehp.00108s1113

Liang, H., Zhou, W., Zhang, Y., Qiao, Q., & Zhang, X. (2015). Are fish fed with cyanobacteria safe, nutritious and delcious? A laboratory study. Scientific Reports, 5(1), 15166. https://doi.org/10.1038/srep15166

Wang, Q., Higgins, B., Ji, H., Zhao, D., 2018. Improved microalgae biomass production and wastewater treatment: Pre-treating municipal anaerobic digestate for algae cultivation, Annual International Meeting of the ASABE. American Society of Agricultural and Biological Engineers, Detroit, MI.

Wang, Q., Prasad, R., Higgins, B.T., 2019. Aerobic bacterial pretreatment to overcome algal growth inhibition on high-strength anaerobic digestates. Water Res 162, 420-426.

#### **Authors Biography**



Al Dean Francisco is a sophomore pursuing a B.S. degree in Biosystems Engineering with the Bioprocess Option at Auburn University. Much of his interests lies within algal-bacteria processes.



Qichen Wang graduated from Auburn University with his Ph.D. in Biosystems Engineering and currently works as a Post-Doc for the department under Dr. Higgins. His research lens includes observing complex bioprocesses for bioremediation and its application.



Dr. Brendan Higgins is an Associate Professor in the Department of Biosystems Engineering at Auburn University. His research focuses on aquaponics and bio-applications for waste remediation, water quality, and production of high-value products. He continues to study combining algal-bacterial processes and their impacts for environmental sustainability.

# Mental Health Among Service Members Who Experienced Military Sexual Trauma: Exploring the Roles of Individual and Social Factors

Melissa A. Garnes<sup>1,\*</sup>, Erin Cooper<sup>2</sup>, and Mallory Lucier-Greer<sup>3</sup>

<sup>1</sup> Undergraduate Student, Department of Departments of Human Development & Family Science, Auburn University
 <sup>2</sup> Postdoctoral Research Fellow, Department of Human Development & Family Science, Auburn University
 <sup>3</sup> Professor, Department of Human Development & Family Science, Auburn University

Military sexual trauma (MST) is sexual assault or harassment that occurs during one's military service and can negatively influence the wellbeing, mental health, and overall readiness of service members (e.g., Livingston et al., 2020). The Department of Defense (2023) received 8,942 reports of sexual assault from active-duty service members in 2022, and it is likely that there were additional instances as some victims chose not to report their assault. MST has been linked to higher rates of a variety of mental health conditions like post-traumatic stress disorder, depression, and anxiety (Klingensmith, 2014).

Ongoing research has examined the antecedents and effects of MST to increase the safety of service members and treat those affected by MST. For example, research indicates a correlation between less organizational influence (e.g., lower rank) as well as sociocultural influences (e.g., being a woman, belonging to a marginalized racial group) and an increased likelihood of experiencing sexual assault or harassment (e.g., Cleveland & Kerst, 1993). Additionally, after a trauma has occurred, several studies have documented that strong unit cohesion and support can buffer the negative effects of traumatic stress (Armistead-Jehle et al., 2011; Mitchell et al., 2012). Such findings demonstrate the complex interplay between MST and individual and social characteristics that influence mental health outcomes.

The current study advances this area of research by assessing depressive and anxiety symptoms, two hallmark indicators of mental health, of active-duty service members who experienced MST while deployed and exploring possible differences in mental health indicators based on individual characteristics and available social

supports. Applying a socioecological theoretical lens that acknowledges the multiple levels of environmental influence (e.g., within-individual factors and social factors) on individual development and functioning (Bronfenbrenner, 1979), this study first tested whether mental health levels differed across demographic groups (i.e., race and sex). Identifying risk factors of elevated distress can help identify those in need of support and treatment planning. To understand the role of social support after experiences of MST, this study explored links between mental health support, unit cohesion, and mental health (depressive and anxiety symptoms). Identifying protective factors, especially ones that are modifiable, may help mitigate negative mental health outcomes. Specifically, this study addressed the following research questions.

Among soldiers who experienced MST while deployed...

- Research Question 1: Are there differences in depressive and anxiety symptoms based on sex and racial minority status?
- Research Question 2: Are there differences in depressive and anxiety symptoms based on unit cohesion and mental health support?
- Research Question 3: Are mental health support and unit cohesion related to lower levels of depressive and anxiety symptoms?

This study utilized secondary data from the Army Study to Assess Risk and Resilience in Servicemembers (STARRS) All Army Study (AAS) to conduct within-group analyses of 114 service members who had experienced sexual assault on deployment. Analyses were conducted in SPSS 29.

<sup>\*</sup> Corresponding author: mag0094@auburn.edu

Analysis of variance (ANOVA) testing was conducted to address Research Questions 1 and 2, exploring differences in mental health symptoms based on sex, race, mental health support, and unit cohesion among soldiers who experienced MST (Table 1). Regarding Research Question 1, there were no statistically significant differences among MST survivors based on sex or race in depressive (sex:  $F_{[1,110]} = .55$ , p = .46; race:  $F_{[1,107]} = 1.53$ , p = .22) or anxiety symptoms (sex:  $F_{[1,111]} = .22$ , p = .64; race:  $F_{[1,108]} = 1.49$ , p = .23). This means that men and women MST survivors reported similar levels of depressive symptoms and anxiety symptoms. Further, those identifying as White and those identifying as a racial minority also reported similar levels of depressive symptoms and anxiety symptoms.

Regarding Research Question 2, MST survivors who received mental health support reported fewer depressive ( $F_{[1,99]} = 11.55$ , p < .001) and anxiety ( $F_{[1,99]} = 12.13$ , p < .001) symptoms compared to those who did not receive mental health support. There were no differences in anxiety symptoms between MST survivors with high and low unit cohesion ( $F_{[1,98]} = 2.17$ , p < .144); however, MST survivors who reported higher unit cohesion reported marginally fewer depressive symptoms ( $F_{[1,97]} = 3.70$ , p < .057) compared to those who reported less unit cohesion.

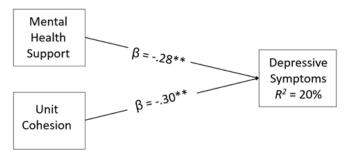
A multiple linear regression model was used to address Research Question 3, exploring the additive associations between having received mental health support and unit cohesion to understand current mental health symptoms. Having received mental health support ( $\beta$  = -0.28, p = .003) and greater unit cohesion ( $\beta$  = -0.30, p = .002) were both associated with fewer depressive symptoms, accounting for 18% of the variance in depressive symptoms (Adjusted  $R^2$  = .18). See **Figure 1** for a visual representation of the model. In a separate model, findings suggested that having received mental health support ( $\beta$  = -0.30, p = .002) and greater unit cohesion ( $\beta$  = -0.24, p < .001) were linked to fewer anxiety symptoms, accounting for 16% of the variance in anxiety symptoms (Adjusted  $R^2$  = .16, p < .001). See **Figure 2**.

Understanding multiple influences on mental health outcomes for service members who have experienced MST is critical for helping professionals working with service members, veterans, and their families, as well as military leadership and policymakers making decisions that will influence them. Our findings underscore the importance of identifying malleable social factors (i.e., mental health support and unit cohesion) that may act as buffers against the negative mental health impacts of MST. These results highlight that social protective factors may be more salient in understanding the current mental health symptoms of service members who experienced sexual assault compared to fixed individual-level factors (i.e., race, sex).

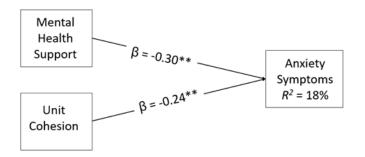
**Table 1.** Subsample descriptive statistics and ANOVA results (N = 114 service members who had experienced MST).

	Depressive Symptoms					Anxiety Symptoms				
Sex	n	М	SD	F	n	M	SD	F		
Women	64	3.67	1.15	0.55	65	3.31	1.31	0.22		
Men	48	3.5	1.23	0.55	48	3.43	1.33	0.22		
Race										
<b>Racial Minority</b>	38	3.4	1.12	1.53	<i>38</i>	3.17	1.36	1 10		
White	71	3.69	1.18	1.55	72	3.48	1.22	1.49		
Mental Health Supp										
Received	64	3.31	1.18	11.55***	64	3	1.27	12 12***		
Not Received	<i>37</i>	4.13	1.11	11.55	<i>37</i>	3.9	1.24	12.13***		
Unit Cohesion										
High	56	3.5	1.26	2 70±	56	3.19	1.32	2 17		
Low	43	3.91	1.07	3.70⁺	44	3.58	1.27	2.17		

*Note.* \*p < .06, \*p < .05, \*\*p < .01, \*\*\*p < .001



**Fig. 1.** Regression results demonstrating the additive associations between mental health support and unit cohesion as predictors of current depressive symptoms.



**Fig. 2.** Regression results demonstrating the additive associations between mental health support and unit cohesion as predictors of current anxiety symptoms.

In addition to MST prevention efforts, stakeholders may consider implementing policies and interventions focused on promoting mental health help-seeking and unit cohesion to mitigate risks to mental health due to deployment MST. Helping professionals can be sources of support, understanding, and compassion and are well-positioned to refer individuals and families experiencing the effects of MST to mental health treatment. Being well-versed in mental health resources for MST will strengthen the ability of all types of helping professionals to best serve service members and veterans. Military leadership can also consider their role in promoting mental health help-seeking and unit cohesion among service members, as cultivating a supportive environment for service members may mitigate negative mental health outcomes. Lastly, this study may also inform policymakers who develop the systems and processes involving MST reporting and support provision. Strengthening the opportunities for service members who have experienced MST to access mental health support and strong unit cohesion may best offset negative mental health effects of MST.

#### **Statement of Research Advisor**

Melissa's research represents a critical development in understanding mental health among survivors of military sexual trauma (MST). Specifically, this study elevated the role of both individual characteristics and social supports to identify needs and leverage points among service members using data from the Army Study to Assess Risk and Resilience in Servicemembers (STARRS) All Army Study (AAS). Findings point to the crucial role of social support mechanisms, such as mental health support and unit cohesion, in promoting mental health among survivors of MST. The implications of this research offer actionable insights for military leadership, policymakers, and helping professionals, namely activating diverse systems of support to promote the wellbeing of survivors.

- Mallory Lucier-Greer, Department of Human Development and Family Science, College of Human Sciences

#### References

- [1] Armistead-Jehle, P., Johnson, S. L., Wade, N. G., & Ecklund, C. J. (2011). Posttraumatic stress in U.S. Marines: The role of unit cohesion and combat exposure. Journal of Counseling and Development, 89(1), 81–88. https://doi.org/10.1002/j.1556-6678.2011.tb00063.x
- [2] Bronfenbrenner, U. (1979). The ecology of human development: Experiments by nature and design. Harvard University Press.
- [3] Cleveland, J. N., & Kerst, M. E. (1993). Sexual harassment and perceptions of power: An under-articulated relationship. Journal of Vocational Behavior, 42(1), 49–67. https://doi.org/10.1006/jvbe.1993.1004
- [4] Department of Defense. (2023). Department of Defense annual report on sexual assault in the military. https://www.sapr.mil/sites/default/files/public/docs/reports/AR/FY22/DOD\_Annual\_Report\_on\_Sexual\_Assault\_in\_the\_Military\_FY2022.pdf
- [5] Klingensmith, K., Tsai, J., Mota, N., Southwick, S. M., & Pietrzak, R. H. (2014). Military sexual trauma in US veterans: Results from the National Health and Resilience in Veterans study. Journal of Clinical Psychiatry, 75(10), e1133-e1139. https://doi.org/10.4088/JCP.14m09244

[6] Livingston, W. S., Fargo, J. D., Gundlapalli, A. V., Brignone, E., & Blais, R. K. (2020). Comorbid PTSD and depression diagnoses mediate the association of military sexual trauma and suicide and intentional self-inflicted injury in VHA-enrolled Iraq/Afghanistan veterans, 2004–2014. Journal of Affective Disorders, 274, 1184-1190. https://doi.org/10.1016/j.jad.2020.05.024

[7] Mitchell, M. M., Gallaway, M. S., Millikan, A. M., & Bell, M. (2012). Interaction of combat exposure and unit cohesion in predicting suicide-related ideation among post-deployment soldiers. Suicide and Life-Threatening Behavior, 42(5), 486–494. https://doi.org/10.1111/j.1943-278X.2012.00106.x



Dr. Mallory Lucier-Greer is a Professor of Human Development and Family Science at Auburn University. Her research is focused on understanding stress and resilience processes in families with a focus on the wellbeing of military families.

#### **Authors Biography**



Melissa Garnes is an undergraduate student pursuing her B.S. in Human Development Child Life at Auburn University. Growing up a military brat, has instilled in her a desire to uplift and give back to the military community. She is also an undergraduate research assistant at Military REACH, helping bridge the gap between research and practice for military families.



Dr. Erin Cooper is a Postdoctoral Research Fellow in Human Development and Family Science at Auburn University. She combines trauma-informed and prevention science approaches to understand adversity and promote resiliency in individuals and families

# Standardizing Success: Creating Gait and Behavioral Markers of Prognosis with Feline Models of Neurodevelopment and Neurodegenerative Diseases

Olivia Grigsby<sup>1,\*</sup>, Jordan Towns<sup>2</sup>, Dr. Emily Graff<sup>3</sup>, and Dr. Douglas Martin<sup>4</sup>

Undergraduate Student, Department of Chemistry and Biochemistry, Auburn University
 Ph.D. Student, Department of Pathobiology, Auburn University
 Associate Professor, Department of Pathobiology, Auburn University
 Professor, Scott Ritchey Research Center, Auburn University

The domestic cat is an important research model to investigate neurodevelopmental and neurodegenerative diseases because feline brains are gyrencephalic and closer in size to that of humans compared to those of mice [1].

Gangliosidoses are lysosomal storage disorders with altered or loss of enzyme functions that cause neuro-degeneration resulting in loss of brain and motor function progressing to death [2]. Both cats and humans develop gangliosidosis that manifest with clinical signs including gait ataxia, tremors, and loss of coordination. Two major forms of gangliosidoses are GM1 and GM2 which are autosomal recessive inherited diseases[3]. Researchers at the Auburn University Scott Ritchey Research Center are working to develop gene therapies that can be used to treat GM1 and GM2 in both cats and humans [2, 3].

For our research we wanted to measure parameters of gait analysis in domestic cats during normal development and in GM1 and GM2 disease progression. The three measurable parameters of gait analysis are front and rear stride length, front and rear base width, and right and left crossover. Each of these parameters gives information on the development of the nervous system. Stride length is expected to increase in proportion to limb growth. Base wide stance is a marker of the development of myelination which occurs postnatally in cats. Finally, crossover is a reliable measure of proprioception, or limb coordination in cats. We hypothesized that the gait parameters will change as the cat

grows, specifically, their stride length and base width stance will increase, and their crossover will get smaller over time. Additionally, we anticipate that these developmental features will be altered in cats with GM1 or GM2 gangliosidosis and align with disease progression. These findings will be used to evaluate treatment groups to measure the effectiveness of various gene therapy approaches.

The groups of cats analyzed included normal unaffected cats, and GM1- and GM2-affected cats. Each cat's gait was measured at 1, 2, 3, 4, 6, and 12 months of age and into adulthood. Gait collection was done by coloring each paw with a different color ink and allowing the cat to freely walk down a straight chute over a piece of paper. That paper was then collected, scanned into adobe acrobat, and analyzed digitally through the measurement tool on adobe acrobat. Analysis of the measurements was done with a repeated measures ANOVA with multiple comparisons to determine the differences between groups.

First, we developed a normal expected timeline of feline gait development. As kittens develop, we found that their stride length for both rear and front base increases significantly at four months of age. For base wide stance the front base wide stance did not change over time. Rear base wide stance however was increased significantly by three months of age. Additionally, there was no change in crossover at all during development. This finding supports the idea that proprioception develops early in kittens shown by previous kitten rolling studies

<sup>\*</sup> Corresponding author: ogg0007@auburn.edu

and that stride length and myelination change with age.

After establishing appropriate developmental controls, we then went on to compare the GM1 and GM2 untreated and treated mutants to their normal age matched controls. We chose to do this comparison for both groups at two months of age. This timepoint falls within the presymptomatic phase of the disease, and we concluded that if changes could be detected here it would allow for quicker detection of the disease. Additionally, this timepoint fell before the diseases became fatal or when the cats became non-ambulatory.

For the GM1 cats there was no change in stride length or in crossover distance at two months but both front and rear base were significantly different, suggesting possible subtle changes in myelination. Front base was significantly decreased (p=0.014) while rear base was also significantly decreased (p=0.011). For GM2 affected cats all parameters of gait changed by two months, which may be associated with rapid disease progression in GM2 gangliosidosis. Rear stride was significantly decreased (p=0.0048), front base width was significantly increased (p=0.0002) and right crossover was significantly decreased (p=0.049).

In the future we hope to collect more data to be able to make comparisons between treatments to see which are the most effective. Additionally, we would like to apply behavioral testing in developing kittens and cats with neurodegenerative disease, specifically novel object testing to test recognition memory and T-maze association tests which will assess learning ability. From these results we hope to put together a multifaceted approach to the effect of neurodegenerative disease on the feline brain as well as to help determine which treatments are most effective and can be the best option for human trials and treatment.

#### Statement of Research Advisor

Olivia Grigsby is a dedicated undergraduate researcher who is committed to better understanding disease processes through the perspective of naturally occurring animal models. Her work is an important contribution that has helped develop and validate new research tools in our lab.

- Emily Graff, Pathobiology, College of Veterinary Medicine

#### References

[1] Griffin B, Baker HJ. Domestic Cats as Laboratory Animals. Laboratory Animal Medicine. 2002:459–82. doi: 10.1016/B978-012263951-7/50015-6. Epub 2007 Sep 2. PMCID: PMC7150223.

[2] Rha AK, Maguire AS, Martin DR. GM1 Gangliosidosis: Mechanisms and Management. Appl Clin Genet. 2021 Apr 9;14:209-233. doi: 10.2147/TACG. S206076. PMID: 33859490; PMCID: PMC8044076.

[3] Lawson CA, Martin DR. Animal models of GM2 gangliosidosis: utility and limitations. Appl Clin Genet. 2016 Jul 20;9:111-20. doi: 10.2147/TACG.S85354. PMID: 27499644; PMCID: PMC4959762.

#### **Authors Biography**



Olivia Grigsby is a senior student pursuing a B.S. degree in Laboratory Sciences and Cell/Molecular Biology at Auburn University. She plans to pursue medical school after graduation.

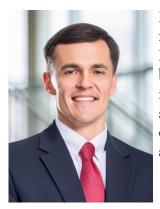


Jordan Towns is a Ph.D. candidate and clinical pathologist at Auburn University College of Veterinary Medicine. In her free time she competes on the national level in the pentathlon.

#### Aubuga University Journal of Undergraduate Scholarship



Dr. Emily Graff is an associate professor and clinical pathologist at the Auburn University College of Veterinary Medicine. She is also an active member of the Boshell Diabetes and Metabolic Disease Research Program.



Dr. Douglas Martin is a professor and director of the Scott Ritchey Research Center at Auburn University. His research in GM1 and GM2 gangliosidoses has had significant impacts on those effected by the disease and their families.

### Phuzzyphase: A Diploid and Polyploid Phasing Tool with Genotype Correction for HIFI and HIC Data

Joshua Horton 1,\*, and Dr. Haynes Heaton2

<sup>1</sup> Undergraduate Student, Department of Computer Science and Software Engineering, Auburn University <sup>2</sup> Assistant Professor, Department of Computer Science and Software Engineering, Auburn University

Accurately haplotype phasing both diploid and polyploid genomes is necessary for getting a precise understanding of organisms' genomes. Pacific Biosciences circular consensus sequencing technology (CCS or HiFi) has revolutionized modern genomics by producing long (10+kb) and highly accurate reads by sequencing circularized DNA molecules multiple times and combining them into a consensus sequence. Additionally, chromosome conformation capture technologies such as Hi-C provide extremely long-range genetic information valuable for haplotype phasing. Here we present Phuzzyphase, a tool using these data types and Bernoulli mixture model clustering to accurately phase both diploid and polyploid genomes. This tool was able to phase human data with a phaseblock N50 with extremely minimal error and virtually no long switch errors, and fully phase most genes.

Diploid organisms such as humans inherit two copies of each chromosome while polyploid organisms such as wheat and potato inherit more than two copies [1]. Haplotype phasing is the process of determining the sequence of alleles that originate from each chromosome [2]. Accurate haplotype phasing of a gene is critical when diagnosing patients especially when multiple deleterious mutations occur on the same gene. If these alleles occur in cis (on the same chromosome), there remains a functional copy of the gene whereas if they occur in trans, there are no functional copies. Historically, haplotype phasing has been done using linkage disequilibrium via statistical phasing. While this has been very useful in studying evolution and population genetics, it does not directly measure the haplotypes of an individual.

For this, we must rely on read-backed phasing in which

\*Corresponding author: jdh0140@auburn.edu

we sequence individual DNA molecules that overlap with multiple genetic variants thus identifying which alleles co-occur on the same haplotype. In read backed phasing, DNA reads are generally mapped to a reference genome, variants are called versus that reference, and heterozygous variants and their associated reads are fed to a haplotype phasing algorithm. This process assumes the upstream genotyping is correct and tries to assign alleles to haplotypes according to the reads on which they occur. However, there is always some level of inaccuracy in variant calling, and variants falsely called as heterozygous cause problems for these algorithms; but if the input data is not taken as truth, the phasing signal can be used to correct these false genotype calls.

In addition to contiguous read backed phasing, emerging technologies such as Hi-C generate long range genetic information which is probabilistically from the same haplotype. Chromatin capture technologies such as Hi-C fix the chromatin and use restriction enzymes and ligation to create small DNA constructs with sequences from the same chromosome but across large genetic distances on that chromosome. This allows direct measurement of haplotype phasing information on the scale of whole chromosomes. However, many popular phasing tools are not able to incorporate this key data type due to either design or algorithmic inefficiencies. For instance, one of the more popular phasing tools, Whatshap, scales with read depth even across regions of missing data such as those common in Hi-C and linked-read technologies [3,4]. Phuzzyphase is able to make use of Hi-C data in order to phase larger portions of the genome and span long homozygous regions of chromosomes which can occur through population bottlenecks and natural selection.

The Phuzzyphase method proceeds by first assigning alleles to HiFi and Hi-C reads, then phasing using HiFi reads, then attempting to combine HiFi phaseblocks into larger meta-phaseblocks using Hi-C reads. In the allele assignment process, in order to overcome alignment edge effects and mapping problems, we align the reads to the reference sequence and to the reference sequence with the alternate allele inserted and assign the read to the allele to which it aligned best. If a read aligns equally well to both sequences, it does not support either allele and is thus unassigned. This is similar to other allele assignment strategies such as those used in WhatsHap, a popular software used for phasing genomic variants.

Phuzzyphase then proceeds to cluster HiFi reads into haplotypes using a cluster center-based technique. Each cluster center represents a haplotype and the values of each loci for that haplotype represent an allele fraction. A true haplotype only has one allele or the other (allele fraction of 0 or 1) but relaxing this discrete constraint allows us to employ numerical optimization strategies such as expectation maximization. The data drives the solution toward the expected discrete outcome of allele fractions of 0 or 1. Normally, expectation maximization starts out with random cluster center initialization. If we proceeded in this way, this would create local optima when fragments originating from haplotype 1 preferred cluster 1 and in another region fragments from haplotype 2 preferred cluster 1. To avoid these local optima, we initialize all allele fractions to 0.5 except the first locus which is randomly initialized. The data then propagates values forward until no data from a previously phase locus spans the next heterozygous site. We use this as a signal for when to end our phase blocks. This cluster center based strategy easily extends to polyploid genomes as we can simply add additional cluster centers to account for the additional haplotypes.

We then use Hi-C data if available to merge phase blocks potentially across whole chromosomes. To do this, for every pair of phase blocks on a chromosome, we calculate a posterior probability of these two-phase blocks being phased together in all possible ways (cis and trans for diploid). We store these in a max-heap sorted by these probabilities. We proceed to pop the maximum probability phase block pairs and merge them into a "meta phase block". We also calculate new

posterior probabilities for phasing this metaphase block with other phase blocks and add these values to the heap. This process continues until the posterior probability of phasing phase blocks falls under some threshold (1-1e4 by default).

Finally, we check for potential long switch phasing errors. Long switch errors are when there is a phase switch that then continues downstream. These are more problematic than short switch errors as all variants downstream will be incorrectly phased with respect to all variants upstream of the long switch error. We do this by taking a breakpoint between every variant and swapping the cluster centers downstream and measuring the likelihood of the data under both the original and swapped versions. Using these two options, we calculate a posterior probability of the data coming from the original or swapped versions. If this probability is less than a threshold (again by default 1-1e4) we break this phase block into two phase blocks.

Demonstrated through the analysis of human genomic data, Phuzzyphase achieves an impressive phaseblock N50 with minimal error rates and virtually eliminates long switch errors. The incorporation of HiFi and Hi-C data enriches the tool with invaluable long-range genetic information, enhancing the accuracy of haplotype phasing. These findings suggest that Phuzzyphase holds promise as a versatile tool with broad applications across various genomic studies. Moving forward, a promising avenue for the Phuzzyphase project involves expanding its testing to the tetraploid genome of the potato. As the potato genome presents unique challenges within its genome and its variant sites, the research could focus on fine-tuning the existing algorithm and parameters to effectively handle polyploid variations. This expansion offers an opportunity to assess Phuzzyphase's adaptability to diverse and challenging genome structures, previously untouched and unsupported by other popular algorithms, providing insights into its performance in scenarios with higher ploidy levels. Additionally, the project could delve into optimizing its speed and accuracy specifically for tetraploid genomes, ensuring that the tool maintains its high accuracy and minimal error rates. Successful testing on the potato genome would not only enhance the tool's applicability but also contribute valuable knowledge to the genomics community.

#### **Statement of Research Advisor**

Josh aided in all aspects of this project from conception to implementation, debugging, and validation. In particular, he contributed algorithmic and statistical ideas for symmetry breaking and criteria for phase block ending.

- Dr. Haynes Heaton, Department of Computer Science and Software Engineering, Samuel Ginn College of Engineering

#### Acknowledgments

I would like to thank Dr Haynes Heaton for their mentorship and expertise. I would also like to thank the department of Computer Science and The Samuel Ginn College of Engineering for this opportunity to further my research ability through their research fellowship program.

#### References

- [1] Tang, D. et al. Genome evolution and diversity of wild and cultivated potatoes. Nature 606, 535–541 (2022).
- [2] Browning, S. R. & Browning, B. L. Haplotype phasing: existing methods and new developments. Nat. Rev. Genet. 12, 703–714 (2011).
- [3] Patterson, M. et al. WhatsHap: Weighted Haplotype Assembly for Future-Generation Sequencing Reads. J. Comput. Biol. 22, 498–509 (2015).
- [4] Zheng, G. X. Y. et al. Haplotyping germline and cancer genomes with high-throughput linked-read sequencing. Nat. Biotechnol. 34, 303–311 (2016).

#### **Authors Biography**



Josh Horton is a senior pursuing a B.S. in Computer Science at Auburn University. As an Auburn Undergraduate Research Fellow, he has contributed to research in the field of Computational Biology, regarding genomics and haplotype phasing algorithms alongside Dr. Haynes Heaton of the Computer Science department.



Dr Heaton is a computational biologist and medical doctor. Interested in computational and algorithmic methods to improve basic science and healthcare. He is an Assistant Professor of Computer Science at Auburn University who completed his PhD in Computational Biology at the Sanger Institute and Cambridge University working with Richard Durbin and Mara Lawniczak on algorithmic and data analysis problems in genomics and single cell transcriptomics

## The Changing Role of Occupational Characteristics in Socioeconomic Health Disparities

Amy E. Hudson<sup>1,\*</sup> and Thomas Fuller-Rowell, Ph.D.<sup>2</sup>

<sup>1</sup> Undergraduate Student, College of Sciences and Mathematics, Auburn University <sup>2</sup> Professor, Human Development and Family Sciences, Auburn University

The relationship between socioeconomic status (SES) and health has long been established. Various factors play a mediating role in the relationship between SES and health, such as neighborhood disadvantage or housing quality. One important factor in the relationship between SES and health is occupation and the characteristics associated with specific jobs. Certain occupational characteristics are associated with better or worse health. Due to the rise in technology and increase in positions requiring higher education, the labor market has shifted dramatically over the past several decades.

The study setting drew from the years 2004 and 2016 of the National Health and Retirement Study (HRS) and the US Occupation Information Network (O\*NET) database. Analyses were drawn from HRS participants younger than 63 years of age and their self-reported occupations to ensure discrete cohorts at each timepoint.

Health outcomes included self-rated health (SRH), body mass index (BMI), Katz's Basic Functional Limitations (ADL), and Lawton's Instrumental Functional Limitations (IADL). These measures were derived from HRS questionnaire items.

Work characteristics considered include substantive complexity, hazardous conditions, and physical demands. Sum scores of O\*NET items were used to create each measure, which were derived from existing literature.

Measures of socioeconomic status include education (a dichotomous assessment of college degree attainment) and income (natural logarithm of lifetime earnings).

Changes in the magnitude of associations between SES

and occupation characteristics, and between occupation characteristics and health, across the two time points were examined using established methods. Results were stratified by race (Hispanic, Black, White) and gender, and adjusted for age and cohabitation status.

The association between substantive complexity and self-rated health (SRH) strengthened for White women ( $\Delta B$ = 0.029, p= 0.0063), Black men ( $\Delta B$ = 0.052, p= 0.046), and Black women ( $\Delta B$ = 0.051, p= 0.0.0075). The average substantive complexity increased significantly for Black women (p < 0.0001), Black men (p < 0.0001), and white women (p < 0.0001), despite the fact that only white women experienced a significant increase in college attainment between 2004 and 2016 (p < 0.0001). From this data, we can conclude that more jobs require a higher level of substantive complexity in 2016 than in 2004, regardless of educational attainment. Additionally, substantive complexity matters more in determining individual health for Black women, Black men, and White women in 2016 than it did in 2004.

For White men, the relationship between income and substantive complexity ( $\Delta B$ = 1.122, p= 0.0001), hazardous conditions ( $\Delta B$ = 0.523, p= 0.0488), and physical demands ( $\Delta B$ = 0.847, p= 0.0071) weakened between 2004 and 2016. The effect of hazardous conditions on BMI ( $\Delta B$ = -0.124, p= 0.0366) and IADL ( $\Delta B$ = -0.008, p= 0.0264) decreased. Physical demands also became less of a determinant of BMI ( $\Delta B$ = -0.141, p= 0.0069) and IADL ( $\Delta B$ = -0.008, p= 0.0264). These findings reflect an overall weakening in the relationship between socioeconomic status and health for white men.

Traditionally, physical demands and hazardous conditions have been characteristic of lower SES occupations

<sup>\*</sup> Corresponding author: aeh0145@auburn.edu

for white men. However, these associations between SES and occupation characteristics among White men appear to be weakening. Furthermore, the average physical demands (p < 0.0002) and hazardous conditions (p < 0.0001) have increased between 2004 and 2016. So, despite increases in physical difficulty and occupational hazards for white men, these factors had less influence on health in 2016 than they did in 2004.

#### Statement of Research Advisor

Amy Beth has conducted detailed analyses to illuminate the changing role of occupation characteristics and work environments in shaping health outcomes across the socioeconomic hierarchy. She has developed a sophisticated skillset to bring complex nationally representative data sources together and address novel research questions with high relevance to society.

- Thomas E. Fuller-Rowell, Human Development and Family Sciences, College of Human Sciences

#### References

Fonda, S., Herzog, A. R., Wallace, R. B., Ofstedal, M. B., Steffick, D., Langa, K., Faul, J. (2004). Documentation of Physical Functioning Measured in the Health and Retirement Study and the Asset and Health Dynamics among the Oldest Old Study. Survey Research Center. Retrieved October 13, 2023 from https://hrs.isr.umich. edu/sites/default/files/biblio/dr-008.pdf (21 December 2004).

Fujishiro, K., MacDonald, L. A., & Howard, V. J. (2020). "Job complexity and hazardous working conditions: How do they explain educational gradient in mortality?," Journal of Occupational Health Psychology, 25(3), pp. 176-186, (2019).

Graf, C., MS, RN, CNS-BC. "The Lawton Instrumental Activities of Daily Living Scale," American Journal of Nursing 108(4): pp. 52-62, (2008).

Paternoster, R., Brame, R., Mazerolle, P. and Piquero, A., "Using the Correct Statistical Test for the Equality of Regression Coefficients," Criminology, 36: 859-866, (1998).

Hadden, W. C., Kravets, N., Muntaner C., "Descriptive dimensions of US occupations with data from the O\*NET", Social Science Research, 33(1), pp. 64-78,

(2004).

#### **Authors Biography**



Amy Hudson is a sophomore-year student pursuing a B.S. in Biomedical Sciences. She has contributed to research on the role of macro-economic context on childhood socioeconomic status gradients in functional limitations by gathering and analyzing statistical data.



Thomas E. Fuller-Rowell is a professor in the Department of Human Development and Family Science at Auburn University and is director of the Health Equity Science Certificate Program. His research examines social determinants of health disparities and how they are evolving in contemporary societies around the world.

### Researching Beneficial Bacteria That Help Plants Survive Cold Environments

Sarah J. Hyde<sup>1,\*</sup> and Kathy S. Lawrence<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Entomology and Plant Pathology, Auburn University <sup>2</sup> Professor, Department of Entomology and Plant Pathology, Auburn University

Plant-growth promoting rhizobacteria (PGPR) are a diverse group of bacteria that have co-evolved with plants, forming symbiotic relationships that can be mutually beneficial [1, 2]. Various geographical locations around the globe are impacted by extended periods of cold on a regular basis. Frost is a plant stressor and crops experiencing short cold periods often produce lower yields[3, 4]. The aim of this research endeavor was to generate a new collection of PGPR strains that will increase cold tolerance in field crops. This was done by evaluating the collection of Alaskan PGPR to determine which would grow at low temperatures, and assessing the potential of the Alaska PGPR strains to enhance corn cold tolerance and plant growth during seed germination.

The bacterial isolate collection was done by Dr. Lawrence in Anchorage, Alaska in September 2022. Twenty-six native plants with roots were collected. In the laboratory, 393 rhizobacteria were identified from the native plants. Cold tolerance experiments were conducted to look for strains that would help plants survive and grown in cold temperatures. Each strain was grown on TSA plates at 4, 22, and 28° C in a randomized complete block design (RCBD) with three replications. The growth of each strain was measured and recorded on a growth scale of 1 to 5, with 1 being no growth and 5 being optimum growth (Fig. 1).



**Fig. 1** The growth pattern of AK386, *Cellulomonas humilata*, was recorded as a 5 or optimum growth at 4, 22, and 28°C.

Corn seeds were treated with 1mL of each individual isolate at 1x107 CFU/ml and grown in Magenta jars, two seeds per jar, in a sandy loam soil at 18°C in a RCBD with three replications (Fig. 2) . Seedling mass was weighed in grams after eight days.







**Fig. 2** (Left) Magenta jars after filling each with sandy loam soil, two corn seeds, and 1mL of each bacterial isolate at 1x107 CFU/ml. (Middle) 50 repetitions in the growth chamber at 18°C. (Right) Corn seeds after inoculation and grown for 8 days.

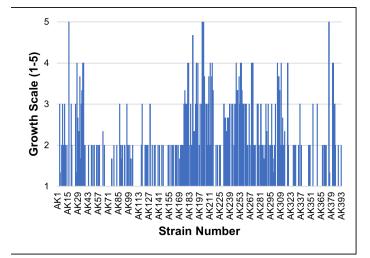
Ten strains of rhizobacteria grew best at 4°C of the 393 strains tested. Specifically, rhizobacteria from the genera *Buttiauxella*, *Erwinia*, and *Pseudomonas* produce the best strain growth at 4°C (Table 1). The average strain growth of the top ten strains was 88.3% more than the average growth of the top ten strains that produced the highest corn biomass (Fig. 3).

Growth chamber evaluations of corn seeding germination and plant biomass ranked the best 10 stains. Rhizobacteria from *Arthrobacter*, *Bacillus*, *Cytobacillus*, *Luteibacter*, *Pseudomonas*, and *Soilibacillus* produced the greatest corn seedling biomass (Table 2). Biomass included the combination of root and shoots. The seedling biomass of the top ten strains grew 37.8% heavier plants than the top ten strains that grew at at 4°C (Fig. 4).

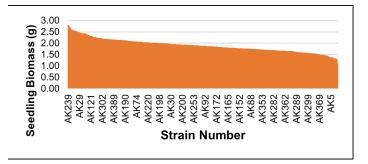
<sup>\*</sup> Corresponding author: sjh0060@auburn.edu

**Table 1** The top ten strain growth at 4°C.

	_		1
Plant	Rhizobacteria	Average	Average
Common	Genus and	Seedling	Strain
Name	Species	Biomass	Growth
		(g)	at 4°C
Wintergreen	Erwinia	1.38	5.0
	billingiae		
Rose	Pseudomonas	2.35	4.0
(native)	yamanorum		
Rose	Erwinia	1.70	4.0
(native)	billingiae		
Devil's club	Pseudomonas	1.40	4.0
	edaphica		
Devil's club	Buttiauxella	1.48	4.0
	agrestis/		
	ferragutiae		
Devil's club	Pseudomonas	1.54	4.7
	neuropathica		
High bush		2.25	5.0
	piscium		
	Pseudomonas	2.16	5.0
	fluorescens		
High bush	Pseudomonas	1.9	5.0
cranberry	piscicola		
Willow	Erwinia	1.64	5.0
	billingiae		
	Common Name  Wintergreen  Rose (native) Rose (native) Devil's club  Devil's club  High bush cranberry High bush cranberry High bush cranberry	Common Name Genus and Species  Wintergreen Erwinia billingiae Rose Pseudomonas (native) yamanorum Rose Erwinia (native) billingiae Devil's club Pseudomonas edaphica Devil's club Buttiauxella agrestis/ferragutiae Devil's club Pseudomonas neuropathica High bush Pseudomonas cranberry piscium High bush Pseudomonas fluorescens High bush Pseudomonas cranberry fluorescens High bush Pseudomonas piscicola	Common NameGenus and SpeciesSeedling Biomass (g)WintergreenErwinia billingiae1.38Rose (native)Pseudomonas yamanorum2.35Rose (native)Erwinia billingiae1.70Devil's clubPseudomonas edaphica1.40Devil's clubButtiauxella agrestis/ ferragutiae1.48Devil's clubPseudomonas neuropathica1.54High bush cranberryPseudomonas piscium2.25High bush cranberryPseudomonas fluorescens2.16High bush cranberryPseudomonas piscicola1.9WillowErwinia1.64



**Fig. 3** The rhizobacteria growth at 4° C of all 393 collected strains.



**Fig. 4** The seedling biomass in grams of all 393 collected strains.

**Table 2** The top ten strains producing the highest corn biomass.

Isolate	Plant	Rhizobacteria	Average	Average	
Number	Common	Genus and Species	Seedling	Strain	
	Name		biomass	Growth	
			(g)	at 4°C	
AK43	Fescue	Bacillus	1.38	5.0	
		paralicheniformis			
AK46	Fescue	Bacillus	2.35	4.0	
		haikouensis			
AK48	Fescue	Arthrobacter	1.70	4.0	
		ginsengisoli			
AK79	Alpine	Luteibacter	1.40	4.0	
	forget-me-	rhizovicinus/			
	not	pinisoli			
AK141	Chocolate	Cytobacillus	1.48	4.0	
	lily	firmus			
AK144	Chocolate	Bacillus	1.54	4.7	
	lily	cabrialesii/			
		inaquosorum/			
		tequilensis			
AK214	Lycopodi-	Pseudomonas	2.25	5.0	
	um moss	neuropathica			
AK239	Marsh	Pseudomonas	2.16	5.0	
	Labrador	piscium			
	tea				
AK318	Bluejoint	Solibacillus	1.9	5.0	
	grass	isronensis			
AK385	Wax myrtle	Pseudomonas	1.64	5.0	
	sweet gale	piscium			

For strain growth at 4°C, the top and bottom ten strains were found to have an 84.2% difference in values. Thus, the best strains were acclimated to cold temperatures and were able to grow and increase bacteria colony size significantly more than the other strains collected. For seedling biomass growth, the top and bottom ten strains had a 67.7% difference in values. This indicated these strains were able to benefit corn plant growth at low temperatures for corn germination. These tests indicated AK strains AK28, AK201, AK202, AK214, and AK239 grew well at 4°C and also increased corn plant growth at 18°C. Strain AK239 Pseudomonas piscium had the best biomass at low temperatures, with AK214 *Pseudomonas neuropathica* closely following. These AK strains should be further tested in the greenhouse and field to determine plant survival benefits in the natural environment.

#### Statement of Research Advisor

Since Summer 2023, Sarah has made significant contributions to our studies on bacteria from cold environments. Her responsibilities have included conducting trials to test bacterial cultures across various temperature ranges to determine their growth temperature tolerances. Additionally, she examined the effects of these bacteria cultures on corn seed germination and plant growth promotion under suboptimal temperatures.

- Dr. Kathy Lawrence, Entomology and Plant Pathology, College of Agriculture



[1] Gouda, S., Kerry, R. G., Samal, D., Mahapatra, G. P., Das, G., & Patra, J. K. (2018). Application of plant growth promoting rhizobacteria in agriculture. In Advances in Microbial Biotechnology (pp. 73-86). Apple Academic Press.

[2] Lyu, D., Msimbira, L.A., Nazari, M., Antar, M., Pagé, A., Shah, A., Monjezi, N., Zajonc, J., Tanney, C.A., Backer, R. and Smith, D.L. (2021). The coevolution of plants and microbes underpins sustainable agriculture. Microorganisms, 9(5), 1036.

[3] Olien, C. R. (1967). Freezing stresses and survival. Annual Review of Plant Physiology, 18(1), 38.

[4] Thakur, P., Kumar, S., Malik, J. A., Berger, J. D., & Nayyar, H. (2010). Cold stress effects on reproductive development in grain crops: an overview. Environmental and Experimental Botany, 67(3), 429-443.

#### **Authors Biography**



Sarah Hyde is a senior-year student pursuing a B.S. in Environmental Science at Auburn University. She has contributed a year to this project and has a passion for improving agricultural systems through research.



Dr. Kathy Lawrence is a professor and researcher in the department of Entomology and Plant Pathology. Areas of expertise are in soil borne and

fungal diseases, specifically plant parasitic nematodes and fungi attacking field crops, vegetables, and ornamentals, with emphasis on nematode and fungal pathogen interactions and host-pathogen relationships in the environment.

### **AAV-mediated Anti-hormone Antibody Therapy** as a Treatment for Alzheimer's Disease

Emma E. Hruska<sup>1,\*</sup>, Arthur D. Zimmerman <sup>2</sup>, and Douglas R. Martin<sup>3,4</sup>

<sup>1</sup> Undergraduate Student, College of Sciences and Mathematics, Auburn University
 <sup>2</sup> Research Fellow, Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University
 <sup>3</sup> Professor, Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University
 <sup>4</sup> Director, Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University

Alzheimer's disease (AD) is the most common form of dementia and is marked by abnormal accumulation of beta-amyloid proteins and the hyperphosphorylation of tau proteins that together lead to neuronal degeneration. It is estimated that by 2025, 7.2 million people aged 65 and older will have AD, a 7% increase from 2023. Additionally, almost two-thirds of Americans with AD are women, and evidence suggests that menopause is a clear driver for AD development. During this period, hormone levels change due to lack of estrogen, and previous studies have implicated altered hormone levels as a potential factor for AD development. Thus, we hypothesize that increased hormone levels may contribute to the neuropathology and memory loss associated with AD. To test this, we used an adeno-associated virus (AAV)-mediated anti-hormone antibody treatment in APP/PS1 mice, a model of AD. We examined changes in estrous cyclicity, hormone levels, and memory and learning behavior. Here, we report that treatment with anti-hormone antibodies significantly disrupts estrous cyclicity, specifically leading to an increased time spent in estrus, and lowers hormone levels 6-9 months post-treatment. Additionally, treated APP/ PS1 mice appear to show improvement in a food choice test that measures social transmission of food preference (STFP), a behavior that deteriorates with neurodegeneration. Overall, AAV-mediated antibody treatments appear to not only disrupt estrous cyclicity likely due to alterations in hormone levels, but also restore learning and memory performance during STFP.

Alzheimer's disease (AD) is a cause of dementia specifically marked by brain changes including abnormal accumulation of amyloid-beta (A $\beta$ ) proteins and degeneration of nerve cells. This disease was named for Alois

Alzheimer, a clinical psychologist and neuroanatomist, who first noted the disease in a 50-year-old woman, namely the  $A\beta$  plaques and neurofibrillary tangles that remain the hallmarks of AD [2].

A $\beta$  protein fragments clump together outside of neurons to form plaques that interfere with neuron communication at synapses, which are connections between neurons where information is transferred. In addition, the basis of neurofibrillary tangles is the accumulation of an abnormal form of the tau protein inside neurons, blocking transport of nutrients and other key molecules [2]. Ultimately, these morphological changes within neurons lead to their damage and destruction and are the primary manifestations of AD.

It is estimated that by 2025, 7.2 million people aged 65 and older will have AD (an 7% increase from 2023). Currently, one in three seniors die from AD or another dementia, and deaths from AD have increased 145% from 2000 to 2019 [1]. One key statistic is that women are twice as likely as men to develop AD, and furthermore, females are more likely to die due to AD than men [3]. Therefore, it is critical that researchers continue to elucidate factors driving the disparity between women and men and to work towards new AD therapies that can stop and/or slow disease progression.

There are a multitude of risk factors for AD ranging from lifestyle choices to genetic pre-dispositions, and some of them can help explain the male/female gender disparity [5]. One key risk factor for AD is alterations in hormone levels, and specifically, our research centers on hormonal fluctuations during menopause and how this relates to disease progression in females. The central

<sup>\*</sup> Corresponding author: eeh0046@auburn.edu

question of our research is whether lowering hormone levels using adeno-associated virus (AAV)-mediated anti-hormone antibodies can slow and/or prevent the progression of AD. Here, we tested whether AAV-mediated antibody treatment was effective by evaluating biologically relevant variables and behavior.

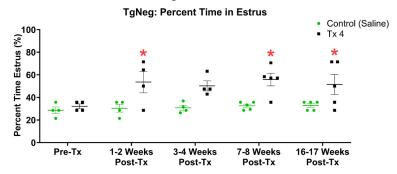
Following anti-hormone antibody treatment, we examined both estrous cyclicity, an important biological factor regulated by this hormone, and serum hormone levels. These are both non-lethal measurements that can be tracked over extended periods of time. In this study, we used APP/PS1 mice and their relevant transgene negative (TgNeg) controls. APP/PS1 mice express a chimeric human/mouse amyloid precursor protein (APP) mutation and a mutant human presenilin 1 (PS1) protein. PS1 plays a major role in processing amyloid precursor protein, and these mice both overproduce and have altered processing of APP. Importantly, they experience early-onset memory loss as well. At 3 months of age, mice were treated intravenously through the tail vein with an AAV expressing one of our four anti-hormone antibodies. Control mice were treated with saline only. Mice were vaginally lavaged daily to track their estrous cycle and determine which phase of the cycle they were in: proestrus, estrus, metestrus, or diestrus. Blood was collected to analyze serum hormone levels, and qPCR was performed to examine AAV vector biodistribution in the brain, heart, and liver.

Lastly, behavioral analysis was performed using an important learning and memory behavior among rodents known as the Social Transmission of Food Preference (STFP). During the acquisition phase of STFP, an observer mouse detects two olfactory cues from a demonstrator mouse: a semiochemical, carbon disulfide, and a general food odor. These two sensory signals are then consolidated into a memory preference. Upon re-exposure to the odor during a food choice test, an observer mouse will recall/retrieve the odor memory and prefer the food containing the demonstrated odor [4]. Food preference is then measured as a ratio of food consumed with the demonstrated odor vs. the total amount of food consumed. A preference ratio above 0.6 has been generally accepted as the preference ratio that signals the mouse has formed a preference for the food containing the demonstrated odor.

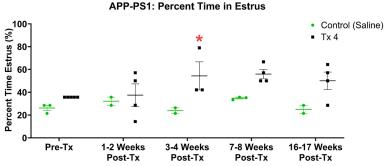
Results focus on mice given Treatment 4, as it was the most consistent of the four AAV-mediated anti-hormone antibodies tested.

Based on the estrous cyclicity data (Figs. 1 and 2), both the TgNeg and APP/PS1 mice receiving Treatment 4 spent an increased percent time in estrus that remained elevated at 16-17 weeks post-treatment, thus indicating a likely effect of the anti-hormone antibody treatment.

When examining serum hormone levels, TgNeg mice given Treatment 4 had lower serum hormone levels at two different terminal time points, either 6 or 9 months post-treatment (Fig. 3). This is also the time point at which there is a natural elevation in hormone level due to age. While not significant, there was a similar trend in the APP/PS1 mice (Fig. 4).



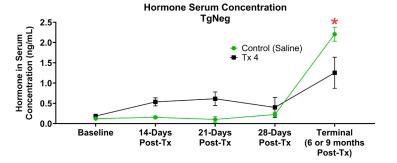
**Fig. 1** Percent time spent in the estrus phase of the estrous cycle in TgNeg mice. \*p<0.05. Data was analyzed using 2-way ANOVA with Tukey post-hoc multiple comparisons.



**Fig. 2** Percent time spent in the estrus phase of the estrous cycle in APP/PS1 mice. \*p<0.05. Data was analyzed using 2-way ANOVA with Tukey post-hoc multiple comparisons.

In addition, AAV vector was distributed throughout the brain, heart, and liver, the only organs evaluated to date (Fig. 5). Finally, results from the STFP show control (saline-treated) TgNeg mice were able to demonstrate

a food preference, which is expected due to their lack of AD pathology. The control (saline-treated) APP/PS1 mice were unable to develop a food preference, which was also expected as they present with cognitive deficits as early as 3 months of age. Strikingly, a food preference was formed in APP/PS1 mice given Treatment 4 the day of odor demonstration and maintained 4 weeks after demonstration (Fig. 6), indicating a restoration of learning and/or memory performance in treated mice.



**Fig. 3** Serum hormone levels in TgNeg mice. \*p<0.05. Data was analyzed using 1-way ANOVA with Tukey post-hoc multiple comparisons.

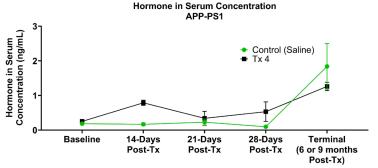
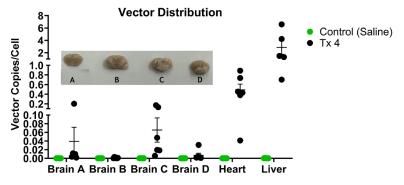


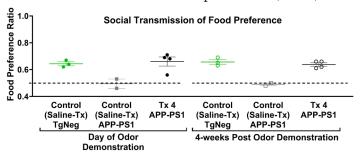
Fig. 4 Serum hormone levels in APP/PS1 mice.



**Fig. 5** AAV vector biodistribution within brain, heart and liver.

Following treatment with an adeno-associated virus (AAV)-mediated anti-hormone antibody, both TgNeg and APP/PS1 mice show altered estrous cyclicity (spe-

cifically more time spent in estrus) as well as lower serum hormone levels at 6 or 9 months post-treatment. In addition, APP/PS1 mice treated with the antibody show maintenance or restoration of learning and memory performance during a food choice test following the social transmission of food preference (STFP).



**Fig. 6** Food choice test following the social transmission of food preference in TgNeg and APP/PS1 mice.

Future studies will examine changes in other memory and learning behaviors to determine which treatment may provide the most benefit for slowing and/or preventing AD progression.

#### **Statement of Research Advisor**

Emma began work on this project in the Spring semester of 2022. Her contributions are extensive and comprise animal treatments, sample and blood collections, and sample analyses including qPCR, ELISA, immunohistochemistry, and STFP behavior. Her work has been instrumental in laying the groundwork for a project that will hopefully provide future therapeutic benefits to prevent or slow AD progression.

- Arthur Zimmerman, Scott-Ritchey Research Center, College of Veterinary Medicine

#### References

[1] Alzheimer's Association. 2023 Alzheimer's Disease Facts and Figures. Alzheimer's Dement 2023;19(4). https://doi.org/10.1002/alz.13016

[2] Hippius, H., & Neundörfer, G. (2003). The discovery of Alzheimer's disease. Dialogues in Clinical Neuroscience, 5(1),101–108. https://doi.org/10.31887/dcns.2003.5.1/hhippius

[3] Holmes, D. J. (2014). Sex and gender differences in life span, aging, and health. Reference Module in Biomedical Sciences.

https://doi.org/10.1016/b978-0-12-801238-3.00161-6

[4] Munger, S. D., Leinders-Zufall, T., McDougall, L. M., Cockerham, R. E., Schmid, A., Wandernoth, P., Wennemuth, G., Biel, M., Zufall, F., & Kelliher, K. R. (2010). An olfactory subsystem that detects carbon disulfide and mediates food-related social learning. Current Biology, 20(16), 1438–1444. https://doi.org/10.1016/j.cub.2010.06.021

[5] Seto, M., Weiner, R. L., Dumitrescu, L., & Hohman, T. J. (2021). Protective genes and pathways in Alzheimer's disease: Moving towards Precision Interventions. Molecular Neurodegeneration, 16(1). https://doi.org/10.1186/s13024-021-00452-5

#### **Authors Biography**



Emma Hruska is a junior-year student pursuing a B.S. degree in Genetics at Auburn University. She has been involved in the Martin Lab at the Scott-Ritchey Research Center for the past two years. As a 2023-2024 Undergraduate Research Fellow, she contributed to research regarding innovative treatments for Alzheimer's disease.



Arthur Zimmerman is a Research Fellow at the Scott-Ritchey Research Center. Arthur received his BS and MS from Auburn University and his PhD from the University of Florida. He returned to Auburn in 2021 where his work focuses on how alterations in hormone levels can influence learning and memory and contribute to neurodegenerative diseases.



Dr. Douglas Martin received both his BS and PhD from Auburn University. He is a Professor in the Department of Anatomy, Physiology and Pharmacology, and is the Director of the Scott-Ritchey Research Center. Dr. Martin's research centers on molecular therapy for neurodegenerative diseases, and specifically, his work has made significant contributions to the development of gene therapy that has been successfully used in ongoing clinical trials for GM1 gangliosidosis, Tay-Sachs disease, and Sandhoff disease.

## Modeling Ammonium To Prepare for a Nitrogen Mass Balance for an Algal-Bacterial System

Ann E. Inskeep<sup>1,\*</sup>, Brendan T. Higgins<sup>2</sup>, and Daniel E. Wells<sup>3</sup>

Undergraduate Student, Department of Biosystems Engineering, Auburn University
 Associate Professor, Department of Biosystems Engineering, Auburn University
 Associate Professor, Department of Horticulture, Auburn University

The purpose of this study was to create models of the transformation of nitrogen in poultry processing wastewater which is treated for irrigation in controlled-environment agriculture. Specifically, this research focuses on ammonium transformations and the phases in which they occur. To reach this objective, the first steps of a Nitrogen mass balance model were created throughout each stage of the treatment system as well as a hydroponic lettuce production system.

The poultry industry is Alabama's largest agricultural industry, producing more than 60 billion gallons of wastewater annually with an estimated processing cost of \$255 million [2,3]. Although the wastewater is a rich source of nutrients, it contains various pathogens such as E. coli and Salmonella [1]. While current treatment techniques show promise for mitigating adverse environmental impacts, there is an increased need for a sustainable approach to treat wastewater which also promotes efficient nutrient utilization.





**Fig. 1 & Fig 2.** Image 1 (left) shows the first bioreactor in the system that filters the poultry wastewater. The second image (right) displays an example of the product of legume lettuce that was produced from the system.

In this system, there is a six-step process starting at a storage tank, through either algae or bacterial bioreactors (Fig. 1), a clarifier, a bag filter, UV treatment, storage tanks, and plant reservoirs (Fig. 2). Two parallel treatment trains were established – one with algal bioreactors and one with bacterial bioreactors. The two systems ran for over 200 days with real poultry processing wastewater at a continuous flow rate of 57 L/day. During this time, four lettuce production campaigns were run, but this research focuses on modeling nitrogen for a portion of the third trial that was conducted. Measurements of ammonium were modeled for 21 days of the 50-day trial. Later, this model will be expanded to cover additional time. The model was developed in Microsoft Excel and enables the determination of net ammonification and nitrification rates in the system. The measured nitrogen concentration data was interpolated using Equation 1 to determine the "actual" values for each alga and bacteria bioreactor on an hourly and daily basis.

$$y = y_1 + (x - x_1) \frac{(y_2 - y_1)}{(x_2 - x_1)} \tag{1}$$

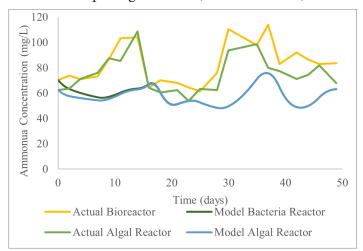
Volume of the Bioreactors and Volume exchange per time step (L) were used to determine a theoretical "model" for each alga and bacteria bioreactor through the storage tank without reaction (Equation 2).

$$C_i = \frac{(C_{i-1} + F)}{V(C_{i-1} - C_f)} \tag{2}$$

New wastewater was introduced to the system on days 0, 9, 17, 28, and 38 which caused oscillation in the data. With the current findings, the actual and no-reaction model had a wider difference starting at the storage tank. This is due to ammonification reactions resulting in higher levels of ammonium in the actual reactor. This same process was repeated for the downstream operations resulting in less ammonification and later nitrifi-

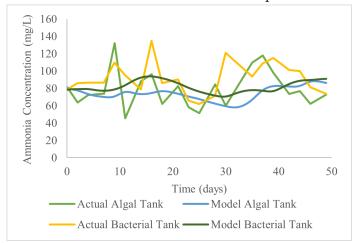
<sup>\*</sup>Corresponding author: aei0006@auburn.edu

cation in the plant grow beds (data not shown).



**Fig. 3.** Above displays the actual and no-reaction model values throughout the algae and bioreactor models. This is the first step in the treatment process.

In the poultry wastewater storage tank, there is a quicker change in the ammonia quantity correlating to the days on which new wastewater is delivered. The ammonia accumulates rapidly in this tank compared to the other parts of the system due to ammonification during storage. Consequently, in Figure 3, the model and actual values converge of ammonium at certain time points because little additional ammonification occurs in the bioreactors. In later stages, (e.g. storage tank before plant reservoirs, shown in Fig. 4), little to no ammonification nor nitrification occurred and consequently, the model and actual ammonium levels are quite similar.



**Fig. 4.** Above displays the actual and model values throughout the storage tank before the fluid enters the plant reservoirs. This is the second to last step in the filtering process.

The process of interpolation of the known ammonium will continue to be used throughout the next phases of the system such as the plant reservoirs. Reaction rates in each compartment will also be calculated. Once this is completed, the same process will occur with nitrite, nitrates, organic nitrogen, and phosphate. When this data is analyzed, we can determine the fate and transformation of nitrogen throughout the entire system. The result will be a comprehensive dataset of all the nitrogen entering and exiting throughout the whole algal-bacteria hydroponic system.

#### **Statement of Research Advisor**

This work embodies the first steps of developing a comprehensive mass balance of macronutrients throughout the "poultryponics" system. Poultryponics is a novel technology to re-use meat processing wastewater to safely grow food. This model enables improvements to the process, aiming to increase nitrification without losses of nitrogen to denitrification.

- Brendan Higgins, Department of Biosystems Engineering, Samuel Ginn College of Engineering

#### References

- [1] Barros LSS, Amaral LA, Lorenzon CS, Junior JL, Neto JGM. Potential microbiological contamination of effluents in poultry and swine abattoirs. Epidemiol Infect. 2007 Apr;135(3):505–18.
- [2] Carawan R, Taylor MC, Curtis PA, Keener K. Survey Shows That Poultry Processors Can Save Money by Conserving Water. Pollution Prevention Pays in Food Processing. 2014;3–4.
- [3] Northcutt, J. K., & Jones, D. R. (2004). A survey of water use and common industry practices in commercial broiler processing facilities. Journal of Applied Poultry Research, 13(1), 48–54. https://doi.org/10.1093/japr/13.1.48

#### **Author's Biography**



Ann E. Inskeep is a senior-year student pursuing a B.S. degree in Biosystems Engineering at Auburn University. She has contributed a year to the project and has played a key role in starting a nitrogen mass balance for a poultry wastewater hydroponic system.



Dr. Brendan Higgins is an Associate Professor in the Department of Biosystems Engineering at Auburn University. His research consists of application in bioenergy, processes that incorporate algae and bacteria for waste remediation, water quality, biofuels, and high-value nutraceuticals. He continues to study combined algal-bacterial processes.



Dr. Daniel Wells is an Associate Professor in the Department of Horticulture at Auburn University. His research focuses on improving controlled environment agriculture in the southeast. With projects including plant nutrition in hydroponic and aquaponic systems, and optimization in temperature-controlled environments.

### **Analyzing the Use of Historical Chairs in Modern Media**

Alissa L. Johnson<sup>1</sup>, Darby Huber<sup>2</sup>, and Anna Ruth Gatlin, PhD.<sup>3,\*</sup>

Second-Degree Undergraduate Student, College of Human Sciences, Auburn University
 Undergraduate Student, College of Human Sciences, Auburn University
 Assistant Professor, College of Human Sciences, Auburn University

Certain artifacts, symbols, philosophies, and other similar items and ideologies have persisted across the rise and fall of different cultures. However, one thing that has impacted humankind's history and a portion of its creation is the design of their residences, workplaces, and entertainment spaces. None has affected these the deepest or the longest than interior furniture pieces' creation and changing structure. What seems like an insignificant piece to a room, or a place to rest your legs or a bag, is more complex regarding its creation and features than a superficial glance allows. This is evident by their repeated use in highly acclaimed movies and TV Shows, gracing the silver screen by being seemingly silent in the background. Upon closer analysis of the structures, materials, historical background, and suggested implementation in modern movies and TV shows, their significance and the meaning they provide as a representation of a feeling or a culture is revealed.

The first step in the analysis process was to learn the chairs that have impacted the design history in a particular way. Whether by the types of materials used, how the chair was produced, or even how the chair reacted to a specific social movement, it provided the basis for looking for pieces of furniture in the media. These furniture options could then be further narrowed down to a select few after later analysis of different media types to see which furniture pieces appeared more frequently than others.

An example of a chair frequently seen in the media upon later analysis was the Barcelona Chair from the Barcelona furniture collection, created and built by Mies van der Rohe and Lily Reich in 1929. While looking like an average, rectilinear leather chair that sits low to the ground, this chair carries more weight than meets the eye. The chair was a component of the site of the Ger-

man Exhibition for the Barcelona Exposition of 1929, otherwise known as The World's Fair of 1929. Mies and Reich were asked personally by the leaders of Germany to put together a collection of modern furniture to represent Germany for the exhibition; a set of furniture that would be fit to" receive a king...the chair had to be monumental." [6]

While receiving applause for the collection during the exhibition, the chair was later manufactured and patented by Knoll International INC. in 1929 and has been produced since. Featured in shows like Cobra Kai, Suits, and much more, it is easy to see why the modern lines and clean looks were so well used upon further inspection. After further consideration, the chair represents power with its robust shape and durable materials. It was not constructed to be a dainty side piece but to be the place fit for royalty to sit. Its size adds even more to this factor of an imposing ruler with a broad base of almost 3 feet wide. By looking through this lens of having the materials carrying through the meaning of a piece of furniture, it becomes clear why the Barcelona chair fits specific situations. And in the words of Mies, it is no wonder why "you couldn't use a kitchen chair." [5, 6]

Carrying on with this theme is another popular furniture piece that was repeated in many historical adaptations, the curule chair. This chair, constructed originally in ancient Egypt but found its closest modern relative in the ancient Romans, was a wooden chair with a rush or woven seat used by the highest-ranking government officials in their respective eras. Later adaptations were found in the Italian Renaissance and the Empire style popular in Western Europe and North America. The chair derives its name from the Greek word for chariot, "cirrus," which was most associated with its use of magistrates and represented a place of judgment. [1]

<sup>\*</sup> Corresponding author: gatliar@auburn.edu

Looking through a more historical lens, we could derive the use of this chair in media like The Crown, Bridgerton, and Downton Abbey. While the chair not only provides an accurate historical representation of furniture that would have been available during that time, but the chair also itself carries a meaning of power, wealth, and justice. This hypothesis is confirmed by what shows the chair is depicted in and what characters are typically seen in the scene with the chair; powerful men either in the dominion of a nation, a corporation, or their estates. [4]

Other furniture types seen less often but depicted in media varied from a historical re-adaptation to a modern film or show that wanted to portray a particular emotion about the character. Such chairs were the Wassily chair constructed in the Modernist movement by Marcel Breuer, the Eames Lounge Chair by Charles and Ray Eames, the Windsor chair of the Georgian and Federalist design movements, and the Klismos chair, which the ancient Greeks built in the 4th Century BC. All these furniture pieces, like the ones previously described, used their structure, materials, and history behind them to convey their use in modern media, whether to convey the accuracy of a film or to reveal a hidden personality trait of a particular character. [2, 3, 7]

By using the repetition of different furniture pieces in other films and dissecting the layers of meaning within the piece's construction and materials, it was viable to assume that furniture carries with it an underlying meaning and significance that impacts humans and the design of spaces long after their initial introduction into the world. The way that furniture is perceived and used in a room is, therefore, also indicative of the historical narrative that the furniture pieces carry, as well as who constructed the furniture piece and why. Through the analysis of these films, it is also clear that the individuals who selected these pieces of furniture were also aware of these facts and used the furniture pieces accordingly. It is safe to assume that furniture carries with it not just a long history of what humans could do at that time, but also how humans perceived and interacted with the world around them and how they continue to interact with it to this day.

#### **Statement of Research Advisor**

One arm of my research is analyzing and critiquing the intersection of interior design and set design. Using the lens of the aesthetic experience of film/television, concept and symbolism, and auteur theory, I explore the mise en scene of stills captured from media. Alissa and Darby are working in this research stream, focusing on analyzing and critiquing the choice of certain chairs in cinema and television..

- Dr. Anna Ruth Gatlin, College of Human Sciences, Auburn University

#### References

- [1] Encyclopædia Britannica, inc. (2023, June 16). *Curule chair.* Encyclopædia Britannica. https://www.britannica.com/topic/curule-chair
- [2] Encyclopædia Britannica, inc. (n.d.-a). *Klismos*. Encyclopædia Britannica. https://www.britannica.com/topic/klismos
- [3] Encyclopædia Britannica, inc. (n.d.-b). *Windsor Chair*. Encyclopædia Britannica. https://www.britannica.com/topic/Windsor-chair
- [4] Furniture, / Mackinnon Fine. (2017, October 10). *The seat of power: The curule form & its origin.* The Source. https://thesourceforantiques.wordpress.com/2017/10/10/the-seat-of-power-the-curule-form-its-origin/
- [5] Knoll. https://www.knoll.com/knollnewsdetail/design-deconstructed-barcelona-chair
- [6] Ludwig Mies van der Rohe. Barcelona Chair. Designed 1929 (this example 1953): Moma. The Museum of Modern Art. https://www.moma.org/collection/works/4369#:~:text=Mies%20van%20der%20Rohe%20designed,%2C%22%20according%20to%20the%20architect.
- [7] Ray, D. (2023, July 5). From the archive: A short history of Marcel Breuer's Wassily Chair. ICON Magazine. https://www.iconeye.com/design/a-brief-history-of-marcel-breuers-wassily-chair#:~:text=His%20 Wassily%20chair%20is%20the%20most%20iconic&text=Hungarian%2DAmerican%20architect%20Marcel%20Breuer,inspired%20by%20a%20bicycle%20

frame.

#### **Authors Biography**



Alissa L. Johnson is a second-degree junior-year student pursuing a B.S. degree in Interior Design through the College of Consumer and Design Science at Auburn University. She has played a vital role in the identification of historical furniture pieces, identifying historical timeframes, and putting together other written research information. Alissa's previous undergraduate degree is in Exercise Physiology from West Virginia University. Alissa came to Auburn University in the Fall of 2021 and has used her passion for interior design with her research-based background knowledge to help further study historical chairs and other furniture pieces. She has a profound interest in history and how people and the designs that they create have evolved in similar but contrasting ways.



Darby Huber is a junior-year student pursuing a B.S. degree in Interior Design through the College of Consumer and Design Science at Auburn University. She has gathered the collection of visual data gained throughout the project and put together all graphic portions of the research. Her research interests lie in how design affects the world and how each interacts with it and in producing modern design ideas that cater to human behavior. Aside from interior design, her interests lie in writing, art, and graphic design.



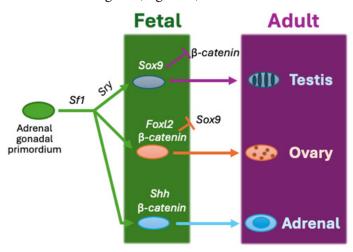
Anna Ruth Gatlin, PhD, is an Assistant Professor of Interior Design at Auburn University. An award-winning interior designer, she also has an established record of traditional and creative scholarly contributions. Many of her creative scholarship works examine aspects of southeastern life through an autoethnographic lens, executed in heritage fiber arts techniques.

### The Role of Anti-Müllerian Hormone Receptor Type 2 (AMHR2)-Expressing Cells in Adrenal Gland Development

Su Jeong Kim<sup>1,\*</sup>, Humphrey Yao<sup>2</sup>, and Chen-Che Jeff Huang<sup>3</sup>

<sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University
 <sup>2</sup> Senior Investigator, Reproductive Developmental Biology Group, National Institute of Environmental Health Sciences
 <sup>3</sup>Associate Professor, College of Veterinary Medicine, Auburn University

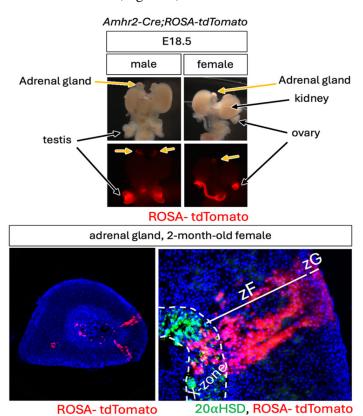
Adrenal gland diseases display a higher prevalence in women than men, and genome-wide studies reveal sexual dimorphism in adrenal gland gene expression. The underlying mechanism of this dimorphism remains unclear, but it is hypothesized to be linked to the shared origin of the adrenal gland and gonads during development. Many key genes are specifically expressed in gonads and adrenal gland to control the development of these three organs (Figure 1).



**Fig. 1** The adrenal gland and gonads share a common embryonic origin. The differentiation and development of these three organs are regulated by specific genes whose expression is tailored to the unique requirements of each tissue.

The receptor for the anti-Müllerian hormone (AMH), encoded by AMHR2, controls the differentiation of secondary sex organs. Mutations in AMH or AMHR2 result in Persistent Müllerian Duct Syndrome (PMDS), a condition in which a biological male develops female sex organs.

Despite primarily expressing in sex organs, the pilot study indicates that AMHR2(+) cells contribute to adrenocortical cells (Figure 2).



**Fig. 2** The image above displays the adrenocortical cells of Amhr2-Cre;ROSA-tdTomato mice. The RO-SA-tdTomato gene (expresses red fluorescence) labels AMHR2(+) cells and their descendants. Note that AMHR2(+) cells and their descendants can be found in fetal gonads and adrenal glands. In the adult adrenal gland, some cells in the adult adrenal gland are either AMHR2(+) or originate from AMHR2(+) cells.

<sup>\*</sup>Corresponding author: szk0189@auburn.edu

The present research project utilizes a mouse model with double genetic modifications, expressing the diphtheria toxin subunit alpha gene (DTA) in AMHR2(+) cells. This causes the elimination of all AMHR2(+) cells and their descendants. A comparative analysis of adrenal glands between double mutant mice and wild-type littermates will elucidate the role of AMHR2(+) cells in adrenal gland development.

Gon	Adrenal Weight (mg)				Body Weight (g)						
Tes	Testis Ovary		Male		Female		Male		Female		
WT	KO	WT	KO	WT	KO	WT	KO	WT	KO	WT	KO
97.1	4.9	3.2	3.0	2.2	1.8	2.8	2.9	27.3	29.3	22.4	23.9
40.8	3.8	3.6	3.4	2.4	2.6	3.0	3.7	43.1	32.3	22.9	27.9
101.1	9.2	3.8	2.5	1.5	2.0	2.3	3.6	35.3	26.3	24.1	34.4
54.0	2.7	4.3	0.1	2.3	2.0	2.5	3.0	31.9	37.9	30.9	32.8
60.7	2.9	2.8	1.2	1.6	1.6	3.4	3.5	30.1	37.7	23.1	27.0
49.2		3.8		2.0		3.5	2.9	35.9		33.4	20.9
88.4				2.4				27.5			
109.0				2.9				39.0			
118.9				2.1				33.9			
	Average										
79.91	4.70	3.58	2.04	2.16	2.00	2.92	3.27	33.77	32.70	26.13	27.81
STD											
28.93	2.66	0.52	1.36	0.43	0.37	0.48	0.38	5.24	5.11	4.75	5.13
P value											
0.00	0.0001 0.0300		0.5098 0.1847			0.73	175	0.5679			

**Table 1** Above displays the weight of the gonads, adrenal gland, and body.

Preliminary results show small testes and ovaries in double mutant mice, emphasizing the importance of AMHR2(+) cells in gonad development. However, despite AMHR2(+) cells being in the adrenal gland, adrenal gland size remains unchanged. (Table 1) This result aligns with lineage-tracing findings that the adrenal gland cortex continuously renews its cell population (Figure 3).

While the preliminary data suggests no impact on adrenal gland size, ongoing research involves double immunostaining to assess the potential effect on adrenal cortex zonation. Marker genes like 3βHSD, β-catenin, CYP2F2, and tyrosine hydroxylase are used to identify specific cell types and zonation patterns (Figure 4). If AMHR2(+) cells play a crucial role, the expectation is a disorganized adrenal cortex in double mutant mice. This study contributes valuable insights into the role of AMHR2(+) cells in adrenal gland development and may uncover connections to sexual dimorphism.

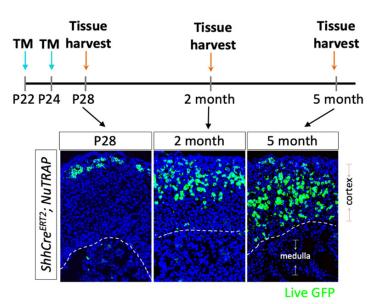


Fig. 3 The image above displays the lineage tracing model showing the renewal of the adrenal gland cortex. Shh(+) cells in the outer cortex (at P28) proliferate and replace the entire cortex in 4 months (from P28 to 5-month-old).

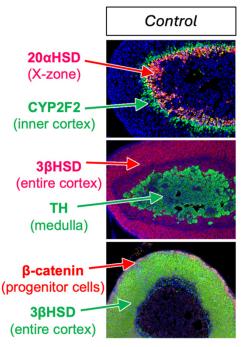


Fig. 4 Double immunostaining shows different types of cells in adrenal glands in control mice.

#### **Statement of Research Advisor**

Su Jeong Kim has made good progress in utilizing double immunostaining techniques to acquire high-quality fluorescent images for the project. Further experiments will be completed to elucidate the role of AMHR2(+) cells in adrenal gland development.

Jeff Huang, Department of Anatomy, Physiology, and

Pharmacology, College of Veterinary Medicine

#### References

Huang, C.C. and Kang, Y., "The transient cortical zone in the adrenal gland: the mystery of the adrenal X-zone," J Endocrinol., 241(1), R51-R63,(2019).

Huang, C.C., Kraft, C., Moy, N., Ng, L., Forrest, D., "A Novel Population of Inner Cortical Cells in the Adrenal Gland That Displays Sexually Dimorphic Expression of Thyroid Hormone Receptor-β1," Endocrinology., 156(6),pp. 2338-2348,(2015).

Zheng, H.S., Kang, Y., Lyu, Q., Junghans, K., Cleary, C., Reid, O., Cauthen, G., Laprocina, K., Huang, C.C., "DHCR24, a Key Enzyme of Cholesterol Synthesis, Serves as a Marker Gene of the Mouse Adrenal Gland Inner Cortex," Int. J. Mol. Sci., 24(2), ijms24020933,(2023).

#### **Authors Biography**



Su Jeong Kim is a senior-year student pursuing a B.S. degree in Microbiology at Auburn University. She has been involved in key research roles in the adrenal gland lab for a year and a half. She is currently working as a student assistant in the diagnostic lab at Wilford & Kate Bailey Small Animal Teaching Hospital.



Dr. Humphrey Yao is a Senior Principal Investigator at the National Institute of Environmental Health Sciences (NIE-HS) at the NIH. He leads the Reproductive Developmental Biology Group in the Reproductive & Developmental Biology Laboratory (RDBL) at NIEHS.



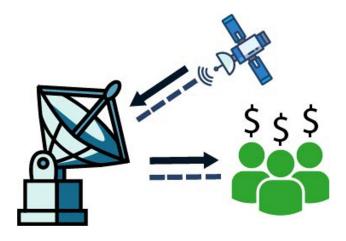
Dr. Jeff Huang is an Associate Professor in the College of Veterinary Medicine at Auburn University. He is the principal investigator for the Laboratory of Developmental Endocrinology at the Department of Anatomy, Physiology & Pharmacology.

## A Centralized User Interface to Display Satellite Mega-Constellations in a Gamified System

Emily Kimbrell<sup>1,\*</sup> and Daniel Tauritz<sup>2</sup>

Undergraduate Student, Department of Computer Science and Software Engineering, Auburn University
 Associate Professor, Department of Computer Science and Software Engineering, Auburn University

Recently, there has been a rise in interest in satellite internet as the world becomes more interconnected than ever before. Satellite-Tycoon (Sat-Tycoon) was developed to understand the dynamics of long-term economic strategies and interactions between satellite internet providers through a gamified model. The focus of this project was to transition Sat-Tycoon from a gamified model to a truly playable and engaging game.



**Fig. 1** Represents the relationship between satellites providing internet to ground stations and potential revenue for players.

User experience testing consisted of collecting feedback about the realism and playability of Sat-Tycoon from a focus group already familiar with the mechanics of the satellite internet industry. The focus group yielded valuable insights into how visual cues influenced players' comprehension of underlying relationships between resource management and profitability. The key finding of this study was that the common challenge faced by users is comprehending the impact of a satellite or a group of satellites on overall constellation performance and downstream business effects for players. This challenge is visually represented in Figure 1. This obstacle

prevented players from developing advanced strategies for resource management.

The team analyzed what characteristics of the old design positively and negatively impacted player experience. The proposed solution to the identified usability challenge was to develop a centralized user interface that intuitively displays significant satellite characteristics such as positioning, orbit design, technology capabilities, and activity status.

Once the playability barriers were clearly identified, a new graphical user interface (GUI) mockup was developed to explore the feasibility of various displays for satellite data and their effects on user experience. Through the design process, the team found that specific areas of interest were (1) combining the mechanics for building and launching satellites, (2) generating an orbit plane graphic that reflected the status of satellites, and (3) implementing a notification system that would encourage players to remain aware of the evolving game environment.

To implement the proposed design, new request calls were created on the frontend that took in relevant orbital positional data and return relevant satellite data to generate an orbit plane graphic that reflects satellite statuses. For the backend, some functionality was refactored to align with the proposed streamlined approach for resource management.

Recently, a stochastic event pipeline was implemented in Sat-Tycoon which models real-world threats to satellite-internet providers' profitability. An example of one such event would be a natural disaster that disrupts a ground station's ability to service customers. The new GUI for satellite characteristics allows developers to ex-

<sup>\*</sup> Corresponding author: eak0043@auburn.edu

pand the types of stochastic events to include magnetic storms that may temporarily disrupt service and satellite collisions that may result in uncontrolled space debris or compromised satellite technology. The inclusion of these new stochastic events has created a more challenging environment that encourages players to formulate more robust strategies.



**Fig. 2** The updated centralized user interface to display satellite mega-constellations data.

The implementation of a centralized GUI for satellite data has fostered a more competitive environment as players can easily analyze the display to determine the placement of their satellite relative to competitors at a given altitude. Players can also check the status of their satellites in each orbit plane by clicking a grid cell and viewing the generated orbit plane graphic pictured to the left in Figure 2. These improvements have enhanced players' capacity to focus on strategic decision-making while remaining informed about the evolving game environment.

#### **Statement of Research Advisor**

Emily started contributing to the Sat-Tycoon project in fall semester 2021, focusing on human-computer interaction. In spring semester 2022, she became the lead developer of the graphical user interface, a significant escalation of responsibility. In academic year 2022-2023, her focus was on designing and implementing a flexible and extensible events pipeline to support environmental events such as natural disasters. Not only did that significantly increase the simulation's realism, it also was an important step toward making the game more fun for human players. In academic year 2023-2024, Emily focused on redesigning the GUI to improve gamification. Through user experience testing – an important and all too often underappreciated phase

in software engineering – she was able to identify the issues preventing the game from being playable. Her subsequent redesign has significantly improved playability, paving the way for its use in the classroom and beyond!

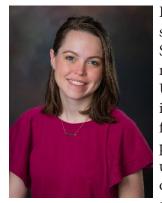
- Daniel Tauritz, Computer Science and Software Engineering, Samuel Ginn College of Engineering

#### References

Rehman S. Qureshi, Cody Roberts, Manuel Indaco, Lucy Bone, Emily Kimbrell, Samuel Mulder, Daniel R. Tauritz, and Davide Guzzetti. Modeling and Gamification Framework of Business Competition Between P-LEO Constellations. In Proceedings AAS/AIAA Astrodynamics Specialist Conference, Charlotte, NC, U.S.A., August 7–11, 2022.

Emily Kimbrell, Daniel Tauritz. Implementing a Flexible Event Pipeline to Improve User Experience in Sat-Tycoon Auburn University Journal of Undergraduate Scholarship 2023 Edition Vol. 12.

#### **Authors Biography**



Emily Kimbrell is a senior-year student pursuing a Bachelor of Software Engineering with a minor in Statistics at Auburn University. She has been working on the Sat-Tycoon project for three years where she has played a key role in improving user experience through developing intuitive user interfaces and expanding game mechanics to create an engaging experience for players.

#### Aubunn6University Journal of Undergraduate Scholarship



Daniel R. Tauritz is an associate professor in the Department of Computer Science and Software Engineering, Interim Director of the Auburn Cyber Research Center, and Director for National Laboratory Relationships in the Samuel Ginn College of Engineering. His research interests include computational intelligence approaches to complex real-world problem solving with an emphasis on national security problems in areas such as cyber security, cyber physical systems, and critical infrastructure protection.

# Internal Erosion in Unsaturated Slopes: Experimental Evaluation and Monitoring

Anna Lancaster<sup>1,\*</sup>, Olaniyi Alfolayan<sup>2</sup>, Dr. Jack Montgomery<sup>3</sup>

- <sup>1</sup> Undergraduate Student, Department of Biosystems Engineering, Auburn University
- <sup>2</sup> Graduate Student, Department of Civil and Environmental Engineering, Auburn University
- <sup>3</sup> Associate Professor, Department of Civil and Environmental Engineering, Auburn University

#### **Background**

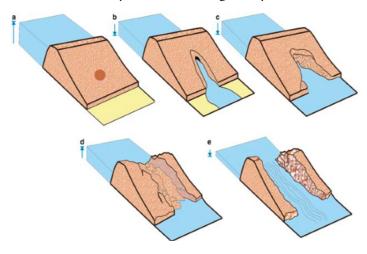
Internal erosion remains a challenging problem for the geotechnical community and is commonly cited as a leading cause of dam failures (Foster et al. 2000, Richards and Reddy 2007, Robbins and Griffiths 2018). Internal erosion is generally divided into four categories: backwards erosion, internal instability, contact erosion, and concentrated leak erosion (Richards and Reddy 2007, Williamson et al. 2015). Significant research has been performed on both backwards erosion and internal instability but evaluating the concentrated leak erosion process still has significant uncertainty. Concentrated leak erosion occurs when flow is concentrated at an opening in the soil (i.e., crack, void, macropore) (Fig. 1), and the previously existing opening enlarges due to erosion by the concentrated flow (Williamson et al. 2015).

Concentrated leak erosion is a multi-stage process and can be subdivided into initiation and continuation of erosion, progression to form an open void or pipe, and finally failure of the structure or slope (USBR and USACE 2015) (Fig. 2). The erosion process is controlled by the combined effects of material properties, hydraulic loading, and effective stress conditions, which Garner and Fannin (2010) termed hydro-mechanical properties.

The objective of this study is to improve understanding of the mechanisms that control erosion rate within voids in earthen dams with unsaturated soil conditions, evaluate and explore factors that control stability of open voids and cracks within earthen dams and track the formation and progress of erosion in earthen dams through the use of low-power, low-cost sensors.



**Fig. 1** Concentrated leak erosion at a site monitored in North Alabama by Dr. Jack Montgomery and students



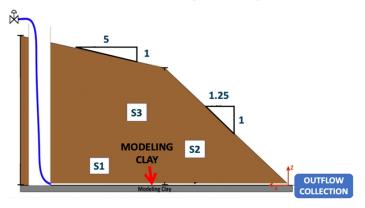
**Fig. 2** Concentrated leak erosion process (Okeke et al. 2013)

#### **Experimental Design**

This research utilized a series of large-scale experiments to examine the response of model unsaturated slopes and existing macropores to combined subsurface flow and precipitation (Figure 3). These experiments are funded through a National Science Foundation grant

<sup>\*</sup> Corresponding author: akl0056@auburn.edu

focused on landslides, but this research builds upon these previous tests to collect data relevant to dams and levees. Tests were conducted to study various hydromechanical properties with various initial conditions such as; varying soil type, initial soil density, initial soil moisture content, and varying slope angles.



**Fig. 3** Cross-section of the model slope showing the location of the void and sensor locations (S1, S2, and S3).

During the experiments, constant head and constant flow conditions were used to initiate and sustain erosion. The discharge at the "toe" of the model dam slope/ pipe was collected and measured to determine the sediment flux and concentration in the water. The time of runoff and seepage flow was recorded, and the runoff rate was measured by collecting runoff at scheduled time durations. Runoff volume was recorded by analysis of sediment content by decanting excess water and then evaporating to oven drying (105 °C). The timing and soil loss by mass wasting was also recorded. At the end of each test, images were obtained from the general view to show the progression of erosion and slope deformation.

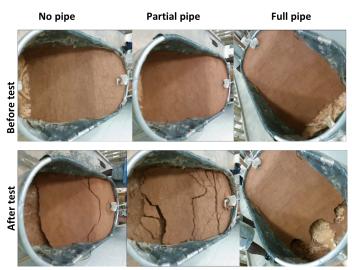
Slopes were constructed in a medium-sized flume with different base angles. Modeling clay was used to form a hydraulic barrier at the base of the slope and a metal pipe (4 mm diameter) was placed on the clay to create a pre-existing void in the slope. Three conditions were considered for the void: (1) a full pipe/void that extended all the way through the slope; (2) a partial pipe that terminated before the toe of the slope; and (3) a slope without a void (no pipe) as a control case. The slope was then compacted in lifts and the metal pipe was removed to form an open void (macropore). Flow into the open void was controlled using a constant head reservoir and a gate valve. Tests were performed at ini-

tial water contents of 9 and 12% and varying dry densities (1.51 and 1.76 g/cm3). Volumetric water content sensors (METER brand Teros 10 or 11) were placed at three locations within the slope and cameras were used to monitor the test.

The slope was constructed of a clayey sand (SC) soil for each of the following tests. This soil was tested using wet sieving analysis and was found to have a fines content of 21.5%. Testing was performed to determine both the liquid and plastic limits for the soil. The soil was found to exhibit a liquid limit of 67 and a plasticity index of 20. Measurement of the soil-water characteristic curve (Fig. 4.) was conducted employing the HYPROP 2 and WP4 equipment (Meter Group) the tests were performed by PhD candidate Olaniyi Alfolayan.

#### **Experimental Results**

An initial series of tests were conducted using slopes compacted at a dry density of 1.51 g/cm3 and 9% water content and varying initial pipe conditions, as previously discussed. Before and after photos of the three tests are shown in Fig. 3 and demonstrate that the presence and condition of the pre-existing voids has an effect on the stability of the earthen slope. For the slope containing the full-length pipe, erosion clearly occurred during the test, but the slope remained stable as the open void allowed the water to drain. This is also shown in the water content results in Fig. 4 where the water content at the downslope sensors (S2 and S3) began to decrease after an initial spike.



**Fig. 5.** Images of slopes with dry density of 1.51 g/cm<sup>3</sup> and 9% water content, before and after testing

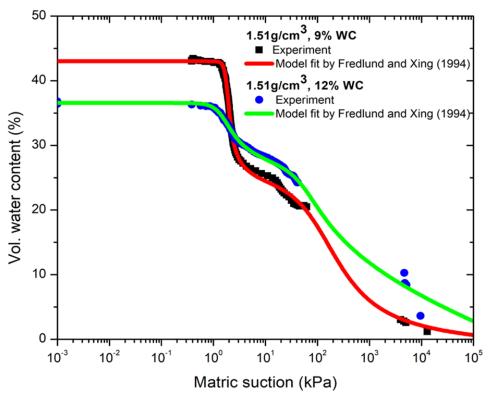


Fig. 4 Soil-water characteristic curve modeled with lines fit by Fredlund and Xing

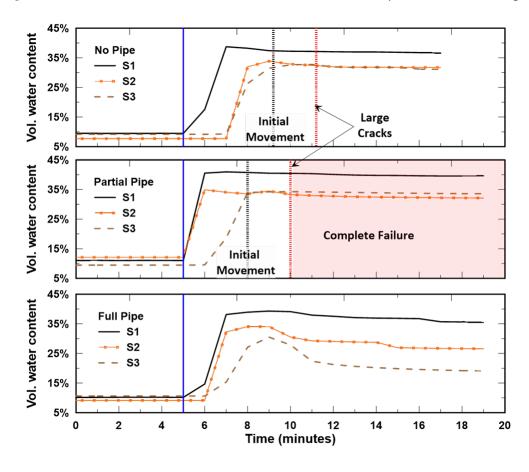
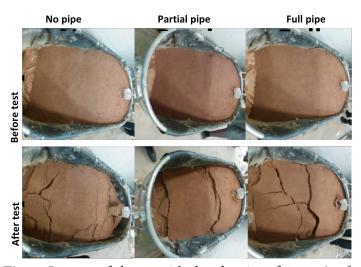


Fig. 6. Results of testing slopes with dry density of 1.51 g/cm³ and 9% water content

The tests with the partial pipe and the slope without a pipe both developed a slope failure, but the magnitude of the movement in the test without the pipe was much smaller (Fig. 5). The water content results showed that the partial pipe allowed the water to reach the downslope sensor very quickly (Fig. 6), which likely triggered the complete slope failure. These results demonstrate that a partial pipe, representing a collapsed pipe or void within a slope, at these initial soil conditions can trigger a slope failure. Without a collapsed pipe or the presence of a void, the slope with no initial pipe formed large cracks throughout testing and a small toe failure but largely remained stable.

The next series of tests were conducted using slopes compacted at a dry density of 1.51 g/cm3 and 12% water content and varying initial pipe conditions, as previously discussed. Before and after photos of the three tests are shown in Fig. 7 and demonstrate that initial moisture content of the soil the presence and condition of the pre-existing voids has an effect on the stability of the earthen slope. For the slope containing the fulllength pipe, contrary to the 9% test, the slope failed to remain stable when the pipe became blocked leading to the entire slope shearing off the impermeable base and therefore collapsing meaning a full slope failure. This collapse shows that having an initial pipe does not always lead to slope stability, but at a higher moisture content, as seen here during the 12% initial moisture content tests, the pipe may not be able to hold the weight of the slope and can collapse.

Through the sensor data in Fig. 8 for the full pipe tests, it can be seen that as S3 spiked the slope collapsed. This observation underscores the role of hydraulic loading in intensifying the structural vulnerability of the slope, resulting in a series of failure mechanisms initiated by the inability of the pipe to withstand the weight of the slope. The tests with the partial pipe and the slope without a pipe both developed a complete slope failure further proving that initial moisture content has great affects on slope stability.



**Fig. 7.** Images of slopes with dry density of 1.51 g/cm<sup>3</sup> and 12% water content, before and after testing.

These results have shown the importance of pipe condition on stability of the slope, but the previous experiments relied on visual observations to detect the development of a slope failure. This leads to some subjectivity in the results. To address this a sensor is currently being developed to improve the monitoring ability of the erosion process and potential failure.

#### **Sensor Design**

The design of the sensor that can detect the presence of internal erosion in earthen structures is currently in the design phase. This sensor utilizes an Arduino Uno system that connects a capacitive soil moisture sensor, an accelerometer, and a pore pressure transducer. After researching similar devices, three sensors were selected, as shown schematically in Fig. 9: (UNO) Arduino Uno, (A) Capacitive Soil Moisture Sensor v1.0 manufactured by DFRobot; (B) ADXL335 Accelerometer manufactured by Adafruit; and (C) a water pressure sensor manufactured by Omron Electronics. With the use of these specific parts the sensor is small, lightweight, and cost effective which will allow many sensors to be created and installed within earthen structures in need of monitoring. The preliminary sensor design (Fig. 9) shows how each component was electrically connected to the Arduino UNO board. The next design stage for this sensor includes the creation of a 3D printed cage for the components. This cage will allow the sensor to be buried within earthen structures and be resistant to corrosion and moisture. The sensor design will be tested in upcoming large experiments planned for summer and fall 2024.

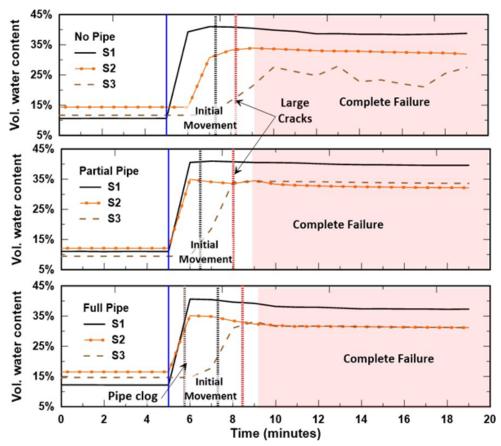
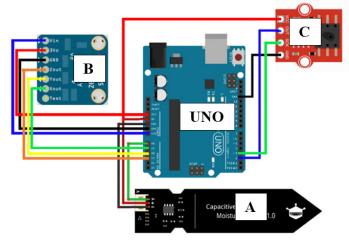


Fig. 8. Results of testing slopes with dry density of 1.51 g/cm<sup>3</sup> and 12% water content



**Fig. 9.** Images of slopes with dry density of 1.51 g/cm<sup>3</sup> and 9% water content, before and after testing

#### **Upcoming Tests**

The upcoming large-scale tests at Auburn University's Advanced Structural Engineering Lab are to increase the understanding of slope stability during internal erosion events while under the influence of modeled weather events such as simulated rainfall. Building upon

previous experiments, these tests will utilize a largescale testing program designed to eliminate boundary effects and provide insights crucial for engineering resilient earthen structures such as dams and levees. One of the primary goals of these large-scale tests is to refine the understanding of hydromechanical properties and erosion mechanisms within earthen structures. By simulating realistic conditions of combined subsurface flow and precipitation on model slopes, comprehensive data can be gathered on various parameters such as soil type, initial density, moisture content, and slope angles.

The testing program will utilize a refined version of the sensor design previously developed (Fig. 5). These sensors, integrated with an Arduino Uno system, and enclosed within a resilient 3D printed case, will provide real-time data on soil moisture, slope deformation, and pore pressure changes. Moreover, the large-scale tests will help to facilitate improvements in sensor resolution, power efficiency, durability, ensuring reliable performance even under harsh environmental conditions. By deploying these sensors at strategic locations with-

in the model slopes, erosion progression, runoff rates, sediment flux, and moisture content can be monitored. This data will not only enhance understanding of erosion mechanisms but also inform the development of predictive models for slope stability assessment. Furthermore, the large-scale tests will offer valuable insights into the behavior of earthen structures under various hydromechanical conditions. By capturing the evolution of erosion patterns and slope deformations through high-resolution imaging techniques, researchers will gain invaluable knowledge to improve the design and maintenance of critical infrastructure.

#### **Conclusions**

From this laboratory study it can be concluded that the presence of pipes in slopes can both affect the timing of landslide initiation and the severity of the sliding compared with slopes without pipes. With the use of METER sensors, it was determined that open pipes within earthen slopes can act as drains, reducing the water content in the slope and increasing the stability, while partial or clogged pipes can trigger rapid loss of suction and failure. This failure occurred more quickly and was more severe than the slope without a pipe.

The experiments performed so far have relied on visual observations to detect slope instability, but this introduces subjectivity into the results and would not be practical at a real dam or levee. This served as motivation for the development of the internal erosion monitoring sensor. Through the integration of a capacitive soil moisture sensor, an accelerometer, and a water pressure transducer, this sensor offers a comprehensive approach to assessing soil conditions crucial for maintaining slope stability. The compact, lightweight, and cost-effective nature of this sensor enables widespread deployment, facilitating extensive monitoring of earthen structures in need. By continuing to innovate and optimize the sensor's capabilities, this design aims to contribute to the advancement of earthen infrastructure resilience and safety in the face of evolving environmental challenges.

#### Acknowledgments

This research was supported by the National Science Foundation under grant number CMMI 2047402. Any opinions, findings, conclusions, or recommendations are those of the author(s) and do not necessarily reflect

the views of the National Science Foundation. The first author also received support from the Kim de Rubertis Student Scholarship Award from the United States Society on Dams. This support is gratefully acknowledged.

#### **Statement of Research Advisor**

Anna worked closely with Olaniyi (PhD student) to complete the experiments and help interpret the results. She took the lead on the entire sensor design, including selecting the components, connecting the electronics, and programming the device. Her research contributions are a major step forward in our understanding of how concentrated leak erosion is connected to landslides and slope instability in dams and levees. Her proposed sensor also has the potential to improve the safety of our existing infrastructure through improved monitoring of this largely hidden failure mode. She will continue this work in her graduate studies with our group.

- Jack Montgomery, Civil and Environmental Engineering, Samuel Ginn College of Engineering

#### References

- [1] Porretta, V. and Tucker, B.V. (2019). Eyes Wide Open: Pupillary Response to a Foreign Accent Varying in Intelligibility. Front. Commun. 4:8. doi: 10.3389/fcomm.2019.00008
- [2] Rathcke, T., Smith, R. (2011). Exploring Timing in Accents of British English. Proceedings of the 17th International Conference on Phonetic Sciences, pp. 1666-1669.
- [3] Van Engen, K. J., and McLaughlin, D. J. (2018). Eyes and ears: Using eye tracking and pupillometry to understand challenges to speech recognition. Hearing Research, 369, 56–66. https://doi.org/10.1016/j. heares.2018.04.013
- [4] Van Engen, K.J. and Peelle, J.E. (2014). Listening effort and accented speech. Front. Hum. Neurosci. 8:577. doi: 10.3389/fnhum.2014.00577

#### **Authors Biography**



Anna K. Lancaster is a senior-year student pursuing a B.S. degree in Biosystems Engineering at Auburn University. She has played key research roles in internal erosion in unsaturated slopes and will be pursuing a M.S. degree in Civil and Environmental Engineering at Auburn University post-graduation.



Olaniyi Alfolayan is a student pursuing a PhD degree in Civil and Environmental Engineering at Auburn University. He has played key research roles in internal erosion and seeks to apply the fundamentals of saturated and unsaturated soil mechanics to the design, construction and performance evaluation of geotechnical and geo-environmental infrastructures



Dr. Jack Montgomery is an Associate Professor in the Department of Civil and Environmental Engineering at Auburn University. His research and teaching focuses on understanding how geotechnical systems respond to natural hazards, including landslides, precipitation, sinkholes, and earthquakes.

# High Dietary Salt Intake Induces Upregulation of Pro-Inflammatory Cytokine Involved with Cognitive Decline Within the Hippocampus

Abigail Law<sup>1</sup> and Vinicia C. Biancardi <sup>2,\*</sup>

<sup>1</sup> Undergraduate Student, Department of Biomedical Sciences, Auburn University
 <sup>2</sup> Associate Professor, Department of Anatomy, Physiology & Pharmacology, Auburn University

Dementia is characterized by cognitive deficits and loss of autonomy and is associated with a higher mortality rate. According to the Centers for Disease Control (CDC), of those at least 65 years of age, there were an estimated nearly 7 million adults with dementia in 2014. Aging is a significant contributor to cognitive decline and dementia. With an increase in the elderly population, CDC projections estimates that in 2026, there will be 14 million individuals living with the disease. As of now, dementia remains a condition without a cure. Pharmacological interventions are available but offer only modest benefits in managing symptoms. Hence, there is a growing consensus on the need for research focused on identifying and implementing preventative measures. Observational research highlights the potential influence of dietary choices on the mechanisms underlying dementia.

In this sense, an increased consumption of table salt (NaCl) has been correlated with cognitive decline and an elevated risk of dementia. However, the mechanisms are not entirely known. Of late, neuroinflammation has been increasingly linked with dementia. Thus, in this work, we investigated whether a high dietary salt (HDS) intake increased pro-inflammatory cytokines within the hippocampus. We studied the effect of HDS in the expression of the pro-inflammatory cytokine IL-6 particularly, given that previous work has suggested a causal link between an elevation of IL-6 and an augmented risk for subsequent decline in cognitive function in human patients.

We used male Wistar rats (150-180 g) divided into two groups: a control group that received regular water in their diet and an HSD-treated group that received 0.3 M of NaCl in drinking water for four weeks. At the end of

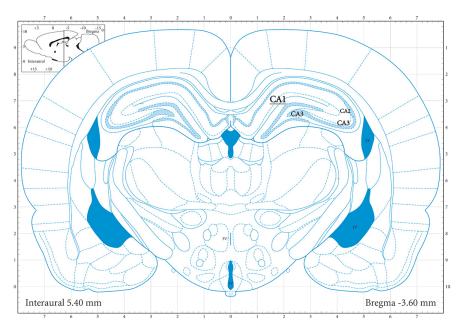
treatment, animals were transcardiacally perfused with a fixative, and the brains were prepared for immuno-fluorescence. 30  $\mu$ m sections of brain tissue containing the hippocampus were cut with a cryostat, and standard immunofluorescence was performed using an anti-IL-6 polyclonal antibody (1:100, Santa Cruz).

Reactions with primary antibody was followed by 4 hours of incubation with a fluorescently labeled secondary antibody. Immunostain was examined with a Confocal Microscope (Nikon A1). Twenty consecutive optical focal planes (1-µm interval) were acquired, and a projection image was generated for quantification. The density of IL-6 staining within the hippocampus was obtained using Image J. We found that HSD-treated animals have a higher density of IL-6 staining (>15 folds) in the CA1 region of the hippocampus than in control rats (Figure 1).

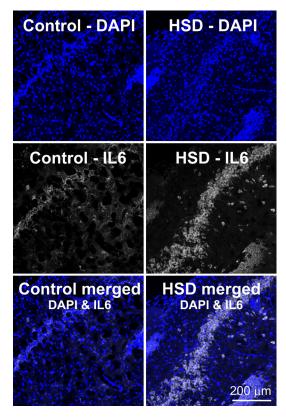
Our data suggest that relatively low but constant HSD consumption increases neuroinflammation, as observed by increased IL6 in the hippocampus (Figure 2). Quantifying the data has shown increased fluorescence intensity and density of the IL-6 protein signaling within the hippocampus of HSD animals compared to control, suggesting the development of brain inflammation in response to a high salt consumption (Figure 3).

Our next aim is to analyze whether microglia, the resident immuno cells of the brain, are also activated and, further, if there is a causal link between microglia reactivity and the increase in pro-inflammatory IL-6 cytokine during HSD.

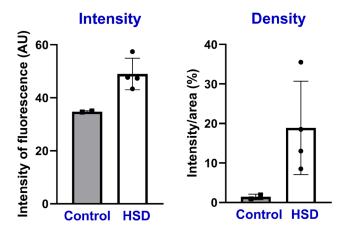
<sup>\*</sup> Corresponding author: vbiancardi@auburn.edu



**Figure 1.** CA1 region of the hippocampus.



**Figure 2.** Confocal Z Stack Image of CA1 Region of control animals (left panel) and HSD animals (right panel) depicting stain with the counterstaining DAPI (blue) and the pro-inflammatory cytokine IL-6 (white). Scale Bar:  $200\mu m$ 



**Figure 3.** Quantification of IL-6 signal within the hippocampus of control and high salt diet (HSD\_. Both density and intensity of fluorescence and density (intensity of fluorescent in a specific area) of the pro-inflammatory cytokine IL-6 is significantly increased within the hippocampus of animals undergoing a high salt diet (HSD) compared to control. \*P<0.05 (Control, n=2; HSD, n=4)

Given the association of neuroinflammation with cognitive decline, our findings, while preliminary, have shown that an increase in IL-6 may be one of the mechanisms involved with the risk of dementia previously associated with an HSD.

#### **Statement of Research Advisor**

Abby Law has done a great job as an undergraduate researcher and was responsible for several technical aspects of the research highlighted here, including handling animals, transcardiac perfusion, tissue sectioning, immunofluorescence staining, and drafting the research summary highlight.

- Vinicia Biancardi, Anatomy Physiology & Pharmacology, College of Veterinary Medicine

#### References

[1] Centers for Disease Control and Prevention., About Dementia. 2019. https://www.cdc.gov/aging/dementia/index.html.

[2] Takamiya S, Shiotani K, Ohnuki T, Osako Y, Tanisumi Y, Yuki S, Manabe H, Hirokawa J, Sakurai Y. Hippocampal CA1 Neurons Represent Positive Feedback During the Learning Process of an Associative Memory Task. Front Syst Neurosci. 2021. doi: 10.3389/fnsys.2021.718619

[3] West MJ, Kawas CH, Martin LJ, Troncoso JC. The CA1 Region of the Human Hippocampus Is a Hot Spot in Alzheimer's Disease. 2006. Annals of the New York Academy of Sciences. https://doi.org/10.1111/j.1749-6632.2000.tb06652.x

[4] Paxinos G & Watson C. The Rat Brain in Stereotaxic Coordinates. 7th edition, Oct.2013, Elsevier. ISBN: 9780123919496.

#### **Authors Biography**



Abigail Law is a senior-year student pursuing a B.S. degree in Biomedical Sciences at Auburn University. She has contributed two years to the project and has a passion for research and work with physiology.



Vinicia Biancardi, MS, PhD, is an Associate Professor at the College of Veterinary Medicine. A native of Brazil, she received her MS and PhD degrees from the Federal University of Sao Paulo. Her postdoctoral training was performed at the University of Cincinnati and the Medical College of Georgia at Augusta University. Before joining the faculty at Auburn, she was an Assistant Research Scientist at the Medical College of Georgia at Augusta University, GA.

# Teaching Away Anxiety: Psychoeducation Interventions for Subclinical Generalized Anxiety in Elementary-Aged Children

Katie E. Lawrence<sup>1,\*</sup> and Linda M. Gibson-Young<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Human Development and Family Science, Auburn University <sup>2</sup> Professor, College of Nursing, Auburn University

Anxiety is one of the most common disorders and developmental struggles in America's youth. 5-12% of children deal with a diagnosable or diagnosed anxiety disorder, with that number increasing to an estimated 31% in adolescence (Merikangas et al., 2010).

Little attention has been awarded to the issues of children's mental health in the school setting, it often being set aside as a secondary priority, or not one at all. However, the research suggests that untreated childhood anxiety and related symptoms could be greatly helped by education-based interventions.

Much like other areas of health and mental health intervention, evidence supports the traditional "the earlier the better" mentality. The earlier evidence-based interventions can be provided to children with anxiety diagnoses or risk factor for disorders, the more likely they are to avoid significant life consequences as a result of mental health difficulties. Children who receive evidence-based intervention and early prevention-based interventions are less likely to suffer immense consequences from anxiety disorders and are less likely to have their disorder persist into adulthood, causing more problems down the line.

This year-long literature review involved answering the following question: Can Cognitive Behavioral Therapy (CBT)-based psychoeducation programs be implemented in schools and afterschool care settings to improve the mental health of elementary-aged youth? The research focused on the potential future applications of such programs for populations of children at-risk for anxiety, but not yet reaching clinical diagnostic levels of symptomology. Finally, the research sought to highlight the elements of such programs that most indicate

effectiveness and long-term improvements in children's mental health.

Many of the studies reviewed and programs analyzed showed that certain qualities lead to more effective anxiety-education programs. These qualities included a small group design, easily implementable by a teacher or other professional who has undergone minimal training, transdiagnostic programs that are broad but still somewhat targeted to children with anxiety symptoms (not a universal intervention), and prevention based (Barrett & Turner, 2001; 2002; Pina et al., 2023).

Programs analyzed in the literature review included the FRIENDS program, Coping Cat, The Cool Kids, CHILLED Program, and COMPASS for Courage. All of these programs, which have all shown some level of efficacy in improving symptomology of anxious children, contain two broad categories of things provided to children. These are (1) awareness of anxiety symptoms for the children experiencing them, and (2) concrete tools provided to assist the children in coping with their own symptoms, whether a product of a diagnosable anxiety disorder or not.

Research has indicated that the most successful programs of previous research involve components of cognitive behavioral therapy and the restructuring of anxious thoughts, caregiver training, relapse prevention, exposure to anxiety triggers, and training on relaxation/mindfulness (Pina et al., 2023).

Future directions for the research to go include involving families and parents in the programs, addressing other mental health issues and symptoms as apart of such psychoeducation programs, and implementing

<sup>\*</sup> Corresponding author: kel0066@auburn.edu

the content of the programs into school curriculum, with targeted groups available for all students.

#### **Statement of Research Advisor**

Katie's role involved assisting with literature reviews, research design, data collection and analysis under my guidance. In higher education research, she contributed to projects exploring topics with pediatric health and school-based interventions. She collaborated on projects investigating child and adolescent mental health, and the relationship of student mental health engagement and academic achievement.

- Dr. Linda Gibson-Young, Nursing, College of Nursing

#### References

Barrett P. & Turner C. (2001) Prevention of anxiety symptoms in primary school children: preliminary results from a universal school-based trial. British Journal of Clinical Psychology. 40(4):399-410. doi: 10.1348/014466501163887.

Pina, A.A., Stoll, R.D., Holly, L.E., Wynne, H., Chiapa, A., Parker, J., Caterino, L., Tracy, S.J., Gonzales, N.A., & Valdivieso, A. (2023). Streamlined pediatric anxiety program for school mental health services. Journal of Anxiety Disorders, 93. https://doi.org/10.1016/j.janx-dis.2022.102655

Merikangas, K. R., He, J.-P., Burstein, M., Swanson, S. A., Avenevoli, S., Cui, L., ... Swendsen, J. (2010). Lifetime prevalence of mental disorders in U.S. adolescents: Results from the National Comorbidity Survey Replication-Adolescent Supplement (NCS-A). Journal of the American Academy of Child & Adolescent Psychiatry, 49(10), 980–989. https://doi.org/10.1016/j.jaac.2010.05.017

#### **Authors Biography**



Katie E. Lawrence is a senior-year student pursuing a B.S. degree in Human Development & Family Science at Auburn University. She has been passionate about psychoeducation for children of all ages, particularly in the areas of relationship health and generalized anxiety.



Dr. Linda M. Gibson-Young is a professor in the College of Nursing with a focus on pediatrics. Her work engages populations across the state of Alabama in programming assisting children in rural areas with their health, particularly in relation to asthma.

## **Improving the Accuracy of Atomic Fluorescence Efficiencies for Solar System Science**

Benjamin Lightfoot 1,\*, John Noonan 2, and Dennis Bodewits3

<sup>1</sup> Undergraduate Student, Department of Physics, Auburn University

#### Introduction

Comets, as the best-preserved remnants of planetary formation within our solar system, are often likened to time capsules: a comet's icy core, or nucleus, contains clues about how the solar system and the Earth emerged, and allows us to study the materials that they formed from[1]. When a comet enters an orbit that brings it close to the Sun, this ice sublimates to space and produces a cloud (or coma) spanning hundreds of thousands of kilometers, which contains chemical information from the early solar system [2]. In order to properly characterize atoms within the cloud, one must first have a quantitative picture of how readily they produce photons in the solar light – a property known as the fluorescence efficiency. Ultraviolet fluorescence efficiency calculations demand access to a high-resolution solar irradiance spectrum, which in turn must be calibrated with daily cadence observations [3]. Unfortunately, solar spectral calibration is a process that is marked by uncertainty, and this directly undermines the precision of such calculations. As a result, the search for accurate fluorescence efficiencies has become a highly cumbersome and customized endeavor, and one that often suffers from ambiguity as to the nature, origins, and subsequent manipulation of spectral data.

In recent years, an attempt was made to construct a model which would avoid some of those pitfalls. By drawing on highly traceable datasets and accommodating for changes in heliocentric conditions, this Python-based prototype took a much more systematic approach to the task at hand. Nevertheless, its reliance on manual data extraction, along with its fragmented approach to regional analysis, hindered its overall effectiveness. To make matters worse, there remained a major hurdle which had so far been neglected – namely, the issue of scaling. The consistent utilization of high-quality,

publicly available data laid a foundation that was sorely needed, but a means of tuning high-resolution spectra to account for solar variability proved elusive. Thus, the primary objectives of our current research have been to implement methods that enhance the model's efficiency and usability, as well as to investigate techniques which could potentially mitigate the difficulties of solar spectral scaling.

#### Methods/Findings

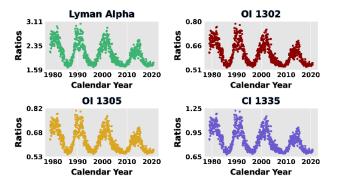
The most immediate impediment to the overall utility of the model stemmed from the absence of any form of automated data extraction from online repositories. In this respect, the need for large data samples extends beyond just solar spectra; information concerning atomic properties is also necessary for obtaining fluorescence efficiencies. For all of our needs on this front, we rely on the NIST Atomic Spectra Database [4]. Unfortunately, NIST does not offer any official API for constructing query requests, but results obtained manually from their website do produce URLs with built-in variables, which can be replicated. Upon discovering this, we quickly set up a script that can take some simple parameterization from the user, create a query request, pull all of the relevant data, and return it in a convenient format. As for solar spectra, the University of Colorado's LISIRD [5] offers daily cadences obtained on numerous missions, covering the present day and going back well over a century. Unlike NIST, LISIRD maintains an API called La-TiS that allows for highly specific query requests – but this freedom comes at the expense of a certain degree of accessibility. As such, we tailored our code to handle most of the finer details under the hood, while still allowing the ability to select dates and wavelengths for a handful of datasets. With both of these scripts operating in tandem, the process of supplying data for analysis has been completely overhauled. One unexpected but

<sup>&</sup>lt;sup>2</sup> Postdoctoral Researcher, Department of Physics, Auburn University <sup>3</sup> Professor, Department of Physics, Auburn University

<sup>\*</sup> Corresponding author: bcl0025@auburn.edu

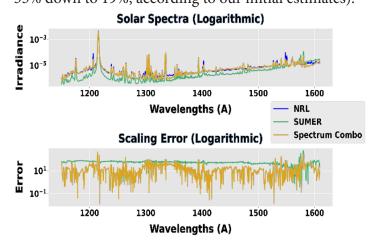
welcome side effect of this change was its immediate impact on our investigation into solar spectral scaling techniques – rapid data retrieval allowed us to examine fluorescence efficiencies using spectra dating from 1978 all the way up to 2020, which offered some particularly illuminating results.

Before delving into our findings, some additional comments are warranted on the challenge posed by solar spectral scaling. At a fundamental level, the dilemma of scaling is that we are forced to compare things which tend to resist comparison. Low resolution observations (NRL)[5], taken daily, paint merely a rough picture of the Sun's fluctuations with time, but they are still the best reference available for calibrating high-resolution spectra (SUMER) [6,7] from years past. In our precursory evaluations, we made the optimistic assumption of a linear relationship – that is, all we would need is a single constant by which to scale SUMER to match the intensities of NRL. In this case, one could take the integrated fluxes of both spectra over all major emission features and compare them directly, taking the median ratio as a reliable scale factor. However, this technique fails to age well when extended over the course of decades. Ideally, the ratios for each emission feature would be fairly similar, but Figure 1 instead illustrates that they can differ by over a factor of 3 on any given date. Even more telling is that none of the plots depict oscillations about a central value of 1, which is what we should see over the completion of entire solar variability cycles (~11 yr. each).



**Fig. 1** Integrated flux ratios as a function of time, plotted for 4 key emission features of SUMER and NRL Having demonstrated that a simple linear scale factor is grossly insufficient, we instead turned to polynomial regression in an effort to capture a more complex relationship. However, in order to perform comprehensive polynomial fitting of one spectrum to another, they

must already be at roughly the same resolution – this hearkens back to the observation that high-resolution and low-resolution spectra are loathe to be compared. Even so, there are ways in which an elaborate spectrum like SUMER can be reduced (or blurred) down to a similar precision to NRL. One such option is to apply a Gaussian convolution, since high-resolution spectroscopy produces approximately Gaussian point spread functions (PSFs). Taking this approach, it still remained to test a myriad of higher order fits, from which a 6th degree polynomial emerged as the most promising candidate. Still, the heavy influence of the primary emission features caused this fit to struggle in quieter regions. To remedy this, one could construct 'masked' spectra where all primary emissions have been removed, and from them produce a masked fit. But naturally, this result falls victim to the opposite problem, often poorly matching the very emission features that had been masked. Undeterred, we instead began to consider the possibility of combining multiple fits together: by taking the values which correspond most closely to NRL at each discrete wavelength, we could theoretically produce a new spectrum with minimal error over the region where SUMER and NRL overlap. The results of this strategy are depicted graphically in Figure 2, where 'Spectrum Combo' represents an amalgamation of datapoints from SUMER itself, a masked polynomial fit, and its unmasked counterpart. In the 1150-1600 Angstrom region, this procedure appears to diminish the relative error by over 30% (from roughly 55% down to 19%, according to our initial estimates).



**Fig 2.** Logarithmic plots of polynomial fitted spectra and their errors as compared to NRL (reference spectrum)

#### **Next Steps**

With the modifications that have been made, we are ready to enter into a period of end-to-end testing, which includes a thorough sweep of the inner workings of the model, as well as external benchmarking of its fluorescence efficiency outputs against several comets with UV spectra in the literature: 46P/Wirtanen, 67P/Churyumov-Gerasimenko, 103P/Hartley 2, C/2009 P1 (Garradd), C/2012 S1 (ISON), and C/2014 Q2 (Lovejoy). Afterwards, we intend to make the model available at large as an open-source software package via the Journal of Open-Source Software (JOSS).

Beyond this, it seems that we may still have only scratched the surface when it comes to the mitigation of scaling inaccuracies. While the most recent polynomial fitting results are quite promising, more rigorous analysis and exploration of alternatives is required before we can draw any definitive conclusions.

Finally, we believe that the next step towards public distribution lies in the creation of a web-based user-interface, which would give users quick and convenient access to the full capability of the model, all without ever having to interact with the underlying code structure.

These last two areas of improvement will serve as the focus of a second research fellowship, beginning in the fall of 2024.

#### Statement of Research Advisor

The rigorous and careful investigation of atomic fluorescence efficiencies that Ben has completed with this project marks a first in the field. To date, no other software has been designed to make calculation of atomic fluorescence efficiencies as easy or as accurate to the day, and this work will have significant implications throughout planetary fluorescence spectroscopy. We are excited to see its continuation and expansion in the coming months.

-Dennis Bodewits, Department of Physics, College of Sciences and Mathematics

#### References

[1] M.F. A'Hearn, "Comets as building blocks." Annual Review of Astronomy and Astrophysics, vol. 49, pp. 281-299, (2011).

- [2] D. Bodewits, B.P. Bonev, M.A. Cordiner, G.I. Villanueva, "Radiative processes as diagnostics of cometary atmospheres," arXiv preprint arXiv:2209.02616 (2022).
- [3] P.D. Feldman, A.L. Cochran, M.R. Combi, "Spectroscopic Investigations of Fragment Species in the Coma," Comets II, pp. 425-448, (2004).
- [4] Atomic Spectral Database, NIST Standard Reference Database #78. Version 5.11. United States of America (US): U.S. Department of Commerce. 1995 [updated 2023 Dec 3; accessed 2024 Apr 11]. https://www.nist.gov/pml/atomic-spectra-database
- [5] Naval Research Laboratory Solar Irradiance (NRLSSI2h). United States of America (US): LASP Interactive Solar Irradiance Data Center, Laboratory for Atmospheric and Space Physics, Uni. of Colorado. 2005 [updated 2024 Apr 12; accessed 2024 Apr 11]. https://doi.org/10.25980/L27Z-XD34
- [6] Solar Ultraviolet Measurements of Emitted Radiation (SUMER). France (Fr): Solar Maximum Mission, BASS2000 Solar Survey Archive. 1994 [updated 2023 Nov 24, accessed 2024 Apr 3]. https://bass2000.obspm.fr/sitemap.php
- [7] W. Curdt et. al. "The SUMER spectral atlas of solar-disk features," A&A, vol. 375, no. 2, pp. 591-613, (2001).
- [8] B. Lightfoot, J. Noonan, D. Bodewits, "Improving Accuracy of Atomic Fluorescence Efficiencies for Solar System Science," [Poster presentation]. 243rd Meeting of the American Astronomical Society, New Orleans, LA, United States, (2024).

#### **Authors Biography**



Benjamin Lighfoot is a junior-year student pursuing a B.S. degree in Physics at Auburn University. He has conducted astrophysics research under Dr. Bodewits since the summer of 2022, working to improve the underlying methodology, utility, and accessibility of the fluorescence efficiency codebase. He has previously interned with The MITRE Corporation, where he worked to enhance the radar-modeling capabilities of PIMS - a Python-based, end-to-end missile defense simulation.



Dr. John Noonan is a Postdoctoral fellow working with Dr. Bodewits in the Physics Department at Auburn University. Dr. Noonan received his Ph.D. in 2022, studying the ultraviolet emission mechanisms of comets, focused largely on emissions from comets 46P/ Wirtanen and 67P/Churyumov-Gerasimenko. In addition to his work in the UV. Dr. Noonan also uses near-infrared observatories to probe comet and asteroid formation theories.



Dennis Bodewits is an Alumni Professor at Auburn University in the Physics Department. His research is focused on cometary emission mechanisms and how observation of comets can provide additional context to understanding their origins. His research goals are spread between testing with laboratory astrophysics facilities, computational modeling, and observations with an array of ground- and space-based assets.

# Effects of Rear-Foot Center of Pressure on the Application of Forces During College Softball Hitting

Aubrie K. Lisenby<sup>1,\*</sup>, Anthony W. Fava<sup>2</sup>, Billy Lozowsk<sup>2</sup>, and Gretchen D. Oliver<sup>3</sup>

Undergraduate Student, Biomedical Sciences, Auburn University
 Doctoral Candidate, School of Kinesiology, Auburn University
 Professor, School of Kinesiology, Auburn University

#### Introduction

Success in the sport of softball, particularly at the collegiate level, requires a high level of technical skill. A critical component of a player's offensive performance, or hitting efficacy, is their ability to make effective ball contact facilitated by their swing mechanics. A hitter's swing is initiated by loading their rear leg. Accordingly, a hitter's ability to create and apply force during rear-leg loading, described as an impulse force, may be influenced by their rear-foot center of pressure (CoP). The impulse force reflects a change in a hitter's momentum, therefore hitting performance could be aided by positioning the CoP on the rear foot to direct momentum during the swing.

To date, the association between rear-foot CoP displacement and impulse force during the softball batting swing has not been established. Previous research has concentrated on broader aspects of the swing, such as kinematics and timing. However, determining a hitter's ability to apply force over swing phases could lead to movement prescription to aid swing mechanics. Therefore, this study aimed to examine the relationship between CoP displacement and impulse force during the load portion of the swing.

Specifically, this research will employ a quantitative approach to measure and interpret hitters' rear-foot CoP displacement during load and its impact on impulse force normalized to their body weight. Insights gained from this analysis are expected to inform innovative training techniques, refine coaching strategies, and elevate the hitting performance of collegiate softball players.

This study recruited 10 female collegiate softball players (19.5 ( $\pm$  0.92) years; with a height of 171.91 ( $\pm$  5.71) cm; and weight of 778.55 ( $\pm$  85.25) N). All participants were actively competing at the Division I level at the time of testing. Upon arrival at the laboratory, testing procedures were explained to each participant and written informed consent was obtained before testing.

Participants were first outfitted with 14 electromagnetic sensors to collect 3D position and orientation data of body segments at 240 Hz. Two force platforms (Bertec Inc., OH, USA) were used to measure CoP and ground reaction force data at 1200 Hz for each lower limb separately. Kinematic and force platform data were time-synchronized using a data acquisition board operating through biomechanical software (Innovative Sports Training Inc., TMM xGen, IL, USA) and was used to record the motion. Global axes were depicted by the positive *X*-axis in the direction anterior to the hitter in stance, the positive *Y*-axis vertically upwards, and the negative Z-axis depicted perpendicular to the *XY* plane on the left in the direction the ball was hit. Locally based coordinate systems for each segment were transformed into the global axis coordinate system.

The experimental setup involved the following: a tee set at waist height directly in front of the lead leg at the end of the stride phase to standardize swing conditions, a hitting net positioned in front of the hitting zone, and an outbound radar gun positioned directly behind the contact point of the ball. The ball was a 400-pound compression NCAA Rawlings softball, and each participant used their competition-standard bat. Participants assumed their natural batting stances with the foot of

**Methods** 

<sup>\*</sup> Corresponding author: akl0064@auburn.edu

the rear and lead leg positioned atop each force plate. Participants performed five maximal effort swings off the tee.

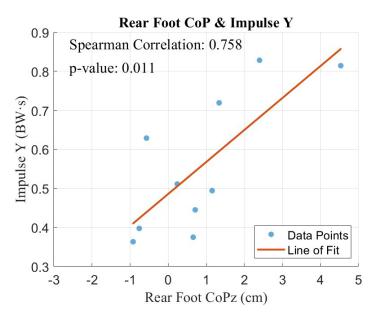
Mechanical variables during the load phase of the hitting motion were extracted and used for analysis. The load phase was defined as the first instance of rear-leg loading to the first instance of lead-leg loading after lead foot movement (stride). The first instance of rear-and-lead leg loading was marked by the hitter shifting their body weight. This was quantitatively defined by a one percent increase in ground reaction force relative to the hitter's body weight, detected via the force plat-form. This specific change was chosen as the threshold to ensure a meaningful weight shift that was robust across loading styles/rates. Post-processing of trials involved biomechanical software (Innovative Sports Training Inc., TMM *xGen*, IL, USA).

The average CoP displacement was measured across the rear foot in the medial-lateral and anterior-posterior planes. A greater positive CoP<sub>z</sub> represented a more lateral position on the foot (5<sup>th</sup> toe), a negative position represented a medial position (inside arch), and zero represented the midline. Impulse forces in three planes of motion were determined by integrating the respective force over the load phase in seconds, normalized to the athlete's body weight. A negative Z impulse represented a propulsive impulse in the direction the ball is hit. A positive Y impulse represented a shear impulse directed upward.

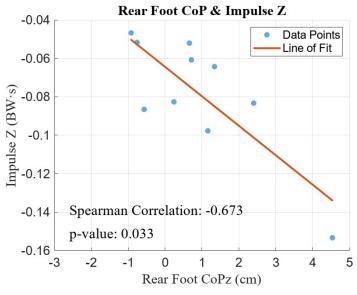
Mean impulses and average CoP in each plane of motion were determined from each participant's five swings. Spearman's rank correlation coefficients ( $r_s$ ) were used to assess the associations between CoP displacement and impulse force in variables that displayed a near-linear relationship. Statistical significance was set at p < .05.

#### Results

The analysis included data from 50 swings, with each of the 10 participants contributing five swings. Normalizing impulse force data to each participant's body weight ensured comparability across individuals. Significant associations were observed between average mediolateral CoP<sub>z</sub> displacement and Y and Z impulse forces during the load phase (Figures 1 & 2).



**Fig. 1.** Association between Rear Foot Center of Pressure Displacement Z and Vertical Impulse Force Y.



**Fig. 2.** Associations between Rear Foot Center of Pressure Displacement Z and Impulse Force Z.

A strong positive relationship ( $r_s = .758$ ) was observed between rear foot  $CoP_z$  displacement and the resultant vertical impulse force. The Spearman correlation coefficient was statistically significant (p = .011). As  $CoP_z$  displacement increases laterally (outside of the foot) there was an increase in vertical impulse force applied during the load phase of the swing (Figure 1).

A moderate negative relationship ( $r_s = -0.673$ ) was observed between rear foot CoPz displacement and propulsive impulse force. The Spearman correlation

analysis was statistically significant (p = .033). As CoP<sub>z</sub> displacement increases laterally (outside of the foot) there was an increase in propulsive impulse force and momentum directed toward the pitcher (direction of swing) during the load phase of the swing (Figure 2).

#### Discussion

The findings of this study reveal significant relationships between the average center of pressure (CoP) displacement in the rear foot and both vertical and propulsive (home plate to pitcher) impulse forces during collegiate softball swings. The strong positive correlation between rear foot CoP displacement and vertical impulse force (Impulse Y), as illustrated in Figure 1, supports the hypothesis that a more lateral displacement of CoP may enhance the vertical component of force during the swing. Elevated swings could potentially be of benefit to hitting performance by allowing for greater ball lift and distance through a more powerful swing. Additionally, Figure 2 demonstrates a strong correlation between lateral CoP displacement and propulsive impulse force (Impulse Z). This indicates that as CoP shifts laterally, it increases vertical lift and enhances forward momentum. This forward momentum is crucial for optimal contact with the ball and effective propulsion, leading to more powerful and controlled swings.

While the findings are compelling, this study is not without limitations. Although sufficient for preliminary analysis, the sample size could be expanded in future studies to enhance the generalizability of the results. Additionally, the use of Spearman's correlation, while appropriate for this analysis, does not account for potential non-linear relationships between variables. Future research could explore these dynamics using more complex statistical models, including regression analysis, to better understand and predict the impact of CoP displacement and rear-leg impulse on additional swing mechanics.

Overall, this research underscores the importance of biomechanical analysis in understanding and enhancing athletic performance in collegiate softball. By better understanding the implications of CoP displacement, coaches, athletes, and sports scientists can refine training protocols to harness the potential benefits of optimized impulse force application during the swing, ultimately leading to improved performance metrics.

#### **Statement of Research Advisor**

Aubrie has taken the initiative to explore the concept of rear foot loading and its significance in softball hitting among NCAA Division I collegiate softball athletes. Throughout this research endeavor, Aubrie has demonstrated growth in her research capabilities, from the initial idea conception to overseeing data collection and collaborating on data analysis and interpretation. Building on the insights from this study, Aubrie intends to pursue further research based on these findings.

- Gretchen D. Oliver, School of Kinesiology, College of Education

#### References

Escamilla, R. F., Fleisig, G. S., Zheng, N., Barrentine, S. W., and Andrews, J. R., Kinematic comparisons of 1996 Olympic baseball pitchers. Journal of Sports Sciences, 19(9), pp. 665-676, (2001).

Haruna, R., Doi, T., Habu, D., Yasumoto, S., & Hongu, N. (2023). Strength and Conditioning Programs to Increase Bat Swing Velocity for Collegiate Baseball Players. Sports (Basel, Switzerland), 11(10), 202.

Inkster, B., Murphy, A., Bower, R., & Watsford, M. (2011). Differences in the kinematics of the baseball swing between hitters of varying skill. Medicine and science in sports and exercise, 43(6), 1050–1054.

Szymanski, D. J., McIntyre, J. S., Madsen, N. H., Pascoe, D. D., and Szymanski, J. M., Effect of torso rotational strength on angular hip, angular shoulder, and linear bat velocities of high school baseball players. Journal of Strength and Conditioning Research, 21(4), pp. 1117-1125, (2007).

Welch, C. M., Banks, S. A., Cook, F. F., and Draovitch, P., Hitting a baseball: A biomechanical description. Journal of Orthopaedic & Sports Physical Therapy, 22(5), pp. 193-201, (1995).

#### **Authors Biography**



Aubrie K. Lisenby is a junior at Auburn University, where she is majoring in Biomedical Sciences with a minor in Sports Coaching. As a Division I collegiate softball player, Aubrie combines her academic pursuits with her athletic endeavors to deepen her understanding of sports performance from a scientific perspective. Her focus on biomechanics and sports performance has led her to explore how physiological and mechanical principles can be applied to enhance athletic training and performance, particularly in softball.



Anthony W. Fava is a fourthyear PhD student in the School of Kinesiology pursuing research related to biomechanics and motor learning strategies to enhance skill development. His work has primarily focused on throwing and hitting-based movements as well as identifying potential risk factors for injury for players participating in throwing and hitting sports. He received his master's degree in human performance and has a background in collegiate strength and conditioning.



Billy Lozowski is a third-year doctoral candidate in the School of Kinesiology. After earning his BSc (Sport Science) and Master of Research (Sports Biomechanics) from St. Mary's University in Twickenham, Billy joined the Sports Medicine and Movement Lab to pursue his PhD. Billy's research primarily focusses on overarm throwing and the mechanics related to performance velocity and accuracy, but also expands into novel statistical approaches to analyzing complex biomechanical data.



Dr. Gretchen D. Oliver, FACSM, ATC, CES is a professor at the School of Kinesiology and director of the Sport Medicine & Movement Laboratory. Youth baseball and softball athlete injury prevention and performance enhancement remain at the core of Dr. Oliver's primary research focus. Among her peers, she is the expert in youth baseball and softball injury prevention and is world-renowned for her research expertise in windmill softball.

### The Impact of Increased Financial Literacy Education and Awareness Through Marketing Approaches

Joseph M. Luria<sup>1,\*</sup> and Dr. Portia Johnson<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Marketing, Auburn University
 <sup>2</sup> Associate Professor, Department of Consumer and Design Sciences, Auburn University

Extension Alabama is a non-profit organization that promotes and trains high school students' use of the financial aid program FAFSA. Higher education is often viewed as unachievable because of the amount of financial cost it takes for an individual to complete their degree. Extension aims to decrease that stigma and encourage students to view higher education as something achievable through the use of financial aid.

Joseph Luria has been interning at Extension Alabama, an organization attached to Auburn University and Alabama A&M University promoting the use of a financial resource management program titled Alabama FAST: FAFSA (ALFAST) for incoming college students.

His research problem dealt with how prospective college students are not often aware of financial aid opportunities available to them. This research can help increase their awareness of these financial aid opportunities and allow them to take advantage of the programs available to them. This project primarily aimed to leverage business marketing techniques to expand and analyze Human Sciences Extension's ALFAST program.

The purpose of this research is to experiment with applied research on the principal, statewide initiatives delivered through Extension AL. These results are geared toward increasing awareness, analytics, and testing efficacy of the ALFAST program using marketing approaches. Luria has collected data on schools in Alabama and the kind of programs they are interested in from Extension AL. All members of the community desire to increase their financial literacy but do not often have the means to obtain it.

From this research, he built skills in executing a mar-

\* Corresponding author: jml0130@auburn.edu

keting campaign that incorporates various forms of business marketing approaches including content marketing (e.g., social media, email marketing, and advertising), brand management/analysis, and public relations. He has yet to examine official results, though it appears that using marketing approaches has paid off for Extension's promotion of ALFAST and that they will continue to create promotions similar to what we have been publishing to increase the reach of their programs. Now that this research has been conducted and after Extension has seen the results, they can troubleshoot previous and future marketing campaigns to more effectively promote the use of their programs such as ALFAST.

#### **Statement of Research Advisor**

Joseph Luria is a third-year Marketing major in the Harbert College of Business at Auburn University. His marketing background, coupled with his experience as a transfer student from Calhoun Community College to Auburn University made him an ideal undergraduate research fellow for applied research on the principal statewide initiatives delivered through the College of Human Sciences Extension.

Luria's personal knowledge and first-hand account of beginning post-secondary education at community college and transition to a 4-year institution greatly informed his work in marketing targeted to high school students.

- Dr. Portia Johnson, Consumer and Design Sciences, College of Human Sciennees

#### References

[1] Johnson, Portia, et al. "4 Steps to Federal Student Financial Aid." Extension, 1 Aug. 2022,

#### Aubung/University Journal of Undergraduate Scholarship

www.aces.edu/blog/topics/finance-ca-reer/4-steps-to-federal-student-financial-aid/. Accessed 30 Apr. 2024.

#### **Authors Biography**



Joseph M. Luria is a senior from Huntsville, AL, finishing his undergraduate in marketing. He has also studied to earn a minor in English and hopes to be able to use his love of writing in the business world working in advertising and marketing.



Dr. Portia Johnson is an Assistant Professor and Extension Specialist of consumer design sciences at Auburn University. She earned her Ph.D. from UGA studying Housing Management and Policy.

## Justice System Contacts, Individual Differences, and Quality of Life

Maddy J. McDaniel<sup>1,\*</sup>, Olivia H. Sawyer<sup>2</sup>, Samantha J. Fede<sup>3</sup>

- <sup>1</sup> Undergraduate Student, Department of Psychological Sciences, Auburn University
  - <sup>2</sup> Research Assistant, Department of Psychological Sciences, Auburn University
  - <sup>3</sup> Assistant Professor, Department of Psychological Sciences, Auburn University

In a review by Porter & Novisky (2016), the authors state that more than two million people are incarcerated in the US while each year around 700,000 individuals are released from prison. Wilper and colleagues (2009) find that roughly 40% of inmates suffer from persistent medical issues while nearly half of prisoners and 60% of jail inmates have mental health concerns during incarceration (Porter & Novisky, 2016).

Past research has shown that incarceration leads to poor outcomes like increased mental health concerns and substance use (Turney et al., 2012; Schnittker et al., 2012), higher mortality rates (Patterson 2013), less community engagement (Porter & Novisky, 2016), and less socioeconomic stability (Mallik-Kane & Visher, 2008; Wakefield & Uggen, 2010). Although general effects of incarceration have been demonstrated across the lifespan (Porter & Novisky, 2016), less is known about the specific effect of prison conditions on these outcomes. Edgemon and Clay-Warner (2019) find that poor prison conditions (over-crowding, exposure to violence etc.) are associated with lower mental health acutely speaking but have not yet examined the effect of these prison conditions on post-release outcomes. Given that current research suggests a high instance of encountering potentially traumatic events while in police custody and during incarceration (Piper & Berle, 2019), this is a particularly important question from the perspective of mental health outcomes.

Past research also fails to consider how individual differences among recently released people might modify potential associations between prison conditions and post release outcomes. Pre-existing conditions and individual differences not only influence the likelihood of incarceration (e.g., traumatic brain injury; McIssac

et al., 2016), but also the experience of incarceration. Previous studies have shown that a wide variety of these features impact poor prison adjustment and/or tendency to have prison disciplinary reports, including cognitive coping, locus of control (Sappington, 2016), criminal subculture identity (Raaijmakers et al., 2017), testosterone levels (Dabbs et al., 1995), anxiety, family status, history of incarceration (Warren et al., 2004), and psychopathy (Thomson et al., 2018). Thus, it is likely that individual differences are an important moderator of how prison conditions might impact post-release functioning.

The current study sought to examine Quality of Life (QoL) changes in a sample of adults residing in the United States before and after contacts with the Justice system. Specifically, we were interested in the association between negative and positive justice system experiences and QoL. We hypothesized that better justice conditions will be associated with QoL improvements and worse justice conditions will be associated with QoL decrements.

Data was collected using self-report questionnaires delivered through Amazon MTurk. To quantify QoL, we used the WHO Quality of Life-Brief questionnaire (Whoqol Group, 1998), which is a 26 self-report questionnaire used to assess an individual's perception of their world, considering the culture and values of their society, alongside their own aspirations, values, and worries. We asked participants to retrospectively report their QoL immediately prior to justice system encounters and currently; the difference between these two values was considered their change in QoL, our DV. To quantify prison experiences, we adapted the questionnaire from Edgemon and Clay-Warner (2019)

<sup>\*</sup> Corresponding author: mjm0180@auburn.edu

to ask about the frequency of negative or positive experiences some people encounter during justice system interactions.

All participants were required to be eighteen years or older, reside in the United States, have a history of justice system involvement as a defendant, and not currently incarcerated. A total of 98 individuals completed the survey; after quality checks on the data, 28 were excluded from analysis for a final N = 70. See Table 1 for Participant Demographics.

We ran a series of separate correlation tests for each pair of QoL subscales and justice system experiences measures. Because some of the variables were non-normally distributed and/or rank ordered, we used Kendall's Tau correlation tests, which are robust to these characteristics.

**Table 1.** Participant Demographics

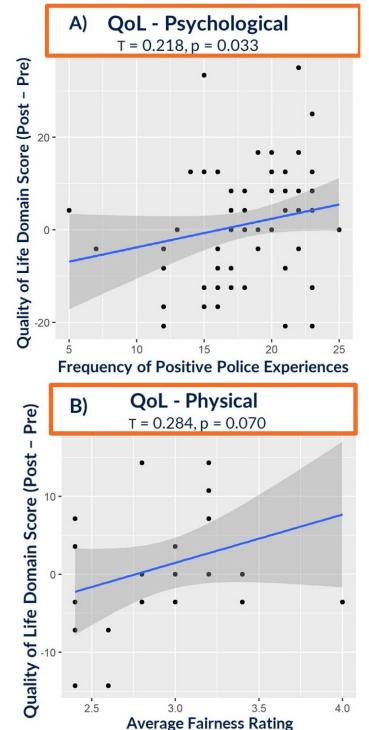
Demographic	History of Justice Involvement	History of Incarceration	
n	45	25	
Age	34.82	30.84	
Years of Edu	16.02	16.32	
Gender (% Men / Women*)	66 / 34 %	84 / 16 %	
Race (% White / Black*)	98 / 2 %	100 / 0 %	
Ethnicity (% Hispanic or Latinx)	16%	12%	
%Rural / Suburban / Urban	33 / 28 / 37 %	84 / 12 / 4 %	

We found one significant association; specifically, there was an association between positive justice system experiences and change in psychological well-being (as measured by the Psychological QoL subscale; T = 0.218, p = 0.033). This association is plotted in Figure 1A.

In individuals who were formerly incarcerated (n = 25), we also found a marginal association between facility fairness and change in physical well-being (as measured by the Physical Health QoL subscale; T=0.284, p=0.070). This association is plotted in Figure 1B.

The results indicate that positive justice experiences predict psychological and physical QoL improvements, which coincides with previous work which found that positive childhood experience reduced recidivism in justice-involved youth (Baglivio & Wolff, 2021). Unexpectedly, there was no association between negative justice experiences and QoL which differs from previ-

ous work which found reduced QoL in people experiencing homelessness following harsher policing (Robinson, 2017) as well as an association between adverse childhood events (but not recidivism) and lower QoL (Van Duin et al., 2020).



*Notes*: Blue line represents linear best fit line with gray shadow representing confidence interval.

**Fig. 1** Linear association between WHOQoL Quality of Life domain score and justice system experiences.

This pattern of results could highlight the need to implement positive policing and beneficial jail initiatives rather than focusing on reforming negative occurrences. However, it is also possible that the associations found in our sample are not representative of typical experiences of justice system contact. For example, our sample does not match typical demographics for justice involved individuals (Pettit & Western, 2004) and we had a large amount of poor-quality data that had to be excluded. Future work should replicate these findings in a larger sample with external validation (e.g., working face-to-face with individuals currently in local jails or prisons as they are released on change in QoL). At that point, individual differences and their impact on these associations can be explored with more confidence.

#### **Statement of Research Advisor**

This study is an important first step in a larger effort to characterize the relationship between prison experiences and outcomes. Maddy played an important role in adapting this overarching goal into a pilot project, collecting data, performing quality assurance, and drafting her presentation of this data at the 2024 Southeastern Psychological Association Meeting. She learned about what designing and running a research project takes from start to finish under the supervision of myself and our research coordinator, Olivia Sawyer. - Samantha J. Fede, Psychological Sciences, College of Liberal Arts

#### References

- [1] Baglivio, M. T., & Wolff, K. T. (2021). Positive childhood experiences (PCE): Cumulative resiliency in the face of adverse childhood experiences. Youth violence and juvenile justice, 19(2), 139-162.
- [2] Dabbs Jr, J. M., [...] Riad, J. K. (1995). Testosterone, crime, and misbehavior among 692 male prison inmates. Personality and individual Differences, 18(5), 627-633.
- [3] Edgemon, T. G., & Clay-Warner, J. (2019). Inmate mental health and the pains of imprisonment. Society and Mental Health, 9(1), 33-50.
- [4] Mallik-Kane, K., & Visher, C. A. (2008). Health and prisoner reentry: How physical, mental, and substance

- abuse conditions shape the process of reintegration (p. 82). Washington, DC: Urban Institute Justice Policy Center.
- [5] Patterson, E. J. (2013). The dose–response of time served in prison on mortality: New York State, 1989–2003. American Journal of Public Health, 103(3), 523-528.
- [6] Porter, L. C., & Novisky, M. A. (2017). Pathways to depressive symptoms among former inmates. Justice Quarterly, 34(5), 847-872.
- [7] Pettit, B., & Western, B. (2004). Mass imprisonment and the life course: Race and class inequality in US incarceration. American sociological review, 69(2), 151-169.
- [8] Raaijmakers, E. A., de Keijser, J. W., Nieuwbeerta, P., & Dirkzwager, A. J. (2017). Changes in the subjectively experienced severity of detention: Exploring individual differences. The Prison Journal, 97(5), 644-668.
- [9] Robinson, T. (2019). No right to rest: Police enforcement patterns and quality of life consequences of the criminalization of homelessness. Urban affairs review, 55(1), 41-73.
- [10] Sappington, A. A. (1996). Relationships among prison adjustment, beliefs, and cognitive coping style. International Journal of Offender Therapy and Comparative Criminology, 40(1), 54-62. Thomson, N. D. (2018). Psychopathy and violent crime. Routledge international handbook of psychopathy and crime, 508-525.
- [11] Turney, K., Wildeman, C., & Schnittker, J. (2012). As fathers and felons: Explaining the effects of current and recent incarceration on major depression. Journal of health and social behavior, 53(4), 465-481.
- [12] Van Duin, L., [...] & Popma, A. (2021). Criminal history and adverse childhood experiences in relation to recidivism and social functioning in multi-problem young adults. Criminal justice and behavior, 48(5), 637-654.
- [13] Wakefield, S., & Uggen, C. (2010). Incarceration

and stratification. Annual review of sociology, 36, 387-406.

[14] Warren, Novero, C. M., Booker Loper, A., & Warren, J. I. (2011). Second-generation prisoners: Adjustment patterns for inmates with a history of parental incarceration. Criminal Justice and Behavior, 38(8), 761-778.

[15] Whoqol Group. (1998). Development of the World Health Organization WHOQOL-BREF quality of life assessment. Psychological medicine, 28(3), 551-558.

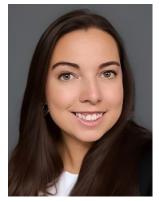
#### **Authors Biography**



Maddy J. McDaniel is a senior-year student pursuing a B.A. in Psychology and Sociology with a concentration in Criminology at Auburn University. Maddy was selected as a college of Liberal Arts Undergraduate Research fellow for 2023-2024. Her research interests include Psychopathology, recidivism risk, trauma exposure, and the assessment and mental health in forensic settings. She is a research assistant within the SCAMPI lab.



Olivia H. Sawyer is a post-baccalaureate research assistant serving as lab manager for the SCAMPI lab. She received a B.S. degree in Neuroscience in 2023 from Auburn University. Her background is in molecular neuroscience, and she plans to pursue a career in medicine after completing her post-baccalaureate training.



Samantha J. Fede is an Assistant Professor in the Department of Psychological Sciences and the Director of the Social Cognition and Affective/Moral Processing Imaging (SCAMPI) lab. She received her PhD in Cognition, Brain, and Behavior from the University of New Mexico in 2017. Her expertise is in cognitive neuroscience, neuroimaging, forensic psychology, substance use disorder research, and decision-making.

## Investigation of Yellow-Pigmented Bacterial Catfish Pathogens

Benjamin A. Marshall<sup>1,\*</sup>, Courtney E. Harrison<sup>2</sup>, and Timothy J. Bruce<sup>3</sup>

<sup>1</sup> Undergraduate Student, Biological Sciences, Auburn University
 <sup>2</sup> Ph.D. Student, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University
 <sup>3</sup> Assistant Professor, School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University

The bacterial genera Flavobacterium and Chryseobacterium are ubiquitous within soil, marine, and freshwater environments and are collectively referred to as yellow-pigmented bacteria (YPB). Flavobacterium spp. are pathogenic to wild and cultured fish species worldwide, and an increasing number of Chryseobacterium spp. have also been reported as emerging pathogens (Loch & Faisel 2015; Bruce et al., 2021). Recently, several YPB were recovered from catfish and other freshwater species following mortality events in Alabama, where YPBs were primarily responsible for causing disease. With the knowledge of YPB as a threat to aquaculture, these isolates were revived on various media types and subject to extensive testing to elucidate the pathogenic potential of these YPB and their susceptibility to antibiotics approved for use in aquaculture. With this information, approaches to mitigating disease outbreaks via YPB infection can be further explored to ensure the well-being of fish in aquaculture.

Eleven different isolates were revived and allowed to grow for 48 h at 28 °C. If strong growth was evident, 3-4 colonies were then transferred into modified Shieh broth (MSB) and allowed to grow for 24-48 h at 28°C. The inocula were then pelleted and subject to DNA extraction utilizing a High Molecular Weight DNA extraction kit. The resulting DNA concentration was quantified using a Thermo Scientific NanoDrop OneC Spectrophotometer and Qubit Flex Fluorometer.

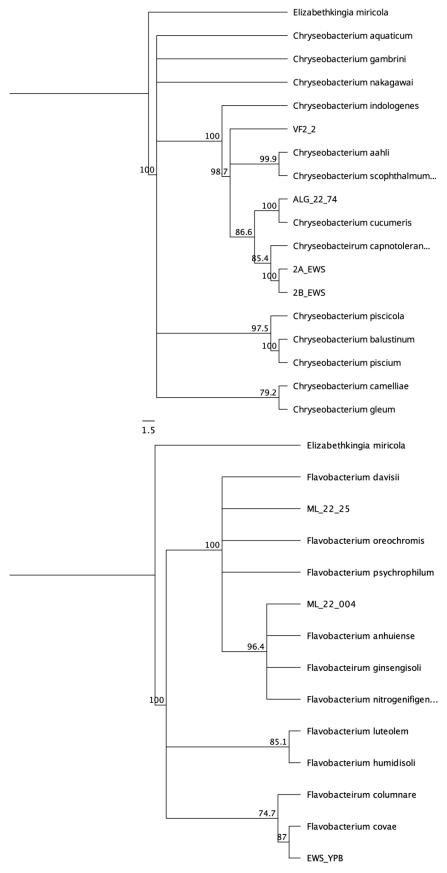
DNA extracted from YPB isolates was assessed for PCR amplification of the gyrase subunit B (*gyrB*) gene, a highly conserved gene in these bacterial genera. Positive and negative controls (*Flavobacterium covae* isolate ALG-00-530 and nuclease-free H2O, respectively) were used to compare fragment size using a 1kb ladder. Amplified fragments were visualized, and products whose

bands were of the appropriate size were purified and sent to Eurofins Genomics for Sanger sequencing.

Phylogenetic trees were assembled utilizing consensus sequences that were aligned using Clustal for Flavobacterium and Chryseobacterium reference sequences downloaded from GenBank. The sequences were trimmed to the same length and a neighbor-joining tree using Tamura-Nei genetic distance model with bootstrap resampling of 1000 replicates was performed. The data generated suggested that four YPB isolates belonged to the genera Chryseobacterium and three to *Flavobacterium*. Three of the four *Chryseobacterium* sp. isolates (2A-EWS, 2B-EWS, VF2-2) were placed into unidentifiable species clades. In comparison, two of the three Flavobacterium sp. isolates (ML-22-004, ML-22-25) were placed into unidentifiable clades, suggesting that these isolates are potentially novel species. The results of the phylogenetic analysis of the gyrB gene can be seen in Figure 1.

Isolates 2A-EWS, 2B-EWS, VF2-2, ML-22-004, and ML-22-25 were placed into unidentifiable clades and considered novel. Thus, these isolates were subjected to whole genome sequencing using the Oxford Nanopore Technologies GridIONx5 platform. Libraries were prepared using the Rapid Barcoding Kit and sequenced on a R9 FLO-MIN106 flow cell. The generated FAST5 files were then re-basecalled in SUP mode using Dorado 0.5.3 De novo assembly of long-read sequences and polishing was performed using Flye 2.9.3 via the Miniconda environment. The results of the whole genome sequencing validated the findings from the phylogenetic analysis, as all isolates were found to be novel except for ML-22-25, which was found to belong to a known species, Flavobacterium humidisoli. However, this isolate was still tested along with the other isolates

<sup>\*</sup> Corresponding author: bam0102@auburn.edu



**Fig. 1.** Chryseobacterium spp. (top) and Flavobacterium spp. (bottom) phylogenetic trees produced after analysis of gyrB gene.

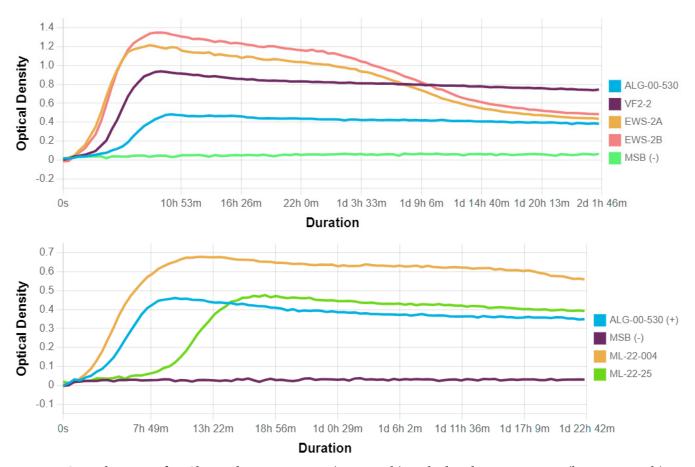


Fig. 2. Growth curves for *Chryseobacterium* spp. (top graph) and *Flavobacterium* spp. (bottom graph).

in growth kinetics experiments and antibiotic susceptibility assays.

After whole genome sequencing was completed, the novel isolates were revived on MSA and allowed to grow for 24-36 h at 28°C. At approximately 12 h before the start of the experiment, a colony from each plate was placed in separate 15 mL tubes with 5 mL MSB and allowed to grow in a shaking incubator at 28°C. After 12 h, 60 μL of each isolate, along with a negative control (MSB) and a positive control (ALG-00-530) were placed into wells containing 2 mL MSB in a 12-well plate. Each isolate was run in duplicate and allowed to grow in a shaking incubator at 350 rpm for ~48 h at 28°C. A Cerillo plate reader was utilized to measure the absorbance of each well every 30 min., and a growth curve for *Chryseobacterium* spp. and *Flavobacterium* spp. was generated as seen in Figure 2.

Finally, a MIC Antibiotic Resistance Assay was conducted on these novel isolates. Novel isolates were revived on MSA and allowed to grow for 24-48 h at 28°C. Overall, this study has revealed the existence of nov-

el Flavobacterium sp. and *Chryseobacterium* sp. that could potentially lead to disease and mortality among freshwater aquaculture species. An in vivo pathogen challenge will be conducted to elucidate the extent of their virulence channel catfish fingerlings and determine the amount of each novel YPB is needed to cause disease and mortality. Furthermore, this study has demonstrated the resistance of these novel YPB isolates to several antibiotics used in aquaculture, stressing the importance of selecting an appropriate treatment for the control and treatment of YPB in catfish aquaculture.

Several colonies from the spread plates were placed into 15 mL conical tubes containing 5 mL of 1X PBS. The mixture was then vortexed and diluted to a McFarland standard of 0.5. Once similar turbidity was achieved, 6  $\mu$ L of the PBS-bacteria mixture was placed in a 15 mL tube containing 6 mL of cation-adjusted Mueller Hinton broth (CAMHB) and vortexed. The MH-bacteria mixture was then poured into a reservoir, and a multi-channel pipette was used to transfer 50  $\mu$ L of CAMHB-bacteria mixture into the wells of a MIC plate

containing various antibiotics, some of which are approved for use in U.S. aquaculture. The MIC plate was incubated at 28°C and allowed to grow for 24-48 h. After this period, a visual examination of bacterial growth was conducted to identify antibiotic susceptibility, and a table was organized that showcased the antibiotic susceptibility of each isolate to the various microbic used. Table 1 is the resulting table generated based on data from the MIC assay.

**Table 1.** MIC assay results. A lighter color indicates susceptibility to the antimicrobial, while a darker color indicates resistance. ENRO: Enrofloxacin; AMP: Ampicillin; OTC: Oxytetracycline; ERY: Erythromycin; FFC: Florfenicol; FLUQ: Flumequine; PRI: Ormetoprim; SDO: Sulphadimenthoxine; OXO: Oxolinic acid; GEN: Gentamicin; TRIM: Trimethoprim; SMZ: Sulfamethoxazole; POS: Positive Control; NEG: Negative Control

Antimicrobics (24 hours post-inoculation)							
Isolate	2A EWS	2B EWS	ML-22-004	ML-22-25	VF2-2	CLSI- approved ECV	
ENRO	0.015	0.015	0.015	0.004	0.06	≤ 0.03	
AMP	> 16	> 16	> 16	0.06	16	≤ 0.5	
ОТС	8	8	2	0.015	> 8	≤ 0.25	
ERY	64	128	4	0.25	64	≤ 2	
FFC	8	8	2	0.12	2	≤ 4	
FLUQ	0.06	0.06	0.06	0.03	0.06	≤ 0.12	
PRI	0.12	0.25	2	0.03	0.12		
SDO	2.38	4.75	38	0.59	2.38	N/A	
ОХО	0.06	0.12	0.12	0.03	0.12	≤ 0.25	
GEN	> 4	> 4	4	0.06	> 4	N/A	
TRIM	0.06	0.12	0.5	0.015	0.06		
SMZ	1.19	2.38	9.5	0.3	1.19	N/A	
POS	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible		
NEG	Resistant	Resistant	Resistant	Resistant	Resistant		
Antimicrobics (48 hours post-inoculation)							
	A	antimicrobics	(48 hours po	ost-inoculatio	on)		
Isolate	2A EWS	antimicrobics 2B EWS	(48 hours po	ost-inoculation	on) VF2-2	CLSI-	
	2A EWS	2B EWS	ML-22-004	ML-22-25	VF2-2	approved ECV	
ENRO	2A EWS 0.030	2B EWS 0.030	ML-22-004 0.015	ML-22-25 0.008	VF2-2 0.06	approved ECV ≤ 0.03	
ENRO AMP	2A EWS 0.030 > 16	2B EWS 0.030 > 16	ML-22-004 0.015 > 16	ML-22-25 0.008 0.12	VF2-2 0.06 16	approved ECV ≤ 0.03 ≤ 0.5	
ENRO AMP OTC	2A EWS 0.030 > 16 > 8	2B EWS 0.030 > 16 > 8	ML-22-004 0.015 > 16 0.5	ML-22-25 0.008 0.12 0.03	VF2-2 0.06 16 > 8	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25	
ENRO AMP OTC ERY	2A EWS  0.030 > 16 > 8 128	2B EWS 0.030 > 16 > 8 128	ML-22-004 0.015 > 16 0.5 4	ML-22-25 0.008 0.12 0.03 0.25	VF2-2 0.06 16 > 8 128	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2	
ENRO AMP OTC ERY FFC	2A EWS  0.030 > 16 > 8  128 8	2B EWS 0.030 > 16 > 8 128 16	ML-22-004  0.015 > 16  0.5 4 2	ML-22-25 0.008 0.12 0.03 0.25 0.25	VF2-2 0.06 16 > 8 128 4	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4	
ENRO AMP OTC ERY FFC FLUQ	2A EWS  0.030 > 16 > 8  128 8 0.12	2B EWS  0.030 > 16 > 8  128 16 0.12	ML-22-004  0.015 > 16  0.5 4 2 0.06	ML-22-25 0.008 0.12 0.03 0.25 0.25 0.03	VF2-2  0.06  16  > 8  128  4  0.12	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2	
ENRO AMP OTC ERY FFC FLUQ PRI	2A EWS  0.030 > 16 > 8  128 8 0.12 0.5	2B EWS  0.030 > 16 > 8  128 16 0.12 0.5	ML-22-004  0.015 > 16  0.5 4 2 0.06 > 4	ML-22-25 0.008 0.12 0.03 0.25 0.25 0.03 0.06	VF2-2  0.06  16  > 8  128  4  0.12  0.12	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4 ≤ 0.12	
ENRO AMP OTC ERY FFC FLUQ PRI SDO	2A EWS  0.030 > 16 > 8 128 8 0.12 0.5 9.5	2B EWS  0.030 > 16 > 8 128 16 0.12 0.5 9.5	ML-22-004  0.015 > 16  0.5 4 2 0.06 > 4 > 76	ML-22-25 0.008 0.12 0.03 0.25 0.25 0.03 0.06 1.19	VF2-2  0.06  16  > 8  128  4  0.12  0.12  2.38	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4 ≤ 0.12  N/A	
ENRO AMP OTC ERY FFC FLUQ PRI SDO OXO	2A EWS  0.030 > 16 > 8 128 8 0.12 0.5 9.5 0.12	2B EWS  0.030 > 16 > 8 128 16 0.12 0.5 9.5 0.25	ML-22-004  0.015 > 16  0.5 4 2 0.06 > 4 > 76 0.12	ML-22-25  0.008 0.12 0.03 0.25 0.25 0.03 0.06 1.19 0.03	VF2-2  0.06  16  > 8  128  4  0.12  0.12  2.38  0.12	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4 ≤ 0.12  N/A ≤ 0.25	
ENRO AMP OTC ERY FFC FLUQ PRI SDO OXO GEN	2A EWS  0.030 > 16 > 8 128 8 0.12 0.5 9.5 0.12 > 4	2B EWS  0.030 > 16 > 8 128 16 0.12 0.5 9.5 0.25 > 4	ML-22-004  0.015 > 16 0.5 4 2 0.06 > 4 > 76 0.12 4	ML-22-25  0.008 0.12 0.03 0.25 0.25 0.03 0.06 1.19 0.03 0.06	VF2-2  0.06  16  > 8  128  4  0.12  0.12  2.38  0.12  > 4	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4 ≤ 0.12  N/A	
ENRO AMP OTC ERY FFC FLUQ PRI SDO OXO GEN TRIM	2A EWS  0.030 > 16 > 8 128 8 0.12 0.5 9.5 0.12 > 4 0.25	2B EWS  0.030 > 16 > 8 128 16 0.12 0.5 9.5 0.25 > 4 0.25	ML-22-004  0.015 > 16  0.5 4 2 0.06 > 4 > 76 0.12 4 > 1	ML-22-25  0.008 0.12 0.03 0.25 0.25 0.03 0.06 1.19 0.03 0.06 0.03	VF2-2  0.06  16  > 8  128  4  0.12  0.12  2.38  0.12  > 4  0.12	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4 ≤ 0.12  N/A ≤ 0.25 N/A	
ENRO AMP OTC ERY FFC FLUQ PRI SDO OXO GEN TRIM SMZ	2A EWS  0.030 > 16 > 8 128 8 0.12 0.5 9.5 0.12 > 4 0.25 4.75	2B EWS  0.030 > 16 > 8 128 16 0.12 0.5 9.5 0.25 > 4 0.25 4.75	ML-22-004  0.015 > 16  0.5 4 2 0.06 > 4 > 76 0.12 4 > 1 > 19	ML-22-25  0.008 0.12 0.03 0.25 0.25 0.03 0.06 1.19 0.03 0.06 0.03 0.59	VF2-2  0.06  16  > 8  128  4  0.12  0.12  2.38  0.12  > 4  0.12  2.38	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4 ≤ 0.12  N/A ≤ 0.25	
ENRO AMP OTC ERY FFC FLUQ PRI SDO OXO GEN TRIM	2A EWS  0.030 > 16 > 8 128 8 0.12 0.5 9.5 0.12 > 4 0.25	2B EWS  0.030 > 16 > 8 128 16 0.12 0.5 9.5 0.25 > 4 0.25	ML-22-004  0.015 > 16  0.5 4 2 0.06 > 4 > 76 0.12 4 > 1	ML-22-25  0.008 0.12 0.03 0.25 0.25 0.03 0.06 1.19 0.03 0.06 0.03	VF2-2  0.06  16  > 8  128  4  0.12  0.12  2.38  0.12  > 4  0.12	approved ECV ≤ 0.03 ≤ 0.5 ≤ 0.25 ≤ 2 ≤ 4 ≤ 0.12  N/A ≤ 0.25 N/A	

#### **Statement of Research Advisor**

Mr. Benjamin Marshall led the research team's efforts in sequencing, data analyses, and MIC testing of novel, yellow-pigmented bacteria isolated from catfish production areas. Benjamin also took a systematic approach to discerning bacterial growth dynamics, which will be used for in vivo pathogen challenge trials. The team will aim to compile all of the project data into a comprehensive manuscript, where Benjamin will help with the journal preparation process.

- Timothy J. Bruce, School of Fisheries, Aquaculture and Aquatic Sciences, College of Agriculture

#### References

[1] Loch, T.P., and Faisal, M. 2015. Emerging flavobacterial infections in fish: A Review. Journal of Advanced Research, 6(3), 283–300.

[2] Bruce, T.J., Ma, J., Knupp, C., Loch, T.P., Faisal, M. and Cain, K.D., 2020. Cross-protection of a live-attenuated Flavobacterium psychrophilum immersion vaccine against novel Flavobacterium spp. and Chryseobacterium spp. strains. Journal of Fish Diseases, 43(8), 915-928.

#### **Authors Biography**



Benjamin Marshall is a junior-year student pursuing a B.S. degree in Microbiology on the Pre-Med Track at Auburn University. As an undergraduate research fellow, he has played a key, primary role in elucidating the characteristics of these bacterial isolates and hopes to carry his research experiences to the field of medicine.



Courtney Harrison is a Ph.D. Student in the School of Fisheries, Aquaculture, and Aquatic Sciences at Auburn University. She received a B.S. in Animal and Nutritional Sciences from West Virginia University and a M.S. in Fisheries and Wildlife from Michigan State University. As a graduate student mentor on this project, she assisted Ben Marshall with the characterization of bacterial isolates and experimental design.



Dr. Timothy J. Bruce is an Assistant Professor of Aquatic Animal Health in the School of Fisheries, Aquaculture and Aquatic Sciences. Dr. Bruce's research program focuses on emerging pathogens, bacterial coinfections, and understanding the fish immune response to disease-causing agents.

## Hemocompatibility of Branched Amphiphilic Peptide Capsules

Emilee Middleton<sup>1,\*</sup>, Ethan Oesterle<sup>2</sup>, and Adriana Avila-Flores<sup>3</sup>

- <sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University
- <sup>2</sup> Undergraduate Student, Department of Biological Sciences, Auburn University
  - <sup>3</sup> Associate Professor, Department of Biological Sciences, Auburn University

#### **Abstract**

Branched amphiphilic peptide capsules (BAPCs) are a peptide-based nanoparticle composed of two unique peptides developed by our group that have recently shown promise as an mRNA-vaccine delivery platform in mice. BAPCs are an attractive alternative to other nanoparticles, such as lipid nanoparticles, due to their long shelf-life, stability at elevated temperatures, and absence of polyethylene glycol (PEG). Additionally, recent studies suggest that targeted delivery to the lungs may be possible when injected intravenously, which specifically makes understanding the interaction of BAPCs with blood a topic of interest. The objective of this study is to explore the hemocompatibility of BAPCs in human whole blood using three criteria - coagulation, hemolysis, and catalase activity. This was done by exposing whole human blood to three concentrations of BAPCs (40, 80, and 120 µM) for 4 hours prior to analysis. The hemocompatibility of BAPC-treated samples were compared to non-treated blood as a control, and all measurements were performed in triplicate using blood from a single donor. The results of this study show that BAPCs do not cause a significant increase in any of the three categories compared to untreated blood samples. These results show promising potential for BAPCs as a future mRNA delivery tool in a clinical setting and open the door for future experiments to analyze additional hemocompatibility criteria, reaction across blood types, and how the addition of nucleic acids impact hemocompatibility.

#### Introduction

Messenger RNA (mRNA) therapy is at the forefront of research to treat various conditions resulting from protein deficiency. When delivered without any aiding molecules, the mRNA tends to degrade before it can reach its desired target. This is largely due to the body's immune response and serum nucleases. Because of this, there has been a movement towards nanoparticles to aid mRNA in delivery.

Currently, there are multiple nanoparticles that have entered commercial use, the main two being lipid nanoparticles (LNPs) and polymeric cationic nanoparticles. Although both nanoparticles have been proven successful, there are still several downsides to them with the main one being shelf life. Without the inclusion of polyethylene glycol (PEG), the previously stated nanoparticles do not remain stable on the shelf (1). However, the inclusion of PEG has led to an increase in toxicity and other negative side effects. In an attempt to solve this issue, our lab uses branched amphiphilic peptide capsules (BAPCs).

BAPCs are composed of two branched amphiphilic peptides [bis(Ac-FLIVI)-K-KKKK-CO-NH2] and bis(Ac-FLIVIGSII)-K-KKKK-CO-NH2] at equimolar concentrations (1). These two peptides contain a cationic C-terminus attached to the protein chain, allowing for the creation of the hydrophilic region of the peptides while the amino acid chains are the hydrophobic region. This amphiphilic nature allows for the peptides to self-arrange into a phospholipid bilayer with a cationic surface. Having the ability to self-arrange leads to BAPCs inherently having a longer shelf life without the inclusion of PEG and further stability in vivo as they can resist degradation by several factors. These factors include proteases, elevated temperatures and more.

Because of the cationic nature of BAPCs, they can interact with the negatively charged mRNA through electrostatic forces. The nucleic acid and nanoparticle form a complex, which is then delivered intravenously to the patient (1). Several in vitro and in vivo assays have shown promising results for BAPCs as an mRNA delivery device.

<sup>\*</sup>Corresponding author: emm0098@auburn.edu

The main purpose of this experiment was to test the hemocompatibility of BAPCs with human blood through measuring hemolysis, catalyst activity, and coagulation. The reasoning behind this experiment is the first contact the nanoparticles would have with a patient is red blood cells, so if BAPCs were to transfer into clinical use, their interaction with blood would be an area of high importance. The rationale behind choosing the three markers in this experiment was hemocompatibility has five main categories (thrombosis, coagulation, platelets, hematology and immunology) (2) before a compound can be classified as hemocompatible, and the three markers tested are the most common ones for the categories. To do this, the nanoparticles were incubated with blood without mRNA for 4 hours inverted every 30 minutes. Following incubation, the samples were centrifuged, and the serum was collected. Appropriate chemicals were added, and the absorbance of the samples were measured by spectrophotometer or plate readers. Our results displayed that BAPCs did not cause a significant increase in any of the three markers tested.

#### Materials and Methods

#### **Materials**

Drabkin's reagent (VWR, Radnor, PA, USA), human whole blood (Human Cell Biosciences, Milpitas, CA, USA), pH 7.4 phosphate buffered saline (PBS) without Ca 2+ and Mg2+ (VWR, Radnor, PA, USA), Complement C5a human ELISA kit (Thermo Scientific, Waltham, MA, USA), Catalyst activity kit (Thermo Scientific, Waltham, MA, USA)

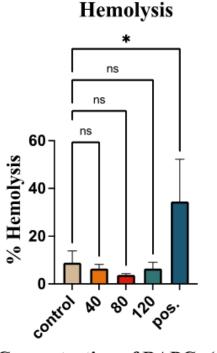
Peptide Synthesis

Peptide synthesis was down as described by (1). The peptides were purified using reverse phase HPLC.

#### **Methods**

BAPCs at three separate concentrations (40  $\mu$ M, 80  $\mu$ M, 120  $\mu$ M) were incubated with human whole blood at 37 °C for 4 hours and inverted every 30 minutes for mixing. For a positive control for all assays, hydrogen peroxide was incubated with human whole blood for 30 minutes. Following the incubation time, all samples were centrifuged, and the serum was collected. For hemolysis, the serum was then placed in microcentrifuge tubes and mixed in a 2:1 ratio with PBS. The resulting diluted solution was then mixed with Drabkin's reagent in a 1:1 ratio. The mixture was then placed in a cuvette,

and the absorbance was measured using a spectrophotometer. The spectrophotometer was blanked with PBS prior to any measurements. For catalyst activity, the protocol specified by the kit was followed with the serum being placed into a 96 well plate, various chemicals added, and appropriate incubation time used. The results of this assay were measured by absorbance in a microwell plate reader. For the ELISA kit, the serum was placed into a 96 well plate and the protocol specified by Thermo Scientific was followed. This involved incubation of various components every 30 minutes and the measuring of the results by a microwell plate reader.



Concentration of BAPCs (mM)

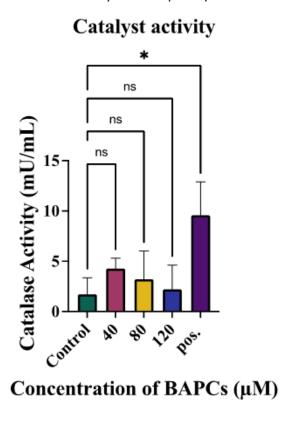
**Fig. 1**- Graph showing the level of hemolysis measured after four hours of incubation with each concentration.

#### **Results and Discussion**

The BAPCs showed no significant increase in any of the assays run. Figure 1 displays the results of the hemolysis assay.

It is shown that all BAPC concentrations were significantly less than the positive control value. The maximum hemolysis value excluding the positive control was 18%, which is out of the suggested range of 6.4-8.6% (3). However, this is likely due to the incubation

time as it affected the control value. Figure 2 displays nanoparticle. the results of the catalyst activity assay.

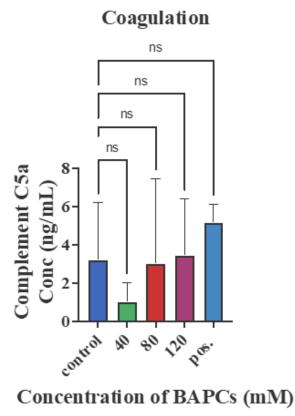


**Fig. 2-** Graph showing the level of catalyst activity measured after four hours of incubation with each concentration.

As seen in the figure, there was no significance in the catalyst activity caused by the various BAPC concentrations in comparison to the human whole blood control. This shows that there is no sign of an increase in oxidative stress by the addition of BAPCs. Figure 3 displays the results of the coagulation assay.

There was no significant increase in the specific complement tested for coagulation in any of the samples. This is likely due to hydrogen peroxide as the positive control, which does not lead to blood coagulation. However, the lack of increase between the control and the concentrations indicates that the BAPCs are unlikely to lead to coagulation within the human blood. Overall, the results show that BAPCs do not cause a significant complexity of the samples.

Overall, the results show that BAPCs do not cause a significant adverse reaction when in contact with human blood. These results are promising for the movement of BAPCs into the clinical aspect. Future testing would move into the other categories of hemocompatibility, and if different blood types interact differently with the



**Fig. 3-** Graph showing the level of coagulation measured after four hours of incubation with each concentration.

#### **Statement of Research Advisor**

Emilee has made significant contributions to our research group, particularly in testing nanoparticle toxicity. She has progressed from following established protocols to developing her own methodologies through diligent research efforts. Thanks to her hard work and dedication, we have achieved promising results indicating the safe intravenously delivery of BAPCs in human models. This advancement represents a significant step forward in moving BAPCs closer to clinical applications.

-Dr. Adriana Avila-Flores, Department of Biological Sciences, College of Science and Mathematics

#### References

[1] N. Kunte, M. Westerfield, E. McGraw, J. Choi, T. Akinsipe, S.K. Whitaker, A. Brannen, P. Panizzi, J.M. Tomich, L.A. Avila Evaluation of transfection efficacy, biodistribution, and toxicity of branched amphiphilic peptide capsules (BAPCs) associated with mRN-

140

ABiomater. Sci., 10 (2022), pp. 6980-6991, 10.1039/ D2BM01314B.

[2] Hemocompatibility. Hemocompatibility- an overview | ScienceDirect Topics. (n.d.). https://www.sciencedirect.com/topics/engineering/hemocompatibility.

[3] Arif SH, Yadav N, Rehman S, Mehdi G. Study of Hemolysis During Storage of Blood in the Blood Bank of a Tertiary Health Care Centre. Indian J Hematol Blood Transfus. 2017 Dec;33(4):598-602. doi: 10.1007/s12288-016-0769-5. Epub 2016 Dec 24. PMID: 29075076; PMCID: PMC5640527.



Adriana Avila-Flores is an associate professor at Auburn University in the department of biological sciences. Dr. Flores and her students' research focuses on several different nanoparticles' toxicity and effectiveness. Her group mainly focuses on branched amphiphilic peptide capsules and their efficacy as a mRNA delivery model.

#### **Authors Biography**



Emilee Middleton is a senior pursuing a bachelor's in chemical engineering at Auburn University. During her time at Auburn, she has served as an undergraduate researcher in the Avila-Flores group and as a learning assistant in the biological sciences department. In the Flores lab, Emilee has contributed to research on toxicity and hemocompatibility of various nanoparticles.



Ethan Oesterle is a senior pursuing a bachelor's in biomedical sciences at Auburn University. During his time at Auburn, he has served as an undergraduate researcher in the Avila-Flores group. In the Flores lab, Ethan has contributed to research on toxicity and hemocompatibility of BAPCs in blood and human lung cells.

### Classifying and Estimating Tremor Frequency via Inertial Measurement Unit

Zachary R. Miller<sup>1,\*</sup>, and Chad G. Rose<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Mechanical Engineering, Auburn University <sup>2</sup> Assistant Professor, Department of Mechanical Engineering, Auburn University

Tremor can generally be defined as involuntary, chaotic twitching motions of various parts of the body, usually caused by neurological impairments [1]. The presence of tremor in the hands and fingers specifically can negatively impact a patient's ability to perform daily tasks such as writing, eating, typing, etc., greatly decreasing the patient's quality of life. Mechanical, non-invasive solutions have been proposed to address the limitations of pharmacological and surgical tremor solutions. One commercially available solution, known as the Readi-Steadi<sup>®</sup>, uses passive, constant damping elements across the whole hand to suppress tremor [2]. Unfortunately, this also suppresses any desired motion, which can unintentionally impede the wearer.

To remedy this over-suppression of movement, a more active solution should be considered. An active tremor solution should utilize a closed feedback loop to detect when tremor is occurring, and then to apply the suppressing effect only when needed. However, such suppression devices must be able to detect when a tremor is happening for active control methods to be viable. The objective of this research is to design a device to accurately study the motion of the index finger to determine and differentiate between voluntary motion and involuntary tremor. It is commonly accepted that most voluntary motion occurs between 1 and 3 Hz, while tremor typically occurs from 3 to 12 Hz [3]. These cutoffs can be used to sufficiently differentiate between tremor and voluntary movement. Validation of this device relies upon achieving similar tremor behavior in the frequency domain as prior research [4]. The device, shown in Fig. 1, uses two inertial measurement units (IMUs), which are attached to the proximal interphalangeal (PIP) joint and the distal interphalangeal (DIP) joint respectively to report translational acceleration data of the left index finger in three dimensions.

The subject's hand is inserted into the glove and placed on a flat surface with the ulnar side of the wrist facing down. The subject then begins flexing and extending their index finger at a constant rate for 20 seconds. The two IMUs interface with a Raspberry Pi Pico microcontroller through a multiplexer using the Inter-Integrated Circuit (I2C) communication protocol. The microcontroller receives translational acceleration measurements from the IMUs using a C++ script through the Arduino IDE, which is then stored in a .csv file by sending the data over a serial connection using PuTTY. The .csv file is processed through a MATLAB script that first calculates the magnitude of the acceleration data which is then processed through a Fast Fourier Transform algorithm to plot the finger's motion in the frequency domain to observe the frequencies present within the motion. The tremor motion is then plotted in both the continuous time domain and the frequency domain, shown in Fig. 2.



**Fig. 1** Tremor measurement device with the (a) IMUs, (b) Raspberry Pi Pico microcontroller, and (c) multiplex

<sup>\*</sup>Corresponding author: zrm0014@auburn.edu

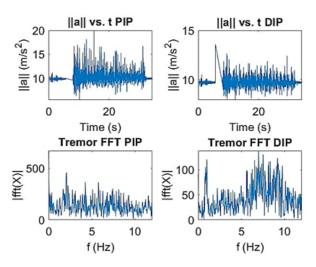


Fig. 2. Tremor acceleration data plotted against time (top) and in the frequency domain (bottom) of the PIP and DIP joints.

The acceleration readings show that the IMU oscillates about 9.81 m/s^2, meaning that the acceleration due to Earth's gravity was the dominant force acting on the IMUs. The frequency readings show that the PIP joint primarily experienced frequencies at 2.1 Hz, 4.3 Hz, and 5.8 Hz. Similarly, the DIP experienced frequencies primarily at 0.5 Hz, 6.5 Hz, and 8.6 Hz. The results show that classifying tremor based upon the frequencies present in the motion is a viable solution. However, some modifications should be made to the device to improve the accuracy of the measurements. The device can then be implemented alongside something like a soft hand exoskeleton to actively suppress tremor.

#### Statement of Research Advisor

Zachary's project was self-motivated, and the work was carried out largely independently. This is a new research area for my group, and addresses a challenge in all wearable robotics, but a particularly challenging issue in wearables for actively compensating tremor – how can a robot best understand and predict what a person intends to do. In this preliminary work, Zachary and I developed greater experience with new measurement modalities and this application area, and I am excited to have Zachary continuing in this research area for his MS work in my group.

- Chad G. Rose, Department of Mechanical Engineering, Samuel Ginn College of Engineering

#### References

[1] Deuschl, G., Raethjen, J., Lindemann, M., and Krack, P., "The pathophysiology of tremor." Muscle & Nerve: Journal of the American Association of Electrodiagnostic Medicine, 24(6) pp. 716-735, (2001).

[2] Readi Steadi<sup>®</sup>, "Readi Steadi<sup>®</sup> Anti Tremor Orthotic Glove System," 2022. https://www.readi-steadi.com/(accessed March. 19, 2024).

[3] Louis, E.D., "Essential Tremor," The Lancet Neurology, 4(2), pp.100-110, (2005).

[4] Zhou, Y., Jenkins, M. E., Naish, M.D., and Trejos, A.L., "The measurement and analysis of Parkinsonian hand tremor," 2016 IEEE-EMBS International Conference on Biomedical and Health Informatics (BHI), Las Vegas, NV, USA, 2016, pp. 414-417, (2016).

#### **Authors Biography**



Zachary R. Miller is a senior-year student pursuing a B.S. degree in Mechanical Engineering at Auburn University. Following graduation, he will be pursuing a M.S. degree in Mechanical Engineering at Auburn University working as a Graduate Research Assistant under Dr. Chad G. Rose's in the Wearable and Bio-Robotics Lab



Chad G. Rose, PhD is an Assistant Professor in the Department of Mechanical Engineering and directs the Wearable and Bio-Robotics (WeBR) Lab. He holds a B.S. in Mechanical Engineering from Auburn University and an M.S. and Ph.D. in Mechanical Engineering from Rice University. Dr. Rose's research focus is on the design and control of robots to rehabilitate, assist, or augment motor and sensory function.

### Understanding the Impacts of Genetic Counseling on Coping Styles of Families with Positive Cystic Fibrosis Carrier Screening

Hannah J. Montgomery<sup>1,\*</sup>, Joshua R. Novak<sup>2</sup>, Kate M. Day<sup>1</sup>, Kaleigh C. Miller<sup>3</sup>, and Katy Marten<sup>1</sup>

<sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University
 <sup>2</sup> Associate Professor, Department of Human Development and Family Science, Auburn University
 <sup>3</sup> Undergraduate Student, Department of Nutritional Sciences, Auburn University

#### Introduction

Cystic Fibrosis (CF), afflicts approximately 30,000 people in the US, with 1 in 30 people being a genetic carrier (American Lung Association, 2022). It is an autosomal recessive genetic disorder, meaning that a person must inherit two affected copies of the CF gene to develop the condition (Endres & Konstan, 2022).

CF affects almost every organ system, causing electrolyte imbalance, decreased pancreatic function, and recurring lung infections among other symptoms (Stern, 1997). Fortunately, there is a high prevalence of carrier screening (Decruyenaere, 1998), particularly around pregnancy and childbearing years, and many patients have a desire for genetic counseling (Ommundsen, 2021) which can better help the individual process and cope with a positive genetic test (Shiloh, 2013).

However, genetics is inherently a family matter; results of genetic tests, favorable or not, often hold implications for biological family members (Rolland, 2006), and a positive CF diagnosis has enormous impacts on the partners (Matire & Helgeson, 2017). Fortunately, some couples do participate in genetic counseling together to navigate genetic diagnoses and concerns (CDC). However, the genetic counseling literature has largely overlooked the dyadic coping processes in reviews of the efficacy and outcomes of counseling, leading to a gap in our understanding of the nuanced effects of genetic counseling on relationships and subsequent coping and adjustment. Thus, the present project seeks to answer the following research question: How does participating in genetic counseling impact the coping styles observed in married partners after a positive cystic fibrosis carrier screening?

The present project will employ a snowball sample method to recruit 20 couples who have attended genetic counseling after receiving a positive CF screening within the last 3 years. Fliers and study information will be posted on social media platforms (e.g., Facebook) where individuals can target and share the information with those that meet the study criteria. Those who meet criteria will click on a Qualtrics link and enter in their email address. Participants will be contacted and a conjoint semi-structured interview will be scheduled. The semi-structured interview will take place on Zoom, be recorded, last approximately 1 hour, and consist of 11 questions about the impact of a positive CF screening and genetic counseling on their individual and relationship (dyadic) coping. Couples will be compensated \$20 for participation.

Next, interviews will be transcribed and the audio/visual recordings will be deleted. Interviews will be read line-by-line and labeled according to conceptual meanings or labels, with a particular focus on gerunds (words ending in "ing"), which are useful in capturing process or action. The final stage is theoretical coding, where the categories will be arranged into a graphical model that is reflective of both the data and experiences of the participants and highlights theoretical ideas or processes that emerge (Charmaz, 2014; Corbin & Strauss, 2008). The results from this project will identify the dyadic coping process so that genetic counselors can better help couples with a positive CF screening and ultimately improve their psychological and relational functioning.

Methods

<sup>\*</sup> Corresponding author: hjm0033@auburn.edu

During their interviews, participants were each asked the following 11 questions about their experiences with CF and their journey through their genetic diagnosis.

- 1. Generally, how do you feel about your genetic counseling session in which you received the news about Cystic Fibrosis?
- 2. How have your genetic results changed your perception of chronic illness?
- 3. How do you view the severity of cystic fibrosis?
- 4. How deep of an understanding do you have of cystic fibrosis as a condition as far as disease process, treatment, and impact on family?
- 5. Have these genetic results impacted your relationship with each other?
- 6. Are there any significant conflicts that have arisen due to the information gained?
- 7. What are some ways you have approached conflict with your partner during this time?
- 8. How do each of you feel the other has handled the positive genetic screening?
- 9. Do you think you would feel differently about the results if you had/had not attended a genetic counseling session?
- 10. How has participating in genetic counseling changed your perception of genetics and/or how you view your own genetics?
- 11. Is there anything you wish your genetic counselor would have explained further?

The first questions focus on the couple's understanding of genetics and CF and how that understanding has grown and changed through attending genetic counseling sessions. The middle section is about how their genetic results, and the process of genetic counseling has impacted their relationships. Specifically, how the couples have approached conflicts that have arisen due to their genetic diagnosis. Finally, the last questions were intended to understand the couples' perceptions of their experience with a genetic counselor and how they believe that experience shaped their view of their genetic results.

#### **Discussion**

While participants are still being interviewed, preliminary data has shown some overarching themes pertaining to the genetic counseling experience of many couples with positive CF genetic carrier screening. Many

couples expressed gratitude for their genetic counseling experience saying that the genetic counselor taught them a lot about genetics and CF in general. Many participants felt that they had a good understanding of CF after genetic counseling but previously had not been provided with much information about the disease.

Moreover, couples described their relationship as growing stronger due to facing hardship together. They talked about having to rely on each other and trust that each one would keep the other informed and included in the treatment process. The biggest issue that couples reported due to their genetic diagnosis was the partner with the positive screen feeling like a burden to the other or being afraid that they have harmed their children by passing down a genetic mutation. The couples felt that the most important part of coping with the news of their genetic diagnoses was communication with each other. Most emphasized that trust in each other stemmed from their openness about their individual experiences which brought them closer as a couple.

#### **Statement of Research Advisor**

Ms. Montgomery, who joined my research lab as a Freshman Genetics Major, has an unmatched passion and enthusiasm for the research process. Upon Hannah's initial literature review, we were surprised to find that not much exists on how a positive Cystic Fibrosis impacts couple relationships and how couples navigate the genetic counseling experience. Thus, she initiated and developed this project, conceptualized the ideas, wrote and submitted the IRB, and is currently underway in conducting the interviews and the qualitative coding process. The results will then be written up, and we expect to discover how couples engage together or separately around this diagnosis, what benefits or issues it may bring up in relationship functioning, and what gender differences may exist. These results will inform how genetic counselors and couples therapists might better tailor the assessment and treatment of Cystic Fibrosis. Way to go Hannah!

- Joshua Novak, Human Development and Family Science, College of Human Sciences

#### References

American Lung Association. Learn About Cystic Fibrosis. Retrieved April 10, 2024, from https://www.lung.org/lung-health-diseases/lung-disease-lookup/cystic-fibrosis,

(November 17, 2022).

Centers for Disease Control. Cystic Fibrosis. Retrieved April 10, 2024 from https://www.cdc.gov/genomics/disease/cystic fibrosis.htm, (May 9, 2022).

Charmaz, K., "Grounded Theory in Global Perspective: Reviews by International Researchers" Qualitative Inquiry. 20(9). (2014).

Corbin, J., and Strauss, A., *Basics of Qualitative Research: Techniques and Procedures for Developing Grounded Theory,* SAGE Publications, (2012).

Decruyenaere, M., Evers-Kiebooms, G., Denayer, L., Welkenhuysen, M., "Upate and Impact of Carrier Testing for Cystic Fibrosis" Community Genetics. 1(1) pp. 23-35, (1998).

Endres, T. M., and Konstan, M. W., "What is Cystic Fibrosis" JAMA Patient Page, 327 (2), pp 191, (2022).

Martire, L. M., and Helgeson, V. S., "Close Relationships and the Management of Chronic Illness: Associations and Interventions." Am Psychol, 72(6), pp. 601-612, (2017).

Ommundsen, R. M., Stromsvik, N., Hamang, A., "Assessing the Relationship Between Patient Preferences for Recontact after BRCA1 or BRCA2 Genetic Testing and their Monitoring Coping Style in a Norwegian Sample" Journal of Genetic Counselling, 31, pp. 554-564, (2021).

Rolland, J. S., McDaniel, S. H., Miller, S. M., Eds., *Individuals, Families, and the New Era of Genetics*, Norton Professional Books, (2006).

Shiloh, S., Koehly, L., Martin, J., Hadley, D., "Monitoring Coping Style Moderates Emotional Reactions to Genetic Testing for Hereditary Nonpolyposis Colorectal Cancer: A Longitudinal Study" Psycho-Oncology, 17, pp. 746-755, (2008).

Stern, R. C., "The Diagnosis of Cystic Fibrosis," New England Journal of Medicine, 13;336(7), pp 487-491, (1997).

#### **Authors Biography**



Hannah Montgomery is a second-year student pursuing B.S. degrees in Genetics and Neuroscience at Auburn University. She loves doing Human Development and Family Science research in the Relationships and Health Lab and is interested in pursuing a career in medical genetics. She is involved in IMPACT, Tri Beta, and the Auburn University Rowing Club.



Josh Novak is an associate professor in the Human Development and Family Science department. He is the principal investigator of the Relationships and Health Lab. See www.Relationships andhealthlab.weebly.com



Kate Day is a sophomore majoring in Biomedical Sciences on the Pre-medicine track. She is also pursuing a minor in Human Development and Family Science and is currently involved in the Relationships and Health Lab. On campus, she is a COSAM Leader as well as being involved in IMPACT and the International Buddy Program.

#### Auburn7University Journal of Undergraduate Scholarship



Kaleigh Miller is an undergraduate junior pursuing a degree in Nutrition Science and a minor in Human Development and Family Science. She is a member of the Honors College, the Relationships and Health Lab, and Auburn University Dance Marathon. Kaleigh is on the pre-med track and hopes to pursue a career as a physician with specific interests in Nutrition and Pediatrics.



Katy Marten is a Senior in Biomedical Science major on the pre-med track and is planning on attaining her master's degree after she graduates with her BS. This is her third semester working with the RH lab. On campus, she is involved in the fraternity Alpha Omicron Pi and Alpha Epsilon Delta. She hopes to start her career as an anesthesiologist or oncologist.

# **Environmental Initiatives in the Lodging Industry: Best Practices, Challenges and Efficacy in LEED Platinum Certified Properties**

Shelby Morris<sup>1</sup> and Dr. Imran Rahman<sup>2,\*</sup>

<sup>1</sup> Undergraduate Student, Horst Schulze School of Hospitality Management, Auburn University <sup>2</sup> Associate Professor, Horst Schulze School of Hospitality Management, Auburn University

The lodging industry does not over-pollute the environment, nor does it consume immense amounts of nonrenewable resources compared to many other industries (Rahman et al., 2012). However, given the enormous size of this industry, the impact it has on the environment can be quite detrimental. On average, hotels in the United States consume approximately 60 billion kilowatt-hours (kWh) of electricity and use 180 billion gallons of water annually. Globally, hotels produce approximately 320,000 tons of waste per year. According to a recent CBRE study (2023), the hotel industry sits at 96 on the CO2 scale exceeding the average line of approximately 80, and hence underscoring a global need to reduce its carbon production. Therefore, the onus is on the lodging industry to be more sustainable in the days to come.

One of the best ways to embrace sustainability is through LEED certification which has become critical to addressing climate change and meeting ESG goals, enhancing resilience, and supporting more equitable communities. It is one of the most comprehensive ways of adopting sustainability, as LEED-certified buildings save money, improve efficiency, lower carbon emissions and create healthier places for people. As of now, there are only 461 LEED certified hotels in the US, with only 65 of them at the Platinum level. Given that there are 166,272 hotels and motels in the country, the percentages of LEED certified, and Platinum LEED certified properties are a meager 0.28% and 0.04% respectively. While most hotels are adopting sustainability in bits and pieces, such as implementing one or more low-cost or no cost practices like the towel reuse program, low flow showerheads, faucets, and toilets, LED lightbulbs, etc., they seem to be shying away from embracing it wholeheartedly (Rahman et al., 2020). Several barriers

exist that discourage hotels from doing so.

First, the concept of a green hotel is not easily grasped by many hoteliers, as the industry bases its business on perceived opulence, luxury, and grandeur (Iwanowski & Rushmore 1994). Other impeding factors include but are not limited to the cost, complexity, varied structure of the industry, low regulatory pressure, lack of information, the need to share best practices (Pryce 2001). Furthermore, there is a substantial number of small and medium-sized hotels that do not see the benefit, cost, or otherwise of adopting environmentally friendly initiatives (Pryce 2001). Needless to say, there seems to be strong consumer demand for green accommodations. About 53% of travelers plan to stay in environmentally friendly accommodations when they travel and about one third travelers research a hotel's environmental and social practices before booking (Booking.com, 2020).

The purpose of this study is to explore best practices, challenges, and efficacy in three LEED Platinum-certified properties in the USA. Through site visits, tours, and interviews of general managers of those hotels, this study provides insights regarding the environmental management processes of those top sustainable properties.

LEED certifications come in multiple levels depending on how many sustainable initiatives a facility has in place. The highest level is platinum, followed by gold, silver, and a base level certification. A hotel earns 'points' on a LEED scorecard after a thorough inspection. Three LEED Platinum properties are involved in this study. Two of them were visited by Shelby Morris and one corresponded through email. Each facility allowed staff members to answer a series of interview

<sup>\*</sup> Corresponding author: izr0004@auburn.edu

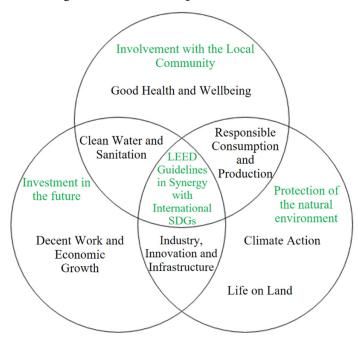
questions about the different facets of the facility and these answers were then backed up by outside sources. The hotels visited are Proximity Hotel in North Caroline, the Len Foote Hike Inn in Georgia and the W in California corresponded through email.

Since water conservation is not only important to the environment but can also save hotels money on their water bill, best practices for water saving are crucial. Some hoteliers might think that sustainability and luxury cannot be compatible as most guests expect unlimited hot water with high water pressure. However, even at an upscale property like Proximity Hotel, they were able to use water by means of efficient faucets costing around 7,000 dollars which were installed by the company Kohler to save 13,000 dollars annually on their water bill. Even with this switch they receive little to no complaints about water temperature or pressure from guests. The W took a similar approach with their own type of water efficient faucets as well as waterless urinals. In addition, they implemented a towel reuse program to lessen the amount of laundry loads thus saving water. This also expands the lifespan of the towels and linens and when they are no longer suitable for the hotel they are donated to local charities.

In terms of energy saving, all three hotels utilize energy efficient LED lighting which requires less maintenance and lasts much longer than traditional light bulbs. Proximity and Len Foote both utilized rooftop solar panels, each having approximately 100 panels in total. At Proximity, these panels are used primarily to heat water while also helping to cover around 60-80% of electricity usage throughout the property. Their goal was to save 20,000 dollars annually which they far surpassed even after installation and payoff.

Companies that have strong Corporate Social Responsibility (CSR) practices or sustainability initiatives often see positive employee responses such as higher retention, increased brand image engagement, increase employee led recruitment, higher job satisfaction and overall increased motivation (Casey and Sieber, 2016). LEED platinum certified properties align with some of the Sustainable Development Goals set out by the United Nations which fall into the three main categories of CSR (Appiah, 2019). See fig. 1. At Proximity, a lot of the employees, including the General Manager,

said that in their job search they looked for companies that aligned with their own personal values and morals, which was why they decided to work there. Other employees stated that even if it was not what led them to the job initially, it was a bonus as it allowed them to have an additional layer of pride in the work they do, especially when day to day it can seem monotonous. This is a crucial aspect of sustainable hotels because employee satisfaction leads to guest satisfaction and satisfied guests are what keep hotels in business.



**Fig. 1** The Three Aspects of Corporate Social Responsibility and How they Interconnect with the Sustainable Development Goals Used to Designate LEED Platinum Certifications

Following traveler market trends and recognizing that 95% of customers believe that the hospitality industry should be moving more towards being more sustainable is important for hotels to be successful (Brazyte, Webber and Schaffner 2017). LEED platinum properties, especially with a well-respected designation from the United States Green Building Council, will be attractive properties to those travelers more inclined to support environmentally friendly hotels. An upper-level staff member at the Proximity hotel reported that some customers said they visited specifically to support a business that aligned with their personal values. Hotels like Proximity and the W provide an option for travelers who want to stay in upscale hotels and still be environmentally conscious. While other unique properties like

the Len Foote Hike Inn provide a more traditional, stereotypical eco-tourism experience for those travelers who want to fully immerse themselves more in nature.

In conclusion, the findings of this study provide insights for and motivation for hotels to embrace environmental management throughout its core operations. By looking into the best practices of the top environmental hotels which justify heavily investing in sustainability guaranteeing a fair return on investment, it is anticipated that other practitioners will follow suit and contribute towards making the hospitality industry more sustainable.

#### **Statement of Research Advisor**

Shelby Morris has been instrumental in developing this timely research project that looks into sustainability practices of leading LEED Platinum certified hotels. Shelby visited some of these properties and interviewed their staff to gain insight into their environmental management processes. Subsequently, she analyzed and interpreted the data and wrote the first drafts of the manuscripts.

- Dr. Imran Rahman, School of Hospitality Management, College of Human Sciences

#### References

Appiah, J. Community-based corporate social responsibility activities and employee job satisfaction in the U.S. hotel industry: An explanatory study. https://www.sciencedirect.com/science/article/abs/pii/S1447677017303297. (March 2019). Vol. 38.

Booking.com (2020). Booking.com predicts the top trends for the future of travel. Retrieved April 20, 2024 from https://partner.booking.com/en-us/click-magazine/bookingcom-news/bookingcom-predicts-top-trends-future-travel

Brazytė, K., Weber, F., & Schaffner, D. Sustainability Management of Hotels: How Do Customers Respond in Online Reviews? https://doi.org/10.1080/1528008X.2016.1230033. Journal of Quality Assurance in Hospitality & Tourism, 18(3), 282–307. (2017).

Casey, D. and Sieber, S., "Employees, sustainability, and motivation: Increasing employee engagement by

addressing sustainability and corporate social responsibility." https://www.ajol.info/index.php/rhm/article/view/141703. (August 10, 2016). Vol. 6. No. 1)

CBRE (2023). Sustainability and ESG Adoption in the Hotel Industry: A Global Status Update. Retrieved September 3, 2023. https://www.cbre.com/insights/reports/sustainability-and-esg-adoption-in-the-hotel-industry

Iwanowski, K., & Rushmore, C. (1994). Introducing the Eco-Friendly Hotel: There are lots of reasons to pay attention to eco-tourism and, let's face it, the main ones have to do with money. Cornell hotel and restaurant administration quarterly, 35(1), 34-38. https://doi.org/10.1177/001088049403500104

Pryce, A. (2001). Sustainability in the hotel industry. Travel & Tourism Analyst, (6), 95-114. https://www.cabidigitallibrary.org/doi/full/10.5555/20023018331

Rahman, I., Chen, H., & Reynolds, D. (2020). Evidence of green signaling in green hotels. International Journal of Hospitality Management, 85, 102444. https://doi.org/10.1016/j.ijhm.2015.09.007

Rahman, I., Reynolds, D., & Svaren, S. (2012). How "green" are North American hotels? An exploration of low-cost adoption practices. International journal of hospitality management, 31(3), 720-727. https://doi.org/10.1016/j.ijhm.2011.09.008

Synergies Between LEED and SDGs. United States Green Building Council. https://www.usgbc.org/resources/synergies-between-leed-and-sdgs. (November 8, 2022).

#### **Authors Biography**



Shelby Morris is a senior pursuing her bachelor's degree in Hotel and Restaurant Management. She is passionate about sustainability and wants to work to make the hospitality and tourism industry more environmentally friendly while providing guests with a unique experience.



Now in his tenth year, Dr. Imran Rahman works as an Associate Professor in the Horst Schulze School of Hospitality Management at Auburn University, Auburn. Dr. Rahman's primary research interest relates to consumer behavior in hospitality and tourism with a focus on sustainability, wines, and special interest tourism. Dr. Rahman has written a major hospitality management textbook and published 42 high-impact research papers in leading journals.

# **Analyzing Data-Driven CR3BP Orbit Representations for Immersive Astrodynamics Catalogs**

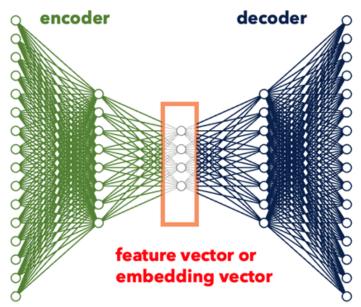
Eirik Mulder<sup>1,\*</sup> and Davide Guzzetti<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Aerospace Engineering, Auburn University <sup>2</sup> Assistant Professor, Department of Aerospace Engineering, Auburn University

Expansion of space exploration and travel into cislunar space can be very challenging due to the highly non-linear dynamics of 3-body systems. One challenge involved is the lack of orbit element sets and catalogs for orbits beyond GEO (above 35,786 km in altitude). We propose the use of a neural network based autoencoder for recognition and identification of critical features in cislunar orbits. To aid this process we utilize the Circular Restricted 3-Body Problem (CR3BP) approximation of 3-body space to allow for simpler orbit computations and analysis.

An autoencoder is a 2-sided neural network composed of an encoder and a decoder [2]. The purpose of an encoder is to reduce a complex data point (an orbit in this case) to a lower-dimensional feature vector (encoding). The decoder serves the reverse purpose, transforming this feature vector back into an approximation of the original data. For traditional data compression, the encoder and decoder will be designed by a human expert to maximize compression, however, this approach is less effective for complex or poorly understood datasets. Using neural networks for both the encoder and decoder allows for training them to encode and decode to a certain compression format. However, for training to be successful, the compression format must be specified and used as a reference for the accuracy of the neural network. An autoencoder pairs the neural network based encoder and decoder together, encoding and then decoding in sequence. This allows for training based on the ability of the autoencoder to reconstruct the original input data, meaning the autoencoder can "discover" a compression format rather than being provided one. Figure. 1 illustrates an example of a neural network based autoencoder, which uses a simple linear network for both the encoder and decoder sections.

For our purposes, we use several variants of neural network based autoencoders, and try to reduce the size of the central feature vector to maximize compression. The design shown in Figure. 1 has a central feature vector, an encoder on the left, and a decoder on the right. The structure used is a feed-forward linear neural network.



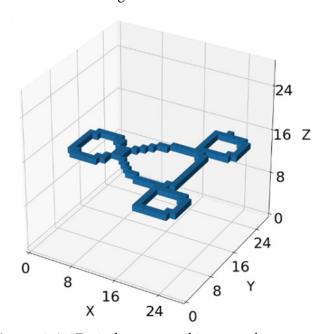
**Figure. 1** An Example Autoencoder. (Diagram created using: https://alexlenail.me/NN-SVG/)

One of the first challenges in neural network design is data representation. We consider a purely positional representation of an orbit (i.e., neglecting any corresponding differential field) and devoid of an absolute location within a global reference system. These two assumptions allow for the use of the autoencoder on orbit drawings without velocity information and at the net of the absolute location of the drawing.

We use a 3-dimensional voxel representation for orbits,

<sup>\*</sup> Corresponding author: ecm0115@auburn.edu

allowing us to use existing image-processing technology like convolutional neural networks for our problem. A voxel is a 3-dimensional unit cube, which is analogous to a pixel in a two-dimension image. Voxel-based volumetric convolutional networks have been used successfully for object identification in the past [1]. A voxel value is zero when the orbit does not pass through the voxel, and the voxel value is one when the voxel contains at least one segment of the orbit. Given a curve in 3D space, we first center the positional data to zero and normalize the position components on their largest dimension. The remaining axes are centered via margins. If position and scale information are needed for an application, the feature vector can be paired with the center and edge length of the voxel grid to add both position and scale back into the prediction. Figure 2 shows a 3:1 resonant orbit that has been converted into a 28 x 28 x 28 voxel grid.



**Figure. 2** A 3D circle converted into voxels.

For this project, we tested both linear neural network structures and a convolutional neural network. The convolutional neural network (CNN) is a common network architecture employed in image recognition tasks, where a "convolution" is swept across the image area, producing multiple feature maps [4]. This can be applied to 3D voxel problems as well.

We conducted several proof-of-concept steps during the research, starting with training neural networks on 3D circles and closed Keplerian orbits, before testing the approach on more complex orbits in the Circular Restricted 3-Body Problem. These initial steps were used to demonstrate the ability of an autoencoder to encode and decode closed curves in 3D space. Initial testing consisted of recreating circles which were randomly rotated in 3D space. After 20 epochs of training, we found the autoencoder was able to reconstruct input data very accurately, based on visual inspection, and any additional pixels were concentrated around the actual curve indicating an "understanding" of the underlying shape, which we considered a successful result.

This early testing showed that the jump from a 3-layer to a 4-layer linear network provided a noticeable jump in performance, but increasing layers beyond that had a marginal impact. Next, we tested training the autoencoders on Keplerian orbits. Specifically, we trained on closed Keplerian orbits, which take the form of ellipses.

Keplerian orbits can be defined by a set of six elements: semimajor axis a, eccentricity e, inclination i, longitude of ascending node  $\Omega$ , argument of periapsis  $\omega$ , and true anomaly v. Due to the scale-invariant nature of our voxel system, the semi-major axis (the size of the orbit) can be ignored and always assumed to be unitary. In addition, true anomaly, which provides the position of an object along the trajectory, can be ignored for our problem, as the network is designed to catalog orbit shapes.

This research ultimately aims to demonstrate the potential of using an autoencoder for extracting and cataloging features of orbits in multi-body dynamics. After demonstrating the encoding and decoding of circles in 3D space, a second stepping stone is demonstrating the technique on Keplerian orbits. Since a consistent way of identifying and classifying Keplerian orbits is already known, it can serve as a benchmark for the results generated by autoencoder architectures.

Since the Keplerian elements are already a known compression algorithms for these orbits, they provide us a good reference point for how small our potential feature vector should be. Using these as a reference, we selected a feature vector in R5.

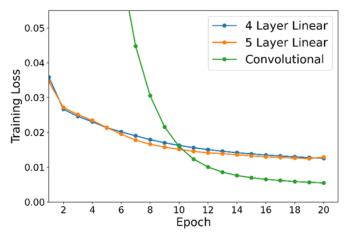
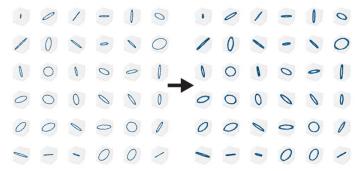


Figure. 3 Training loss on Keplerian orbits.

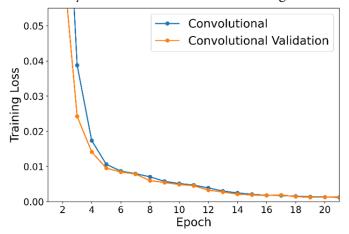


**Figure. 4** Results of the convolutional network when trained on Keplerian orbits.

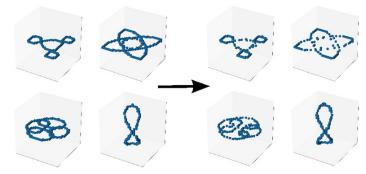
Figure 3 reports the training loss on Keplerian orbits over twenty epochs for four- and five-layer linear networks and a convolutional network, with the previously discussed architectures. The linear network we used struggles to handle the more complex feature domain of the elliptical orbits and produces subpar results. However, the convolutional network continues to perform well, and Figure 4 shows the input/output pairs for the convolutional network over 20 epochs. These results demonstrate the ability of a convolutional network to rapidly learn and recreate closed 3D paths, which is critical for work in more complex dynamical systems.

The circular restricted three-body problem (CR3BP) simplifies the general cislunar multi-body dynamics. The CR3BP formulation utilizes a rotating reference frame centered on the Earth's and Moon's barycenter and assumes both celestial bodies travel in circular paths around the barycenter. This simplification allows for the dynamical exploration of cislunar space and generation of orbits which can be converted into the full dynamical model.

We trained the convolutional autoencoder on a set of CR3BP orbits on the Jet Propulsion Lab's 3-Body Orbit Catalog [3]. The 4- and 5-layer linear networks trained on this catalog were unable to produce high quality results, so they were excluded from this testing.



**Figure. 5** Training loss over 21 epochs on CR3BP orbits.



**Figure. 6** Inputs and Outputs from a convolutional autoencoder trained on CR3BP orbits.

Figure 5 shows the training loss from training the convolutional neural network on a dataset composed of CR3BP orbits from JPL's 3-body catalog. Figure 6 shows the resulting input/output pairs and encoding vectors produced by the autoencoder after training.

From this research we learned that convolutional machine-learning models are able to recreate complex 3D orbits using voxels. We found that autoencoders show promise for labeling CR3BP orbits with small feature vectors, and believe that future research has the potential to expand on the human-readability and usability of these encodings.

#### **Statement of Research Advisor**

Eirik is developing and training auto-encoders of periodic orbits in the circular restricted three-body prob-

lem based on voxel information. Such auto-encoders will be crucial for visualizing orbit properties and designing spaceflight solutions effectively, potentially enabling more intuitive and immersive trajectory design pipelines. Eirik's research holds a significant impact, as multi-body gravitational interactions are fundamental to the orbital dynamics of unique scientific missions, human return missions to the moon, and national security applications.

- Davide Guzzetti, Department of Aerospace Engineering, Samuel Ginn College of Engineering

#### References

[1] D. Maturana and S. Scherer, "VoxNet: A 3D Convolutional Neural Network for real-time object recognition," in 2015 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS), Sep. 2015, pp. 922–928. doi: 10.1109/IROS.2015.7353481.

[2] D. Bank, N. Koenigstein, and R. Giryes, "Autoencoders," arXiv preprint arXiv:2003.05991, 2020.

[3] Jet Propulsion Lab, "Three-Body Periodic Orbits." Available: https://ssd.jpl.nasa.gov/tools/periodic\_orbits.html

[4] Z. Li, F. Liu, W. Yang, S. Peng, and J. Zhou, "A Survey of Convolutional Neural Networks: Analysis, Applications, and Prospects," IEEE Transactions on Neural Networks and Learning Systems, vol. 33, no. 12, pp. 6999–7019, Dec. 2022, doi: 10.1109/TNN-LS.2021.3084827.

#### **Authors Biography**



Eirik Mulder is a sophomore-year student pursuing concurrent bachelor's degrees in Aerospace Engineering and Computer Science. He works as a VR and astrodynamics researcher in the 3i Space Dynamics Laboratory and as the Command and Data Handling team lead for the Auburn University Small Satellite Program.



Since 2019, Dr. Davide Guzzetti is an assistant professor in the Department of Aerospace Engineering at Auburn University. Dr. Guzzetti's research focus is astrodynamics and space mission design in complex space environments. He obtained a PhD in astrodynamics from Purdue University in 2016, and he holds a Master's degree in space engineering from Politecnico di Milano, Italy. He is also an alumnus of the Italian honor society Alta Scuola Politecnica.

### **Captive Coral Breeding Methods Influence Restoration Success**

Madeleine Park<sup>1,\*</sup>, Kelly Dunning<sup>2</sup>, and Janna Willoughby<sup>3</sup>

<sup>1</sup> Undergraduate Student, Department of Forestry and Wildlife Sciences, Auburn University
 <sup>2</sup> Associate Professor, Haub School of Environment and Natural Resources, University of Wyoming
 <sup>3</sup> Assistant Professor, Department of Forestry and Wildlife Sciences, Auburn University

#### **Abstract**

ACoral reefs are some of planet Earth's most biologically diverse and important ecosystems, but coral reefs face many threats including pollution, human development, and most destructive, climate change. In this study, we investigated the relationships between differing coral captive breeding restoration techniques and their success in terms of survival once planted into wild habitats. We first extracted data from 50 scientific publications focused on coral reef restoration in the Florida Keys National Marine Sanctuary and Great Barrier Reef. We found that the average number of colonies collected and used in captive breeding was 816, while the average number of replicated fragments was 1,777. Overall, studies that used more colonies and had more fragments tended to have different techniques of out-planting and varied in environmental traits. We also found that the number of reef samples collected averaged ~3 reefs, while the average depth averaged 5.0m. Overall and across most captive breeding descriptions, corals adapted to increasing temperatures. In addition, both on-shore and off-shore nurseries as well as out-planted coral growth displayed positive results, but coral survival was dependent on specific nursery environmental qualities. Coral restoration through captive breeding adaptation to current and future climate conditions is a progressing field with extraordinary conservation potential for marine ecosystems.

Key Words: captive breeding, conservation, coral restoration, adaptation, evolution

#### Introduction

Coral reefs are the most biologically diverse ecosystem on planet Earth, rivaled only by tropical rainforests (coral reef ecosystems, n.d.). There are thousands of years of history held by these extraordinary organisms. Due to the immense diversity among corals, reef ecosystems provide for about 25% of our ocean's fish species and over 1 million aquatic vertebrates and reliant organisms (coral reef ecosystems, n.d.). Coral ecosystems include hard and soft corals, as well as sponges, crustaceans, mollusks, fish, sea turtles, sharks, dolphins, and other aquatic species. Due to the diversity amongst the coral polyps, thousands of species of coral encompass our tropical seas (NOAA, 2016b). These ecosystems are vital for the survival of these marine species and provide benefits to human communities as well (The Nature Conservancy, 2022). Coral reefs provide coastline protection from storms and erosion and over half a billion people depend on reefs for food and income (coral reef ecosystems, n.d.). Marine species depend on healthy coral reefs for locating food resources and as a place to reproduce and rear their young in the many nooks and crannies formed by corals (Global threats, 2021). Without reef ecosystems, the function of life underwater will perish.

According to the National Oceanic and Atmospheric Administration (NOAA), coral has more depth to its definition than one may assume (NOAA, 2019). Corals are marine invertebrate organisms composed of hundreds to thousands of individuals which are known as polyps. Polyps play a vital role in the coral's function as a whole; each has one singular opening with a casing of tentacles that allows them to consume small organisms for food as well as act as a defense with stinging cells on the tentacles. These thousands upon thousands of polyps work together to develop a singular functioning coral. Fringing reefs protect shorelines from storms, protect seagrass and mangrove habitats, and project seaward directly from the shore, forming borders along

<sup>\*</sup> Corresponding author: mep0114@auburn.edu

the shoreline and surrounding islands. Barrier reefs hold the most biodiversity than any other reef structure and border shorelines and are separated from their land mass by a lagoon of deep water. Atolls are circular with a central lagoon where parts of the reef platform may emerge as one or more islands, and gaps in the reef provide access to the central lagoon. Patch reefs are small, isolated reefs that grow up from the open bottom of the island platform or continental shelf. They usually occur between fringing reefs and barrier reefs. They vary greatly in size, and they rarely reach the surface of the water.

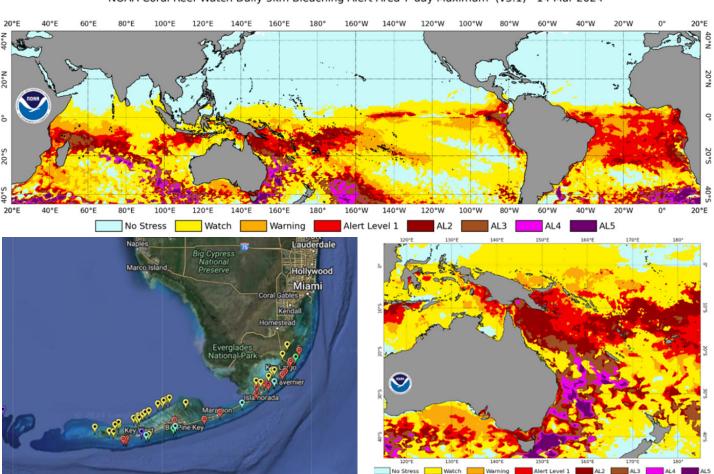
Coral Reefs occupy tropical seas surrounding the entirety of the equator and are incredibly sensitive to change in the climate including temperature, ocean acidification, toxins that may enter the water, precipitation, and so on. If these conditions are not met, corals become incredibly stressed, and diseased and will inevitably die. Whether this cause is from disease or climate change, the resulting state of these individuals is decaying and stripping of their vibrant flesh color which leaves behind the white skeleton many of us have seen on today's reefs, a prime example being Cahuita National Park in southern Costa Rica. This ecosystem is highly delicate, any shift externally can cause a catastrophic collapse in species survival. One of the most prominent and direct threats to coral reef ecosystems is climate change. Due to corals being so incredibly delicate to external shifts, rising and falling temperatures stress coral polyps, causing them to lose the algae and zooxanthellae that live within them and allow them to survive. Once the corals lose their tissue, their color is stripped from their skeleton resulting in coral bleaching. This includes changes in sea levels, changes in storm patterns, rainfall, ocean currents, and ocean acidification. According to the United Nations Environment Programme, 14% of corals have been lost due to climate change since 2009.

There is a diverse span of threats that face our planet's coral reefs today. These include physical damage and destruction, varieties of pollution, overfishing, coral harvesting, and most substantially, climate change (US EPA, 2017). Physical damage occurs via development across coastlines, dredging, fishing practices, boating, and recreational accidents or misuse. In regards to pollution, many of these toxins make their way into coastal

waters including sedimentation, nutrients, pathogens, toxic substances, and microplastics (US Department of Commerce, n.d.). Overfishing creates an imbalance in ecosystem function which has devastating impacts on the reef and the species that depend upon it. Coral harvesting involves the illegal trade of these endangered species for aquarium trade, jewelry, etc. Coral reefs are incredibly delicate species and stressors such as these stated above have led to their population decline and risk of extinction, along with the species which rely on the reef for survival. Global threats lead to warming temperatures that result in coral bleaching, rising sea levels, stronger storms, and increased ocean acidification, each having a devastating impact on these reefs (Figure 1). In fact, climate change is the most detrimental threat to coral reef ecosystems across the world which is continuing to proceed quicker than our changes (Fisheries, 2019). The Great Barrier Reef Foundation has reflected that alongside each of the threats facing reefs, climate change has led to catastrophic outbreaks that disrupt the ecosystem such as the crown-of-thorns starfish (Great Barrier Reef Foundation, 2020).

There are various programs across the globe committed to participating in coral reintroduction and restoration efforts to fight against climate change and human activity. Coral restoration can take many forms such as simple growing, gardening, and outplanting. For example, The Coral Reef Conservation Program takes part in coral mapping, monitoring, and modeling for on-the-ground and in-water restoration activities. This includes improving habitat quality, preventing the loss of corals and their habitat, enhancing coral population resilience, and improving coral health and survival (Fisheries, 2021). Similarly, the U.S. Coral Reef Task Force is partnered with the U.S. government on coral reef protection to enhance coral reef ecosystem management. Mission Iconic Reefs has restored corals throughout the Florida Keys National Marine Sanctuary and produced 30,000 branching corals; these survivors recovered from bleaching, proving that they may be more resilient to the stressors that initially caused the bleaching.

In terms of coral restoration methods, there are a variety of approaches being performed across the map (Restoration Program | Coral Restoration Foundation, 2018). For example, the Pacific Island regions collect detached



NOAA Coral Reet Watch Daily 5km Bleaching Alert Area 7-day Maximum (v3.1) 14 Mar 2024

**Figure 1.** NOAA Coral Reef Bleach Watching Map, focusing on the Great Barrier Reef region and NOAA's map of the Florida Keys National Marine Sanctuary.

coral fragments to grow in dense coral nurseries; these corals are then reattached to reefs piece by piece. Similar strategies are currently put into place along Puerto Rico's coral reef to restore the ecosystem subject to hurricane disturbance. Micro Fragmentation is a technique that has been popularly used throughout the Florida Keys. Corals can reproduce sexually through spawning and asexually via micro fragmentation (SECORE International | SECORE's Coral Restoration Approach, 2023). Corals are fragmented into finger-like pieces and often hung on coral trees underwater to propagate large numbers of corals to combat the quickening and catastrophic effects of climate change. Once these corals reach a substantial size, they are then attached to the reef, restoring more than 34,000 square feet of habitat. Australia's Great Barrier Reef has also participated in the 'coral farming approach' of nurseries as well as coral IVF. These farming trees can take many different forms, including some diverse constructs currently used in the

coral triangle.

Some organizations have taken the next step in growing and planting genetically diverse corals, which can adapt to climate change (Melbourne, 2022). Captive breeding can be used to help increase the population of a threatened species for wild release; many coral species currently reside under the Endangered Species Act. Several institutions across the globe have begun captive breeding for coral reefs. Researchers have begun using frozen sperm to fertilize live eggs to breed elkhorn; they use two elkhorn corals from different geographic locations for the goal of rescuing endangered species, making them more resilient to climate change, and restoring other coral species (Ault, 2018). Elkhorn is often chosen due to its quick-growing nature and is a very important ecological habitat, which has declined 90% in the Caribbean region. Freezing the genetic material of corals allows them to be viable

for longer to generate new specimens and strengthen smaller populations with the addition of genetic diversity (Banaszak et al.). For example, teams have created a bank in Australia's Great Barrier Reef during annual spawning.

When it comes to restoring captive breeding approaches, many issues may arise in terms of species survival. However, we do not have a complete understanding of how these methods influence survival. Here, we used a systematic review of primary literature to collect and synthesize data to fill in this research gap.

#### **Methods**

We used a systematic review of primary literature to collect our data. First, we identified fifty scientific articles focused on captive coral breeding efforts in the Florida Keys National Marine Sanctuary and Australia's Great Barrier Reef using Google Scholar and the following search terms: coral breeding, breeding coral, coral restoration, micro-fragmentation, micro-fragmenting corals, coral captive breeding, coral propagation, Florida coral restoration, coral fragmentation, sexually producing corals, heat resistant coral, coral genetics, coral breeding Florida, coral breeding great barrier reef, and coral heat-resistance Florida were used to find related journal articles.. From each paper, we extracted location of colony collection, target species, number of target colonies collected from the wild, corals surveyed, corals collected opportunistically, number of control colonies collected, number of ramets collected, number of fragments collected, number of replicates collected, number of different reefs collected from, number of coral nurseries, reefs surveyed, depth collected from, distance between parental reefs, out-planting and offshore nursery conditions, experimental and on-shore nursery conditions, asexually versus sexually bred, collection components, techniques, analysis type, and onshore versus off-shore growth. This also included the number of replicates, genets, ramets, potential parents, sequences, sexual recruits, familial crosses, spawning colonies, and duration in captivity. We also characterized the results of each article into coral systems that displayed adaptation to increasing temperatures under sexual or asexual breeding, on-shore nursery growth of asexually versus sexually bred corals, off-shore nursery growth of asexually versus sexually bred corals, and survivorship of out planted asexually versus sexually

bred corals.

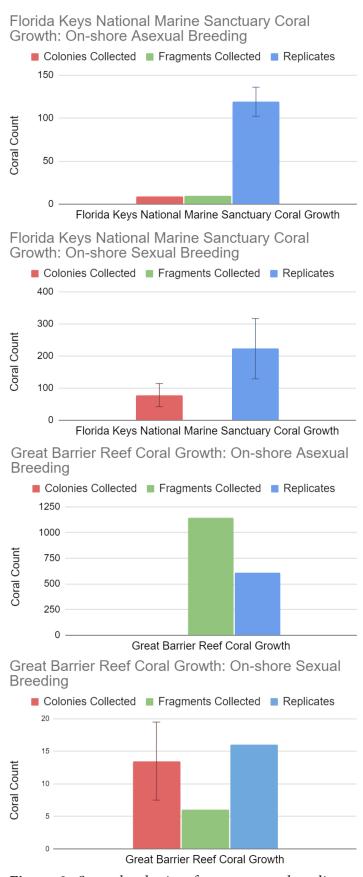
Using the data collected from the literature review, we analyzed the similarities and differences in output between Florida and the Great Barrier Reef on-shore and off-shore nursery growth. To do so, we calculated the mean number of colonies, fragments, replicates, days in captivity, and depth of collection used in each of these breeding systems. as well as species most commonly used, days in captivity, depth collection, and nursery conditions. We then compared these values across categories using a 95% confidence interval. We followed the same analysis approach for comparing out-planted corals and their captive-bred method was compared along with days in captivity, growth methods, and out-planting conditions and for the detection of corals displaying heat tolerance characteristics.

#### Results

We characterized the on-shore coral growing characteristics for Florida National Marine Sanctuaries and the Great Barrier Reef both sexually and asexually. We found that there were several differences amongst these groups that were related to growth, and that these varied between geographic areas (Figure 2).

We also characterized the off-shore coral growing characteristics for the Florida Keys and the Great Barrier Reef, and again found geographic differences between our coral captive breeding approach categories. (Figure 3).

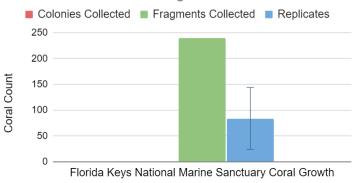
We characterized coral growing characteristics for out-planted corals both sexually and asexually and compared among categories (Figure 4). We found that there were no significant differences amongst the groups for either sexually or asexually bred corals.

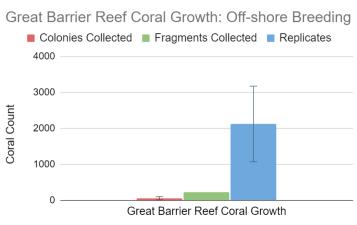


**Figure 2.** Several colonies, fragments, and replicates were collected for on-shore nursery rearing in the Florida Keys and Great Barrier Reef. Corals were asexual-

ly reared and sexually reared. Bar height indicates the mean number in each category, and several studies are shown below each bar. Error bars indicate variance around the number of colonies, fragments, and replicates used across the breeding efforts.

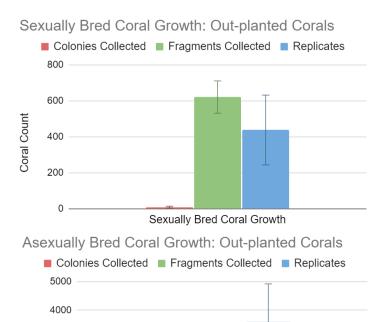
Florida Keys National Marine Sanctuary Coral Growth: Off-shore Breeding





**Figure 3.** Several colonies, fragments, and replicates were collected for off-shore nursery rearing in the Florida Keys and Great Barrier Reef. Bar height indicates the mean number in each category, and several studies are shown below each bar. Error bars indicate variance around the number of colonies, fragments, and replicates used across the breeding efforts.

We also characterized the days of captivity for on-shore reared corals, off-shore reared corals, out-planted corals, and corals displaying heat tolerance and how these values were represented in the literature. This analysis was done considering the days corals spent in captivity because this value influences adaptation potential, which can be important as corals adapt to warming temperatures in the wild environment.



**Figure 4.** Several colonies, fragments, and replicates were collected for out-planted corals grown sexually and asexually. Bar height indicates the mean number in each category, and several studies are shown below each bar. Error bars indicate variance around the number of colonies, fragments, and replicates used across the breeding efforts.

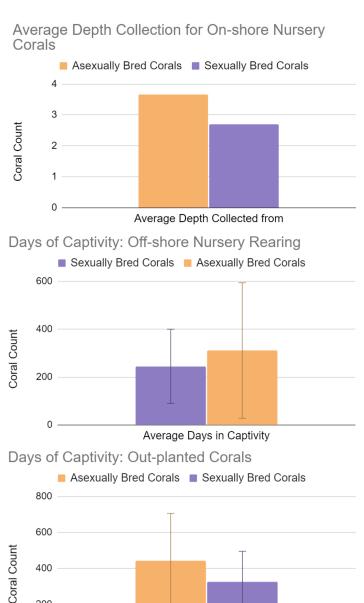
Asexually Bred Coral Growth

Coral Count

3000

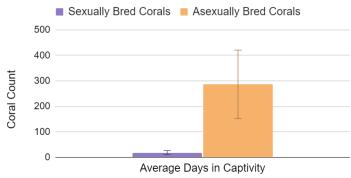
2000

1000



Average Days of Captivity: Corals Displaying Heat Tolerance

Average days in captivity

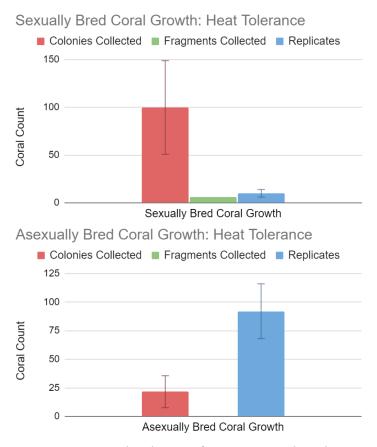


**Figure 5.** Days of captivity were collected for corals that were both asexually (orange) and sexually (purple) bred. Days collected for out-planted corals, corals

200

displaying heat tolerance, on-shore reared corals, and off-shore reared corals. Bar height indicates the mean number in each category, and error bars indicate variance around the number of colonies, fragments, and replicates used across the breeding efforts. The average collection depth for on-shore nursery corals is displayed asexually (orange) and sexually (purple.)

Finally, we characterized coral growing characteristics of corals displaying heat tolerance grown both asexually and sexually. These values were compared among heat tolerance outcomes for captively bred corals (Figure 6).



**Figure 6.** Several colonies, fragments, and replicates were collected for corals displaying heat tolerance growth asexually and sexually. Bar height indicates the mean number in each category, and several studies are shown below each bar. Error bars indicate variance around the number of colonies, fragments, and replicates used across the breeding efforts.

We found that the average number of colonies collected and used in captive breeding was 816, while the average number of replicated fragments was 1,777. Overall, studies that used more colonies and had more fragments tended to have different techniques of out-planting and varied in environmental traits. We also found that the number of reef samples collected averaged ~3 reefs, while the average depth averaged 5.0m (Figure 5). Overall and across most captive breeding descriptions, corals adapted to increasing temperatures. In addition, both on-shore and off-shore nurseries as well as out-planted coral growth displayed positive results, but coral survival was dependent on specific nursery environmental qualities.

#### **Discussion**

In terms of on-shore nursery-bred corals, a greater count of colonies was collected for sexually bred corals than asexually bred in comparison to fragments collected. There was a greater count of fragments collected for coral restoration in the Great Barrier Reef, as opposed to the Florida Keys. In terms of the Great Barrier Reef, an outstanding number of fragments were collected for asexual breeding. A substantial count of replicates were generated across all boards, especially those of the Florida Keys and the Great Barrier Reef's sexually bred corals. The greatest replicate generation came from the Great Barrier Reef's asexually bred corals. In terms of off-shore nursery-bred corals, a significantly high count of fragments were collected for the Florida Keys in comparison to colonies; neither the Florida Keys nor the Great Barrier Reef displayed very high colony collection. More replicates were produced by the Great Barrier Reef than by the Florida Keys. In terms of out-planted corals, asexually bred corals produced far greater numbers across all boards. However, sexually bred coral replicates were a product of primarily fragment collection, while asexually replicated corals were a product of both colony and fragment collection. Sexually bred corals had a greater duration in captivity for on-shore nursery rearing, while the opposite was true of off-shore nursery rearing. Both out-planted corals and corals displaying heat tolerance both displayed higher days in captivity for asexually bred corals. In terms of corals that displayed heat tolerance characteristics, there were far more replicates for those that were asexually bred than sexually bred. This data can be incredibly useful in evaluating common methods and successes in coral restoration both in the Florida Keys and the Great Barrier Reef.

#### Statement of Research Advisor

Madeleine successfully secured an undergraduate fellowship for her coral reef conservation research, and turned it into an interesting project that will have a positive impact on the Florida Keys National Marine Sanctuary.

- Dr. Kelly Dunning, Department of Forestry and Wildlife Sciences, School of Forestry. Research advisor with the College of Forestry, Wildlife, and Environment at Auburn University during the 2023-2024 Undergraduate Research Fellowship period.

#### References

Ault, Alicia. To Help Corals Fight Back, Scientists Are Breeding Populations Separated by Hundreds of Miles. Smithsonian, Smithsonian.com, 19 Dec. 2018.

Banaszak, Anastazia T., et al. Applying Coral Breeding to Reef Restoration: Best Practices, Knowledge Gaps, and Priority Actions in a Rapidly Evolving Field. Restoration Ecology, 27 Apr. 2023

Coral Triangle | Places | WWF. World Wildlife Fund, www.worldwildlife.org/places/coral-triangle#:~:text=Overfishing%2C%20destructive%20fishing%20 methods%20and.

Fisheries, NOAA. National Academies Study Offers Framework for Coral Reef Research and Recovery | NOAA Fisheries. NOAA, 29 Nov. 2021.

---. Restoring Coral Reefs | NOAA Fisheries. NOAA, 9 Dec. 2019.

Great Barrier Reef Foundation. "Threats to the Reef." Great Barrier Reef Foundation, 2020.

Melbourne, MD. Captive Breeding to Prevent Extinction. Pursuit, 7 Dec. 2022.

NOAA. Coral Reef Ecosystems | National Oceanic and Atmospheric Administration. Www.noaa.gov, NOAA, 1 Feb. 2019.

---. Coral Reefs: Essential and Threatened | National Oceanic and Atmospheric Administration. Noaa.gov, 2016.

Nordborg, M. Gene Editing Is Revealing How Corals Respond to Warming Waters. It Could Transform How We Manage Our Reefs. The Conversation, 10 Nov. 2020.

Reef Resilience Focus Area | Florida Department of Environmental Protection. Floridadep.gov, 2023.

Restoration Program | Coral Restoration Foundation." Coral Restoration Foundation | United States, 2018, www.coralrestoration.org/restoration.

SECORE International | SECORE's Coral Restoration Approach." Secore.org, 2023.

The Nature Conservancy. 8 Easy Ways You Can Help Coral Reefs. The Nature Conservancy, 2021.

NOAA. What Is Coral Spawning? Noaa.gov, 2019.

Department of Commerce, National Oceanic and Atmospheric Administration. Anthropogenic Threats to Corals - Corals: NOAA's National Ocean Service Education. Oceanservice.noaa.gov, 2017.

#### **Authors Biography**



Madeleine Park is a senior-year student pursuing a B.S. degree in Wildlife Sciences at Auburn University. She has played key research roles investigating coral restoration method success and techniques in the Florida Keys National Marine Sanctuary and Great Barrier Reef.



Kelly H. Dunning is an associate professor at the Haub School of Environment and Natural Resources at the University of Wyoming. She has many years of experience researching the conservation and governance of coral reefs around the world.

#### Aubunn4University Journal of Undergraduate Scholarship



Janna R. Willoughby is an assistant professor in the College of Forestry, Wildlife, and Environment at Auburn University at Montgomery. She has experience in the evolutionary genetics of captive populations.

# Investigation of the Tumor-Promoting Characteristics of the STK11 and MSK1 Genes in Canine Mammary Cancer

Caroline Parrish<sup>1,\*</sup>, Deepika Goyal<sup>2</sup>, R.Curtis Bird<sup>3</sup>

 <sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University
 <sup>2</sup> Graduate Student, College of Veterinary Science, Department of Pathobiology, Auburn University
 <sup>3</sup> Professor of Molecular and Cancer Genetics, Department of Pathobiology, College of Veterinary Medicine, Auburn University

MSK1 and STK11 are key regulatory genes in the signal transduction cascade controlling cell proliferation in cancer cells. The open reading frames of the genes encoding MSK1 and STK 11 expressed in mammary cancers of domestic dogs, Canis lupus familiaris, were analyzed to find highly conserved sequences for designing an rtPCR reaction that can specifically amplify the targeted region of each of the coding sequences, as well as using gel electrophoresis to verify the results. Once the successful primers were synthesized, they were utilized in an rtPCR reaction to find the targeted sequences (Figure 1).

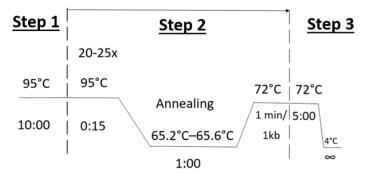
The PCR protocol that is shown is the optimized protocol (Figure 2). Optimization was based on the strongest amplification with the least apparent background. To optimize the PCR protocol for amplifying the targeted sequences, first, rtPCR primers were created by using the genomic sequences of the MSK1 and STK11 genes in Canis lupus familiaris. The canine genome was then analyzed to identify primer pairs also conserved in Homo sapiens that were within protein coding sequence and spanned an intron-exon junction.

There are various parameters and factors that went into optimizing the rtPCR reaction. The rtPCR protocol was optimized by analyzing the amount of RNA and MgSO4 added to each reaction, and then changing these amounts based on the results obtained when the bands were observed through non-denaturing agarose gel electrophoresis (Figure 3). Additionally, DMSO was added to the reaction to improve the specificity of the primers. The annealing temperatures of the primers were also adjusted accordingly by increasing incremen-

tally by 0.5° C along with cycle number to optimize amplification (Figure 2).

Primer Set	Forward Primers	Reverse Primers
STK11	5' GGTTCTCTATCCAGCAGATCAGAC 3'	3' AAGTCCTGAGTGTAGTGATGTCG 5'
	1007-1030	1200-1223
MSK1	5' ATCTATGTTGGAGAGATCGTGCTTG 3'	3' ATCCGGTGCCATGTATTCAATAGTC 5'
	690-714	869-893

**Fig. 1** Primers that will be used to amplify the sequences on each gene with the forward and reverse primers, and the location in which the primer will amplify.



**Fig. 2** The temperature curve for both STK11 and MSK1 genes for the 30 cycles for PCR.

The successful cell culturing of the canine mammary tumor cell lines that would be used to extract RNA was done through careful passage allowing the cells to go through a few passages before extracting. This allowed us to obtain a relatively high concentration and purity

<sup>\*</sup> Corresponding author: cgp0040@auburn.edu

of RNA to be used in rtPCR reactions.

The theoretical length determined from the sequence of the MSK1 gene is between 203-230 nucleotides and for STK11 is between 230-336 nucleotides in length (Figure 1). The gel electrophoresis results showed this length of nucleotides for each gene sequence (Figure 3).

STK11 MSK1

Fig. 3 Gel electrophoresis results for MSK1 and STK11 genes with 2, 5, and 10 µL RNA.

Primer Sequence Alignment

1 - 3601-359

60 18 61 118 18 18 119 178 18 18 179 238 18 18 239 298 18 18 299 18 18 360 359 A C

Fig. 4 Preliminary sequence that will be targeted by the primers created.

By creating two high quality primer pairs using the rt-PCR design protocols, we believe that we have found primers that will amplify the targeted sequence ranges that are found in canine mammary cell lines (Figure 4).

This approach has produced a highly optimized rtPCR protocol that will allow specific amplification of the target sequences encoding MSK1 and STK11 and can then be used further for gel extraction and sequencing to verify the results.

#### Statement of Research Advisor

Caroline Parrish has performed all of the research on this project with supervision from doctoral student Deepika Goyal and her research Supervisor Dr. Bird. She has, in addition written all of the report of this research which was reviewed by Dr. Bird.

- Dr. R. Curtis Bird, Professor of Molecular and Cancer Genetics in the Department of Pathobiology, College of Veterinary Medicine

Target Gene Sequence Alignment

#### References

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 2012 Jun 18;13:134. doi: 10.1186/1471-2105-13-134. PMID: 22708584; PMCID: PMC3412702.

Madeira F, Pearce M, Tivey ARN, et al. Search and sequence analysis tools services from EMBL-EBI in 2022. Nucleic Acids Research. 2022 Apr:gkac240. DOI: 10.1093/nar/gkac240. PMID: 35412617; PMCID: PMC9252731.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990 Oct 5;215(3):403-10. doi: 10.1016/S0022-2836(05)80360-2. PMID: 2231712.

https://en.vectorbuilder.com/tool/sequence-alignment.html

RPS6KA5 ribosomal protein S6 kinase A5 [Homo sapiens (human)] Gene ID: 9252, updated on 10-Mar-2024. NIH, National Library of Medicine.

RPS6KA5 ribosomal protein S6 kinase A5 [Canis lupus familiaris (dog)] Gene ID: 490831, updated on 5-Mar-2024. NIH, National Library of Medicine.

STK11 serine/threonine kinase 11 [ Homo sapiens (human)]. Gene ID: 6794, updated on 16-Apr-2024. NIH, National Library of Medicine.

STK11 serine/threonine kinase 11 [Canis lupus familiaris (dog)]. Gene ID: 485088, updated on 5-Mar-2024. NIH, National Library of Medicine.

#### **Authors Biography**



Caroline Parrish is a junior majoring in Genetics and minoring in Medieval and Renaissance/Early Modern Studies. She is a 2023-2024 Auburn University Undergraduate Research Fellow. Her research under Dr. Richard Curtis Bird looks at identifying the tumor promoting characteristics of two genes, MSK1 and STK11, in canine mammary cancer. After graduation, she hopes to attend graduate school to obtain a Ph.D. in cancer biology.



Deepika Goyal is a Ph.D. candidate in Cancer Biology at Auburn University's Department of Pathobiology, Biomedical Sciences. Her research focuses on identifying microRNAs for both diagnostic and therapeutic applications in canine mammary tumors.



Dr. R. Curtis Bird is Professor of Molecular and Cancer Genetics in the Department of Pathobiology, College of Veterinary Medicine, and Director of the University Core Flow Cytometry and High-Speed Cell Sorting Laboratory. He earned his Ph.D. from the University of Toronto in Molecular and Cellular Biology in 1982 and trained as a Medical Research Council Post-Doctoral Fellow at Memorial University and the University of Guelph before joining the faculty at Auburn University in 1985.

### A Multimodal Study of Vocal Function and Upper Airway Temperature in Individuals with Asthma Versus Controls

Annie L. Pauley<sup>1,\*</sup> and Mary J. Sandage, PhD, CCC-SLP<sup>2</sup>

<sup>1</sup> Undergraduate Student, Department of Speech, Language, & Hearing Sciences, Auburn University <sup>2</sup> Lanier Endowed Professor, Department of Speech, Language, & Hearing Sciences, Auburn University

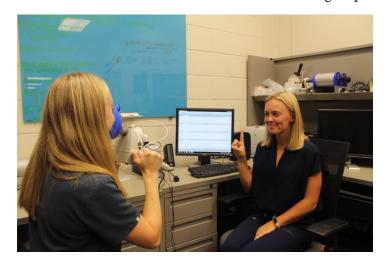
Chronic inhaler use for asthma and its effects on vocal physiology and function are not well understood. Medication taken for asthma is known to have a negative effect on vocal function [1]. This preliminary study aimed to understand upper airway temperature and vocal function differences in individuals with asthma versus healthy controls.

Using a prospective between group design, 3 participants with asthma and 3 controls were recruited. Inclusion criteria: nonsmokers or not pregnant; no breastfeeding prior six months; no diagnosis of other respiratory disease, reflux, diabetes, neurological disease, or hormonal imbalance; take a maintenance inhaler daily (asthma group); and no drying medications (other than an inhaled corticosteroid). Following consent, posterior pharyngeal wall temperature was collected after 20 minutes of equilibration to lab environment with an infrared thermometer. Videolaryngostroboscopic imaging, acoustic, aerodynamic, and perceptual measures were collected as seen in Figure 1.

Average upper airway temperature (UAT) for asthma volunteers pre-trial was 34.6°C and post-trial 35.28°C. Average UAT for control volunteers pre-trial was 34 °C and post-trial average was 35.2°C. Upper airway temperature change (ΔUAT) increased by an average of 0.68°C in the volunteers with asthma and an average of 0.53°C in controls. Average phonation threshold pressure (PTP) for asthma volunteers was 7.79 cm H\_2 O. Average PTP for controls was 5.73 cm H\_2 O. The average Voice Handicap Index (VHI) score for the asthma group was 22.67. Average VHI for controls was 3.67. The average OMNI-VES score for asthma volunteers was 3 out of 10, and the controls' was 0 out of 10. The

average Rate of Fatigue (ROF) Scale score in asthma volunteers was 2.33, and the average for controls was 0.33

UAT average, range, and differences pre- and post-trial were essentially the same in both groups. PTP values were higher in the asthma group by 2.06 cm H\_2 O, which is consistent with clinical observations of more vocal effort reported secondary to voice use with asthma and from inhaler use. Average perceptual measures from the VHI were 19 points higher in participants with asthma than those who were in the control group.



**Fig. 1** Annie Pauley collecting phonation threshold pressure, an aerodynamic measure of minimal lung pressure required to produce voice.

Since this is a preliminary study, data collection is still ongoing and will be described further in future published work.

<sup>\*</sup> Corresponding author: alp0112@auburn.edu

#### **Statement of Research Advisor**

Annie Pauley conducted a complex, multimodality research protocol with volunteers. To date, she has gathered all of the data for the control population and plans to wrap up data collection for the rest of the asthma group as part of her masters thesis project.

- Mary J. Sandage, PhD, Department of Speech, Language, & Hearing Sciences, College of Liberal Arts

#### References

[1] Sandage, et al. "Passive upper airway thermoregulation and high-speed assessment for conventional versus menthol cigarette: implications for laryngeal physiology." Journal of Voice, Jan. 2020; 34(1): 25-32

#### **Authors Biography**



Annie L. Pauley is a senior-year student pursuing a B.S. degree in Speech, Language, and Hearing Sciences (SLHS) at Auburn University. She has played key research roles in recruiting participants, collecting data, and presenting research at conferences and symposiums. She will attend graduate school in the Department of Speech Language & Hearing Science in Fall 2024.



Mary Sandage, PhD, CCC-SLP, F-ASHA, is a Lanier Endowed Professor in the Department of Speech, Language, & Hearing Sciences. Her clinical and research expertise merges the disciplines of exercise science and upper airway/laryngeal physiology.

### Distress Tolerance, Anxiety Sensitivity, and the Use of Cannabis as a Coping Mechanism

Madeleine E. Rein<sup>1,\*</sup>, Mallory J. Cannon, B.A.<sup>2</sup>, Julia Y. Gorday, M.S.<sup>3</sup>, Richard Macatee Ph.D.<sup>4</sup>

Rates of cannabis use and Cannabis Use Disorder (CUD) have continued to grow in the United States as the legalization of cannabis has increased (Hasin et al., 2016; Compton et al., 2019). CUD has been linked to poorer quality of life and worse health outcomes, making it important to identify who may be at increased risk for developing this condition (Connor et al., 2021; Gutkind et al., 2021). One potential risk factor of CUD is distress tolerance (DT), or one's ability to tolerate negative affective states. Those with low DT have evidenced increased rates of cannabis use, cravings, use-related problems, and dependence, all of which are associated with CUD (Peraza et al., 2019; Buckner et al., 2020). Increased cannabis use in those with low DT may be a way to regulate negative internal experiences (e.g., anxiety). For individuals with low DT, increased cannabis use has been identified as a mechanism to regulate negative internal experiences and cope with sleep- related problems found in individuals with anxiety and post-traumatic stress disorders (Bonn-Miller et al., 2010; Buckner et al., 2020; Potter et al., 2011). While initial use may aid the individual in coping with distress in the short term, the repeated use of cannabis as a coping strategy may increase the individual's risk for CUD in the long term, resulting in increased distress levels and functional impairment (Potter et al., 2011). The current study aims to explore the relationship between DT, cannabis-use related problems, coping motives, and anxiety symptom severity.

We hypothesized that the strength of the positive relationship between distress intolerance (DI) and greater cannabis use-related problems would increase as reports of cannabis use for coping increased. Secondly, we hypothesized that DI would moderate the relationship between anxious arousal and the use of cannabis for coping such that the strength of the positive relationship between anxious arousal and coping motivated cannabis use would increase as DI increased.

PARTICIPANT DEMOGRAPHICS		
N	163	
Age- M (SD)	21. 5 ( <i>2.55</i> )	
Sex- N (%)	Male- 68 (41.5%)	
Race- N (%)	White- 135 (82.3%)	

Fig. 1 Participant Demographics

Data was collected from a sample of English-speaking, young weekly cannabis users aged 18-30 (N=163; uses cannabis at least 2 days a week for the past year; Figure 1). Participants completed a battery of self-report measures. The Distress Intolerance Index (DII) is a 10-item self-report measure that assesses one's ability to experience or withstand negative emotional, physical, or psychological states (McHugh & Otto, 2012). The Marijuana Problems Scale (MPS) is a 19-item self-report that assesses the impact of marijuana use on several areas of the participant's life (e.g., interpersonal problems, financial troubles; Stephens et al., 1994). The Marijuana Motives Measure (MMM) is a 25-item self-report measure that assesses several different marijuana use motives (Simons et al., 1998). We used the 4-question Coping subscale of this measure. Finally, the Mood and Anxiety Symptom Questionnaire (MASQ-D) is a 30item questionnaire that assesses for general anxiety and depressive symptoms (Clark & Watson, 1991). We used the Anxious Arousal subscale of the measure.

<sup>&</sup>lt;sup>1</sup>Undergraduate Student, Department of Psychological Sciences, Auburn University

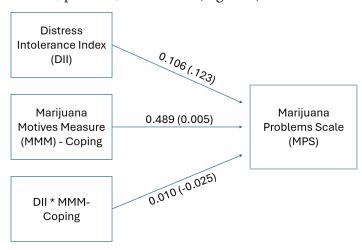
<sup>&</sup>lt;sup>2</sup> Graduate Student, Department of Psychological Sciences, Auburn University

<sup>&</sup>lt;sup>3</sup> Doctoral Candidate, Department of Psychological Sciences, Auburn University

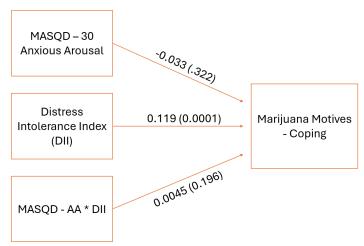
<sup>&</sup>lt;sup>4</sup> Associate Professor, Department of Psychological Sciences, Auburn University

<sup>\*</sup> Corresponding author: mer0084@auburn.edu

Linear regression was conducted to evaluate the relationship between DI and increased cannabis-use related problems with coping motives as a moderator. The overall model was significant, F(3, 159) = 5.068, p = .002, R2 = 0.873, but the interaction was not significant, F(1, 159) = .322, p = .571,  $\Delta R2 = .002$  (Figure 2). Linear regression was conducted to evaluate the relationship between increased anxious arousal and coping cannabis use motives with DI as a moderator. The overall model was significant, F(3, 160) = 6.571, p = .0003, R2 = .110, but the interaction was not significant, F(1, 160) = 1.690, p = .196,  $\Delta R2 = .009$  (Figure 3).



**Fig. 2** Interaction Term Model between DII, MMM, and MPS with beta weights (f) and p values, p < .05



**Fig. 3** Interaction Term Model between MASQ-D, DII, and MMM with beta weights ( $\mathfrak{G}$ ) and p values, p < .05

Results indicate that coping motives do not moderate the relationship between DI and cannabis-use related problems. Additionally, DI does not moderate the relationship between greater reported anxious arousal and cannabis use for coping motives, contrary to Potter et al. (2011).

There are several potential explanations for why our analyses were not statistically significant. Firstly, the MMM may not be the best measure for this study as it may exclude self-report data from individuals with lower levels of insight into the motives of their cannabis use. Additionally, the MMM may not effectively assess the relationship between respondents' experienced anxious arousal and their use. For example, MMM Item #17, "I use marijuana to...forget about my problems" does not specifically measure how much these problems are negatively impacting the respondent. It may be beneficial for future research to investigate the relationship between anxiety-related cannabis cravings on coping motivated use (Farrelly et al., 2022). Further research on the relationship between DI, anxiety, cannabis use for coping motives, and CUD is needed to inform and develop individualized treatment modalities for individuals with comorbid CUD and anxious disorders.

This study is not without limitations. Within the sample, White young adults were overrepresented, which does not provide a representative sample of the Southeast region of the United States; Further research should focus on recruiting a generalizable sample. Finally, this research is correlational and cross-sectional in nature; longitudinal study designs are needed to understand temporal relationships among study constructs.

#### **Statement of Research Advisor**

For the past year, Madeleine has worked in the BRAINS Lab as a lead undergraduate research assistant. Madeleine led the development of the present project by identifying variables of interest, reviewing the existing literature, and crafting her research question. Under the supervision of graduate students, Mallory Cannon and Julia Y. Gorday, Madeleine has gained experience in conducting and interpreting statistical analyses. Recently, Madeleine has presented this data at the Auburn University Student Symposium (April 2024).

- Richard Macatee, Department of Psychological Sciences, College of Liberal Arts

#### References

Bonn-Miller, M. O., Babson, K. A., Vujanovic, A. A.,

& Feldner, M. T. (2010). Sleep problems and PTSD symptoms interact to predict marijuana use coping motives, Journal of Dual Diagnosis, 6, 111–122. doi:10.1080/15504261003751887

Buckner, J. D., Walukevich, K. A., & Zvolensky, M. J. (2019). Distress tolerance and cannabis craving: The impact of laboratory-induced distress. Experimental and Clinical Psychopharmacology, 27(1), 38–44. https://doi.org/10.1037/pha0000231

Clark, L. A., & Watson, D. (1991). Tripartite model of anxiety and depression: Psychometric evidence and taxonomic implication. Journal of Abnormal Psychology, 100(3), 316–336.

Connor, J. P., Stjepanović, D., Le Foll, B., Hoch, E., Budney, A. J., & Hall, W. D. (2021). Cannabis use and cannabis use disorder. Nature Reviews. Disease Primers, 7(1), 16. https://doi.org/10.1038/s41572-021-00247-4

Farrelly, K. N., Romero-Sanchiz, P., Mahu, T., Barrett, S. P., Collins, P., Rasic, D., & Stewart, S. H. (2022). Post-traumatic stress disorder symptoms and coping motives are independently associated with cannabis craving elicited by trauma cues. Journal of Traumatic Stress, 35(1), 178–185. https://doi.org/10.1002/jts.22715

Gutkind, S., Fink, D. S., Shmulewitz, D., Stohl, M., & Hasin, D. (2021). Psychosocial and health problems associated with alcohol use disorder and cannabis use disorder in U.S. adults. Drug and Alcohol Dependence, 229, 109-137. https://doi.org/10.1016/j.drugalcdep.2021.10913

Hasin, D. S., Kerridge, B. T., Saha, T. D., Huang, B., Pickering, R., Smith, S. M., Jung, J., Zhang, H., & Grant, B. F. (2016). Prevalence and correlates of DSM-5 cannabis use disorder, 2012-2013: Findings from the National Epidemiologic Survey on Alcohol and Related Conditions–III. American Journal of Psychiatry, 173(6), 588–599. https://doi.org/10.1176/appi.ajp.2015.15070907

McHugh, R. K., & Otto, M. W. (2012). Refining the measurement of distress intolerance. Behavior Therapy, 43(3), 641–651. https://doi.org/10.1016/j. beth.2011.12.001

Peraza, N., Smit, T., Garey, L., Manning, K., Buckner, J. D., & Zvolensky, M. J. (2019). Distress tolerance and cessation-related cannabis processes: The role of cannabis use coping motives. Addictive Behaviors, 90, 164–170. https://doi.org/10.1016/j.addbeh.2018.10.047

Potter, C. M., Vujanovic, A. A., Marshall-Berenz, E. C., Bernstein, A., & Bonn-Miller, M. O. (2011). Posttraumatic stress and marijuana use coping motives: The mediating role of distress tolerance. Journal of Anxiety Disorders, 25(3), 437–443. https://doi.org/10.1016/j.janxdis.2010.11.007

Simons, J., Correia, C. J., Carey, K., & Borsari, B. (1998). Validating a five-factor marijuana motives measure: Relations with use, problems, and alcohol motives. Journal of Counseling Psychology, 45(3), 265–273.

Stephens, R. S., Roffman, R. A., & Simpson, E. E. (1994). Treating adult marijuana dependence: A test of the relapse prevention model. Journal of Consulting and Clinical Psychology, 62(1), 92–99. https://doi.org/10.1037/0022-006X.62.1.92

#### **Authors Biography**



Madeleine E. Rein is a senior-year student graduating summa cum laude in May 2024 with a B.A. in Psychology from Auburn University. She was awarded a Research Fellowship for the 2023-2024 school year. Her research interests include how mental health issues such as posttraumatic stress and suicidal thoughts and behaviors are evaluated and treated in a forensic setting and the impact these factors have on competency.

#### Aubunn3University Journal of Undergraduate Scholarship



Mallory J. Cannon, B.A. is a second-year graduate student in the Department of Psychological Sciences at Auburn University. Mallory's research interests are focused on studying how higher-level processes, such as emotional regulation and decision-making, relate to anxiety and fear-related disorders and problematic substance use - and how delineating these processes may help us implement strategies to improve therapeutic techniques and interventions.



Julia Y. Gorday, M.S. is a doctoral candidate in the Department of Psychological Sciences at Auburn University. She received a M.S degree in Clinical Psychology at Auburn University. Julia's research interests are focused on cognitive factors that impact the etiology and maintenance of anxiety and fear-related disorders and problematic cannabis use (e.g., metacognition, information processing biases).



Dr. Macatee is the director of the BRAINS Lab and an aassociate professor in the clinical psychology department at Auburn University. Dr. Macatee earned his Ph.D. in clinical psychology from Florida State University in 2018 after he completed a pre-doctoral clinical internship at the Department of Psychiatry at University of Illinois - Chicago. At the BRAINS Lab, Dr. Macatee focuses on the multi-method measurement and treatment of biobehavioral risk and maintenance factors for internalizing and substance use disorders, with a particular emphasis on co-morbid presentations. and substance use disorders, with a particular emphasis on co-morbid presentations.

## Localizing the Movement Protein (NSm) of Tomato Spotted Wilt Virus in Nicotiana Benthamiana

Alexandra C. Rios<sup>1,\*</sup>, Abdelaal H.A. Shehata<sup>2</sup>, Amanda Strayer-Scherer<sup>3</sup>, Kathleen M. Martin<sup>3</sup>

Undergraduate Student, Department of Entomology and Plant Pathology, Auburn University
 Graduate Student, Department of Entomology and Plant Pathology, Auburn University
 Assistant Professor, Department of Entomology and Plant Pathology, Auburn University

#### Introduction

Tomato Spotted Wilt Virus (TSWV) is an ambisense RNA- containing virus of the genus, Orthotospovirus [1], and a member of the 30K superfamily [2]. TSWV was discovered in Australia in 1915 and is now one of the most economically devastating viruses in the world [1]. It was first found in the U.S. in Texas in 1971 and has since spread throughout the southeastern region, causing several severe outbreaks in one of its many hosts, peanuts [3]. Despite the introduction of resistant field strains, TSWV remains a threat to Alabama peanuts. TSWV's viral genome consists of three single stranded RNAs, referred to as the genomic large (L), medium (M), and small (S) RNA, that are all contained within a spherical membrane [4].

The protein of interest in this project is the non-structural movement protein (NSm), which is located on the M segment. NSm functions in the construction of tubular structures within the plasmodesmata of the plant cell, allowing for cell-to-cell movement of the ribonucleoprotein [5]. The NSm protein of a well-characterized strain, TSWV-MT2, has been shown to localize in the plasmodesmata of plant cells at 2 days post infiltration (dpi) and is fully aggregated in the cell periphery by 4 dpi [6]. Our hypothesis, based on mutation mapping from a protein alignment with Alabama isolates and MT2, is that localization patterns would be unchanged from MT2. Therefore, the objective of this study to identify natural variations in the NSm open reading frame and determine how these mutations impact protein localization.

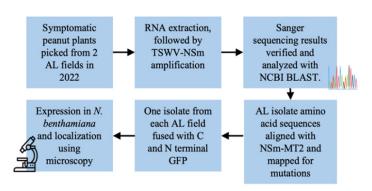
#### **Materials and Methods**

Symptomatic peanut plants originating from two field

locations in Alabama, including Brewton Agricultural Research Center (BARU) and Wiregrass Research and Extension Center (WGREC) were collected in 2022. RNA extraction of the leaves was performed, and primers specific for NSm proteins were used for amplification. The sequences of NSm isolates were verified and analyzed using NCBI BLAST [7], and one isolate from each location was translated and aligned against seventeen other closely related TSWV-NSm sequences from Florida, Georgia, North Carolina, South Carolina, and Virginia (KU179544.1, KU179542.1, KU179550.1, KU179546.1, KU179592.1, KU179516.1, KU179598.1, AY744487.1, AY744489.1, AY744488.1, KU179528.1, OP832374.1, AY744490.1, KU179600.1, KU179616.1, AY870390.1, and KU179524.1) and NSm-MT2 to map their mutations [8]. The sequence of NSm-MT2 matches the TSWV-NSm-7-1 (AY956380.1) exactly so it was used when creating alignments. Each isolate was fused to either the C or N terminal of green fluorescent protein (GFP) according to Chakrabarty et.al. [9]. Vectors were then transformed into Agrobacterium tumefaciens and expressed in wild-type Nicotiana benthamiana leaves, as described by Martin and Whitfield [10].

Infiltrated leaves were stained with aniline blue fluorochrome to highlight the plasmodesmata according to Widana Gamage and Dietzen [11], except the concentration was increased by a factor of 10. Images were taken under a Nikon Eclipse Ts2R microscope at 2, 3, and 4 dpi at 40X magnification with NIS-elements viewer. Images of at least three plant cells were taken for each of three replicates, and subsequent figures were constructed using Microsoft PowerPoint. A summary of the methods is described in Figure 1.

<sup>\*</sup> Corresponding author: acr0117@auburn.edu



**Fig. 1** A map of the procedure described in the methods section.

#### Results

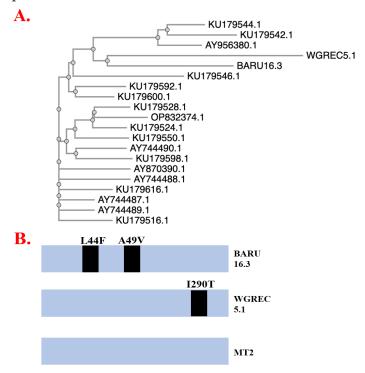
The phylogenetic tree in Figure 2A demonstrated that BARU 16.3 and WGREC 5.1 are most closely related to each other within their own clade. The tree also revealed that the two AL isolates are more closely related to isolates originating from Florida (KU179544.1, KU179542.1, KU179546.1). The two AL isolates had variable relatedness with isolates from North Carolina. South Carolina, Georgia, and Virginia. Furthermore, the alignment in Figure 2B involving the two TSWV-NSm AL isolates and TSWV-NSm-MT2 as a reference revealed no conserved mutations. However, each isolate did have unique point mutations. BARU 16.3 had two amino acid mutations toward its N terminus, while WGREC 5.1 had a point mutation toward its C terminus. Because there were no conserved mutations, it was then hypothesized that localization patterns would be unchanged between AL isolates and MT2.

While TSWV-NSm-MT2 localized in the plant cell as expected, the results of localization proved different than the original hypothesis for the AL NSm isolates. NSm isolates fused to the C terminus of GFP, represented by GFP-NSm-BARU16.3 in Figure 3, aggregated in the plant cell at 2 dpi, whereas the NSm isolates fused to the N terminus of GFP, represented by NSm WGREC5.1-GFP in Figure 3, aggregated in the plant cell at 4 dpi, like MT2.

#### Discussion

Because there were no conserved mutations, it was hypothesized that the AL isolates would have unchanged localization patterns when compared to MT2. However, the results of localization microscopy did not affirm the original hypothesis. The mutations in BARU 16.3

do occur in a domain that is required for tubule formation in protoplasts and cell-to-cell movement (5). However, this does not explain why the same pattern would be observed in both AL isolates. Due to the contrasting results of NSm isolates fused to the N and C terminuses of GFP, it is possible that the orientation of the GFP may impact aggregation in the plant cell. GFP is a 238 amino acid protein and therefore may sterically hinder sites that impact posttranslational modifications [12]. Previous data indicates that mutations in domains close to both the N and C terminals, specifically amino acids 19 to 159 and 209 to 283, alter NSm movement from cell-to-cell [5], and it is possible that disrupted function of sites at either terminus may impact tubule formation and NSm distribution [5]. However, more investigation is required to determine whether these differences are due to GFP orientation or due to the protein itself.



**Fig. 2** 2A is a phylogenetic tree of the two chosen NSm isolates from AL and NSm-MT2, as well as seventeen other closely related strains of TSWV-NSm. 2B is a map of non-conserved amino acid mutations in the two AL NSm isolates chosen for localization when compared to NSm-MT2.

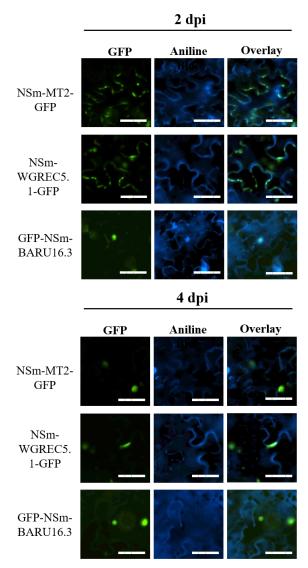


Fig. 3 Display of the images taken at 2 and 4 dpi. From top to bottom, the rows are NSm-MT2-GFP, NSm-WGREC5.1-GFP, and GFP-NSm-BARU16.3. Each day, from left to right, has images of GFP, aniline blue, and an overlay. The scale bar is  $100~\mu m$ .

#### **Statement of Research Advisor**

Alex conducted all the experiments in this work demonstrating her excellence in both cloning and microscopy to answer molecular questions. She was able to determine that although these natural mutations were occurring in the field, these did not impact how the protein localized. This was new information as we work towards understanding which mutations Tomato spotted wilt virus NSm has in the field and how they impact the infection cycle. This represents a critical first step in the determination of why symptoms are increasing in the field over time.

- Kathleen Martin, Department of Entomology and Plant

Pathology, College of Agriculture

#### References

- [1] Goldbach, R., Peters D., "Possible causes of the emergence of tospovirus diseases," Seminars in Virology, 5(2), pp. 113-120,(1994).
- [2] Melcher, U., "The '30K' superfamily of viral movement proteins," The Journal of general virology, 81(Pt 1), pp. 257–266,(2000).
- [3] Strayer-Scherer, A., Graham, S., Tomato Spotted Wilt Virus on Peanuts Alabama Cooperative Extension System. Retrieved April 17, 2024, from https://www.aces.edu/blog/topics/crop-production/tomato-spotted-wilt-virus-on-peanuts/, (August 11, 2021).
- [4] Pappu, H.R., "Tomato Spotted Wilt Virus," In B. W.J. Mahy and M. H.V. Van Regenmortel, Eds., Encyclopedia of Virology, (Third Edition), University of California: Elsevier Science, pp. 133-138, (2008).
- [5] Li, W., Lewandowski, D. J., Hilf, M. E., Adkins, S., "Identification of domains of the Tomato spotted wilt virus NSm protein involved in tubule formation, movement and symptomatology," Virology, 390(1), pp. 110-121,(2009)
- [6] Martin, K.M, Chen, Y., Mayfield, M.A., Montero-Astúa, M., Whitfield, A.E., "Visualizing tomato spotted wilt virus protein localization: Cross-kingdom comparisons of protein-protein interactions," bioRxiv,(2023).
- [7] Altschul, S. F., Gish, W., Miller, W., Myers, E. W., Lipman, D. J., "Basic local alignment search tool," Journal of molecular biology, 215(3), pp. 403–410,(1990).
- [8] Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., Higgins, D. G., "Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega," Molecular systems biology, 7(539),(2011).
- [9] Chakrabarty, R., Banerjee, R., Chung, S. M., Farman, M., Citovsky, V., Hogenhout, S. A., Tzfira, T., Goodin, M., "PSITE vectors for stable integration or

transient expression of autofluorescent protein fusions in plants: probing Nicotiana benthamiana-virus interactions. Molecular plant-microbe interactions," MPMI, 20(7), pp. 740–750,(2007).

[10] Martin, K.M. and Whitfield, A.E., "Cellular localization and interactions of nucleorhabdovirus proteins are conserved between insect and plant cells," Virology, 523, pp. 6-14,(2018).

[11] Widana Gamage, S. M. K., Dietzgen R.G., "Intracellular Localization, Interactions and Functions of Capsicum Chlorosis Virus Proteins," Frontiers in Microbiology, 8,(2017).

[12] Uckert, W., Pedersen, L., Günzburg, W., "Green fluorescent protein retroviral vector: generation of high-titer producer cells and virus supernatant," Methods in molecular medicine, 35, pp. 275–285,(2000).





Alexandra C. Rios is a senior-year student pursuing a B.S. degree in Biomedical Sciences at Auburn University. She has been working in the Martin lab since Fall '22 and has since developed an interest in pathology and virology. After graduation, she plans to expand her experience working with plant pathogens into working in human medicine and pathology.



Abdelaal H.A. Shehata is a graduate student in the Department of Entomology and Plant Pathology at Auburn University.



Dr. Kathleen Martin is an Assistant Professor in the Department of Entomology and Plant Pathology. She started her lab in vector entomology at Auburn in 2019. She works on the molecular aspects of insect transmission of plant viruses in the field. Her work focuses on Cotton leafroll dwarf virus, Soybean vein necrosis virus and Tomato spotted wilt virus. She started working on plant viruses during her Master's program at the University of Arizona and continued to work on viruses that also infect their insect hosts/vectors at the University of Kentucky where she completed her PhD in 2011.



Dr. Strayer-Scherer is an Assistant Professor and Extension Plant Pathologist in the Department of Entomology and Plant Pathology. She joined Auburn University in 2019. The goal of her extension program is to provide research-based integrated pest management (IPM) through extension programing to help stakeholders control diseases in cotton, peanuts, smalls grains and forages, ornamentals, and turfgrass production systems. Her research program largely focuses on the areas of integrated pest management and diagnostics of economically important diseases of cotton and peanut.

### Isolating the Effects of Individual Nest Characteristics on Offspring Phenotypes of Brown Anole Lizards

John M. Rodgers<sup>1,\*</sup>, M. Christopher Norris<sup>2</sup>, and Daniel A. Warner<sup>3</sup>

- <sup>1</sup> Undergraduate Student, Department of Biological Science, Auburn University
  - <sup>2</sup> Graduate Student, Department of Biological Science, Auburn University
  - <sup>3</sup> Associate Professor, Department of Biological Science, Auburn University

#### **Abstract**

The influence of the external environment on offspring phenotypes and survival is well described for a variety of oviparous (i.e., egg-laying) species. Much of this previous work has focused on replicating natural incubation environments in the laboratory, based on the characteristics of maternally chosen nest sites in the wild. Although several environmental factors affect offspring phenotypes (e.g., substrate type, soil moisture, temperature), most studies do not assess the relative contribution of each factor to variation in offspring phenotype. To understand how multiple nest characteristics interact to affect offspring phenotypic variation, we studied the brown anole lizard (Anolis sagrei) and evaluated three major factors that typically vary among their nest sites: substrate type, incubation temperature, and soil moisture. We show that relatively moist soil increases egg mass during development at a greater rate than other treatments. Egg incubation temperature strongly affected the developmental rate of embryos, and moisture explained most of the variation in hatchling body size. Substrate had no effect on any phenotype. We found no interactive effect among incubation substrate, moisture, or temperature on variation in hatchling morphology.

#### Introduction

Developmental plasticity is a phenomenon where a single genotype can express multiple phenotypes depending on their developmental environment (West-Eberhard 2003). Most organisms exhibit sensitivity to their developmental environment; therefore, conditions experienced during embryonic development can have large effects on offspring phenotypes. Oviparous organisms with little to no parental care are great study organisms for developmental plasticity because their

Developmental plasticity has been documented in a wide range of taxa, but many studies that examine developmental plasticity do not use ecologically relevant environmental conditions. Understanding the influence of natural conditions on the phenotype of offspring can provide insight into the variation within natural populations and how natural selection acts on that variation. Reptiles are commonly used in experiments characterizing phenotypic variation across differing temperatures (While et al. 2018). However, these studies rarely consider the effects of other nesting variables and how they interact. Natural variation in one environmental variable can have an impact on the state of another environmental variable, so understanding how these natural conditions interact is critically important. For example, both temperature and moisture conditions can affect the phenotypes of offspring (Packard & Packard 1988; Deeming 2004; Warner et al. 2012), and these two variables can potentially influence each other. Decoupling the effects of multiple variables is important for understanding the relative contributions of each variable on phenotypic development. Our goal is to take ecologically relevant nest environments and tease apart certain environmental factors (i.e., temperature, moisture, substrate) to quantify the individual effects of each variable and their interactions.

eggs are subject to their surrounding environment for the majority of their development without any buffering due to parental care (Noble et al. 2018). Reptiles are particularly well suited for studies of developmental plasticity since eggs that are left unattended within nests are exposed to a wide range of environmental variations, including variations in temperature, moisture, and substrate type (Warner et al. 2018).

<sup>\*</sup> Corresponding author: jmr0125@auburn.edu

#### Methods

To address our goals, we used the brown anole lizard (Anolis sagrei) (Fig. 1). Adult Lizards were collected from our study population at Tomoka State Park in Florida in April 2023. A breeding colony of 50 pairs was established at Auburn University. Over the summer, eggs were collected from the breeding pairs and placed in 1 of 8 different treatments. The treatments are set up in a full factorial design with two levels of temperature (hot, cold), two types of substrates (sand, soil), and two levels of moisture (wet, dry). All eggs were placed in incubators programmed to mimic hot (Mean 29.17°C, Max 40.33°C, Min 24.83°C) and cold (Mean 26.81°C, Max 29.67°C, Min 24.33°C) nest environments measured from the field. The dry and wet conditions were manipulated by placing eggs in substrates set at water potentials of either -120 kPa or -50 kPa, respectively. Incubation substrate was either sand or soil collected from our field site in an area where eggs have been previously found (Pruett et al. 2020).

Eggs were weighed when they were collected and again once every week until they hatched to record egg growth over time in the different treatments; change in mass reflects water uptake by the eggs. When the eggs hatched, snout-vent length (SVL), tail length, and mass of the hatchlings were recorded as well as their sex.

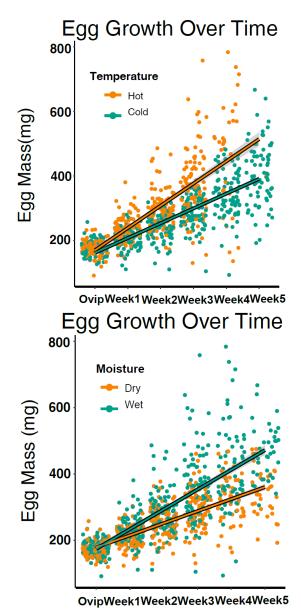


Fig. 1 Male (left) and female (right) brown anole lizard.

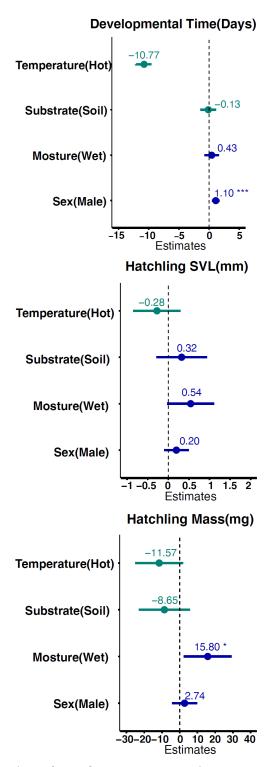
#### Results

Egg growth was significantly influenced by the moisture and temperature treatments (Fig. 2). Eggs from the hot treatment had increased growth over time compared to the cold and exhibited faster development resulting in early hatching. Eggs from the moist treatment also had increased growth over time but did not hatch earlier than those from the dry treatment.

Most of the variation in hatchling traits was influenced by incubation temperature or moisture (Fig. 3). The hot incubation treatment shortened the developmental time by about 11 days compared to the cool treatment. Hot temperatures also reduced body size, but these effects were not statistically supported. Wet incubation conditions generated relatively large offspring compared to dry conditions. The substrate had no significant effect on hatchling phenotypes, and we found no significant interactions between the three incubation variables. Male offspring had slower embryonic developmental rates than females, but the difference was only by about 1 day.



**Fig. 2** Change in egg mass over the incubation period for each temperature treatment (top graph) and the two moisture treatments (bottom graph).



**Fig. 3** The effect of temperature, substrate, moisture, and sex on developmental time (top graph), hatchling snout-vent length (SVL) (middle graph), and hatchling mass (bottom graph). Data points are effect sizes and bars represent standard errors. The treatments in parentheses are the reference treatments for calculating effect sizes. Values indicate effect sizes, and asterisks denote statistical significance: \*<0.05, \*\*<0.001, \*\*\*<0.0001.

#### Discussion

The nest environment that brown anole eggs experience before hatching has significant impacts on the development of the hatchlings. We show that different aspects of the natural nest environment can influence different traits of the embryos and the hatchlings. Interestingly, the effects of temperature and moisture appear to operate in isolation, at least under the conditions used in our experiment. Indeed, we found no interactive effects among temperature, moisture, and substrate type. To our knowledge, this is the first study that has simulated natural nest conditions to isolate the individual effects of these different factors.

The phenotypic effects of different components of the natural nest environment will likely have consequences on offspring fitness. Higher temperatures increase the rate of development of the embryos, thereby enabling hatchings to emerge earlier in the season compared to those experiencing cooler temperatures (Pearson & Warner 2018). Higher levels of moisture increased the mass of offspring at hatching, likely due to increased metabolic rates under relatively moist environments resulting in more yolk conversion to tissue (Miller & Packard 1992). These effects of the nesting environment on phenotypic development can have fitness consequences, and possibly have life-long impacts on their survival and future reproduction (Mitchell et al. 2018). Accelerated development and larger body size are conditions that could be beneficial in different scenarios (Delaney & Warner 2016; Pearson & Warner 2018). For instance, an organism produced early in the breeding season would have more time to grow after hatching so it may benefit from a hotter nest environment. On the other hand, offspring from late-produced eggs would emerge towards the end of the season and therefore have less time to grow and have a reduced chance of achieving a larger body size before winter. Moreover, late-hatched offspring emerge into a highly competitive environment with the early-produced cohorts. Understanding these patterns raises further questions about the effects of the behavioral choices that mothers make when selecting microhabitats for egg laying as well as other potential factors that could influence the nest environment like rainfall patterns or changes in temperature due to climate change.

#### **Statement of Research Advisor**

John Rodgers was instrumental in the early design of this experiment and helped develop the protocols for measuring different phenotypic traits of lizards. He took care of the lizard colony, collected/incubated eggs, and performed all the measurements. He was closely involved with the statistical analyses, and he wrote the early draft of this report.

- Daniel Warner, Department of Biological Sciences, College of Science and Mathematics

#### References

Deeming, D.C. Reptilian incubation: Environment, evolution and behavior. Nottingham, England: Nottingham University Press. (2004)

Delaney, D.M., & Warner, D.A. Age- and sex-specific variation in micro and macrohabitat choice in a territorial lizard. Behavioral Ecology and Sociobiology 70, 981-991. (2016)

Miller, K., & Packard, G.C. The influence of substrate water potential during incubation on the metabolism of embryonic snapping turtles (Chelydra serpentina). Physiological Zoology 65, 172-187. (1992)

Mitchell, T.S., Janzen. F.J., & Warner, D.A. Quantifying the effects of embryonic phenotypic plasticity on adult phenotypes in reptiles: a review of current knowledge and major gaps. Journal of Experimental Zoology A, 329, 203-214. (2018)

Noble, D.W.A., Stenhouse, V., & Schwanz, L.E. Developmental temperature and phenotypic plasticity in reptiles: A systematic review and meta-analysis. Biological Reviews, 93, 72–97. (2018)

Packard, G.C., & Packard, M.J. The physiological ecology of reptilian eggs and embryos. In C. Gans & R.B. Huey (Eds.). Biology of the Reptilia, New York, NY: Alan R. Liss. (Vol. 16, pp. 523–605). (1988)

Pearson, P.R., & Warner, D.A. Early hatching enhances survival despite beneficial phenotypic effects of late-season developmental environments. Proceedings of the Royal Society B, DOI: 10.1098/rspb.2018.0256. (2018)

Pruett, J.E., Fargevieille, A., & Warner, D.A. Temporal variation in maternal nest choice and its consequences on lizard embryos. Behavioral Ecology 31:902-910. (2020)

Warner, D.A., Du, W.G., & Georges, A. Introduction to the special issue – Developmental plasticity in reptiles: Physiological mechanisms and ecological consequences. Journal of Experimental Zoology A, 329, 153-161. (2018)

West-Eberhard, M.J. Developmental plasticity and evolution. Oxford University Press. (2003)

While, G.M., Noble, D.W.A., Uller, T., Warner, D.A., Riley, J.L., Du, W.G., & Schwanz, L.E. Patterns of developmental plasticity in response to incubation temperature in reptiles. Journal of Experimental Zoology A 329, 162-176. (2018)

#### **Authors Biography**



John M. Rodgers is a senior-year student pursuing a B.S. degree in Organismal Biology: Conservation/Biodiversity at Auburn University.



Daniel A. Warner is an Associate Professor in the Department of Biological Sciences at Auburn University. He received his PhD in evolutionary biology from the University of Sydney in 2007. He has been studying reptile ecology and evolution for over 25 years and has published >150 papers in this area.

#### Aubung University Journal of Undergraduate Scholarship



M. Chris Norris is a PhD candidate in the Department of Biological Sciences at Auburn University.

# Studying the Effect of Drought on Peanut's Stomatal Characteristics: Development of an Automatic Detection Method

Spencer Rubin 1,\*, Sajid Hanif<sup>2</sup>, Alvaro Sanz-Saez<sup>3</sup>, Jónathan Heras<sup>4</sup>, and Ángela Casado García<sup>5</sup>

<sup>1</sup> Undergraduate Researcher, Department of Crop, Soil, and Environmental Sciences, Auburn University

Approximately 65% of peanuts in the U.S. are produced without irrigation due to their tolerance to short-term drought. However, the American Peanut Council has identified drought damage as the most serious challenge facing peanut sustainability in the U.S. Improved resistance to abiotic stress such as drought is crucial for the long-term viability of U.S. peanut production. Stomatal characteristics of stomatal density and size affect the overall plant transpiration efficiency and have been identified as possible drought tolerant characteristic. We expect to find smaller and less dense stomata on more drought tolerant cultivars allowing the plant to preserve more water during drought stress. Measuring stomata density and size is very time consuming because it requires that a person identifies them one by one and measures the size with an imaging software. We are working on adapting an existing AI software (Label Stomata) that measures stomata characteristics in soybean, common bean, and grasses.

Five peanut cultivars known for their drought tolerance were grown in the rainout shelter facility at EV-Smith in 2023, using a randomize complete block design with 4 replications (Figure 1). 70 days after planting, drought was imposed on the peanuts by closing the shelters when it was raining. Twenty days after the drought started, leaves were sampled, and super glue was used to take prints of the stomata from the leaves. 320 leaf samples were imaged using an Olympus Slide View VS200 microscope (Figure 2) in automatic collection of 5 images per leaf sample, totaling 1600 peanut images.



**Fig. 1** Rain-out shelter used to grow the peanuts under well water and drought conditions

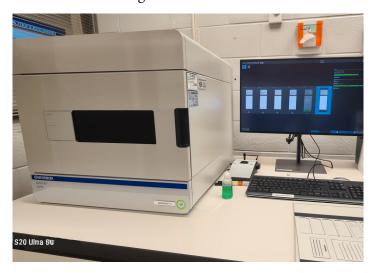


Fig. 2 Image of Olympus microscope used to take images of stomata.

Once the images were taken, ten were selected based on the quality and clarity of the image, with two images

<sup>&</sup>lt;sup>2</sup> Graduate Researcher, Department of Crop, Soil, and Environmental Sciences, Auburn University

<sup>&</sup>lt;sup>3</sup> Assistant Professor, Department of Crop, Soil, and Environmental Sciences, Auburn University

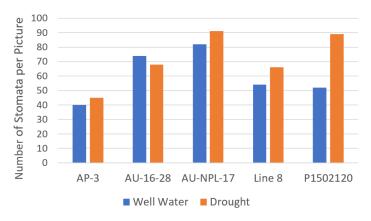
<sup>4</sup> Lecturer, Department of Mathematics and Computer Science, University of La Rioja

<sup>&</sup>lt;sup>5</sup> Research Trainee, Department of Mathematics and Computer Science, University of La Rioja

<sup>\*</sup> Corresponding author: sjr0032@auburn.edu

from each cultivar, one being grown with well water, and the other being grown in drought. Due to unforeseen problems adapting the software Label Stomata to work with our images, we have not been able to automatically count stomata number and size. Therefore, the stomata were hand counted in each image.

Based on this small sample size, as seen in the Figure 3, drought sensitive cultivar AP-3 showed lower stomata density in comparison with drought tolerant ones. This could mean that drought tolerant cultivars could control better the amount of water that is lost through the stomata. The PI502120 cultivar under drought tends to increase stomata number and reduce size. In the future, we will continue to work with the University of La Rioja to adapt the Label Stomata to analyze our images, and further confirm our results.



**Fig. 3** Graph of stomatal counts from five different cultivars

#### Statement of Research Advisor

Spencer studied how plants modify their stomata, pores in the leaves that are used to capture  $\mathrm{CO}_2$  and lose water under drought stress. To do this, Spencer teamed up with a graduate student, Sajid Hanif, to take stomatal prints from a peanut drought experiment. After that, he collaborated with University of La Rioja, Spain to automatically count the number and size of stomata. Although there is still a lot that needs to be done to run the software that is able to recognize and count the stomata in peanut, Spencer has helped to start a very exciting line of research that my lab will continue in the future.

- Alvaro Sanz Saez de Jauregui, Department of Crop, Soil, and Environmental Sciences, College of Agriculture

#### References

Casado-García, Á., Del-Canto, A., Sanz-Sáez, Á., Pérez-López, U., Bilbao-Kareaga, A., Fritschi, F., Miranda-Apodaca, J., Muñoz-Rueda, A., Sillero-Martínez, A., Yoldi-Achalandabaso, A., Lacuesta, M., & Heras, J. (2020). LabelStoma: A tool for stomata detection based on the YOLO algorithm. *Computers and Electronics in Agriculture*, 178, 105751. https://doi.org/10.1016/j.compag.2020.105751

#### **Authors Biography**



Spencer Rubin is a senior-year student pursuing a B.S. degree in Environmental Science at Auburn University. He has currently been involved in Plant Physiology research for the past two years as a student worker and research fellow, in Dr. Alvaro's Lab. He has contributed to research in the development of A.I. system for measuring and counting stomata.



Sajid Hanif is a current Ph.D. student at Auburn University, in the department of Crop, Soil, and Environmental Sciences. His research interests are in improving crop growth and performance.

#### Aubuna5University Journal of Undergraduate Scholarship



Dr. Alvaro Sanz Saez de Jauregui is an Assistant Professor at Auburn University, in the Crop, Soil, and Environment Science Department. Dr. Alvaro's research is focused on detecting genotypic variation to abiotic stress, like high temperatures, elevated carbon dioxide (CO<sub>2</sub>), and drought. His studies are also looking for new and improved methods of measuring plant responses to environmental stress, as it is a difficult process to do in the field.



Dr. Jonathan Heras is a Lecturer of Applied Computer Sciences at the University of La Rioja, Spain, who specialized in image recognition and development of user-friendly Apps.



Ms. Angela Casado is a PhD Student of Computer Sciences at the University of La Rioja, Spain, who has developed a Software to detect images of stomata in soybean and wheat.

# Maximum Thermal Tolerance of the Pinfish (*Lagodonrhomboides*) Across Different Populations of the Western Atlantic

Jacob E. Samenuk<sup>1,\*</sup>, Katie Eaton<sup>2</sup>, and Moisés A. Bernal<sup>3</sup>

Pinfish (Lagodon rhomboides) are vital to coastal ecosystems in the Gulf of Mexico and Western Atlantic Ocean. These fish are highly abundant and serve as a prey source for many commercially relevant fishes, seabirds, and marine mammals [1]. Despite their ecological importance, not much is known about their thermal tolerance. It is particularly important to understand the thermal performance of marine fishes because ocean temperatures are increasing, and marine heatwaves are intensifying due to human-mediated climate change [4]. Most marine species are ectotherms, and they are particularly vulnerable to temperature changes. In the case of the pinfish, juveniles occupy shallow water between the spring and summer seasons, making them susceptible to ocean warming. Therefore, it is crucial to study the thermal tolerance of the species across different populations to understand their sensitivity, and their ability to tolerate warming oceans.

For this project, pinfish were collected from Wilmington, NC, Marathon, FL, Perdido Bay, AL, and Port Aransas, TX (Fig. 1). In total, 9 individuals from Alabama, 13 individuals from Florida, 23 individuals from Texas, and 18 individuals from North Carolina were collected. We measured the maximum thermal tolerance (CTMax) of all the pinfish per locality, by exposing them to a 0.3°C/min temperature increase until loss of equilibrium was observed. This represents the point at which typical swimming function is impaired and physiological functions start failing.

**Fig. 1** A map showing the 4 sampling sites: Wilmington, NC (Blue), Marathon, FL (Yellow), Perdido Bay, AL (Red), and Port Aransas, TX (Orange) and a photo of a pinfish (*Lagodon rhomboides*) by Zachary Randall [5].

Additionally, genetic analyses on pinfish were completed by examining two mitochondrial markers (CytB and CO1). For this, 8 samples from Alabama, 13 samples from Florida, 8 samples from Texas, and 12 samples from North Carolina were analyzed. Gill tissue was tak-

<sup>&</sup>lt;sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University

<sup>&</sup>lt;sup>2</sup> Graduate Student, Department of Biological Sciences, Auburn University

<sup>&</sup>lt;sup>3</sup>Assistant Professor, Department of Biological Sciences, Auburn University

Minnesota

Misconsin

Michigan

Pennsylvania

New York

Massachusetts

Prode Island

Connecticut

New Jersey

Ohio

Macylant

Delaware

Nebraska

Illinois

Indiana

West Virginia

Virginia

Kansas

Missouri

Kentucky

North Carolina

Oklahoma

Arkansas

Mississippi

Alabama

Georgia

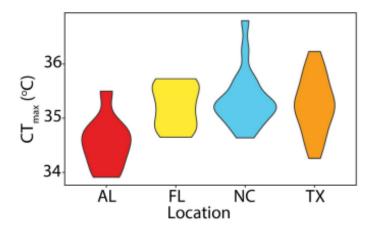
Louisiana

Texas

<sup>\*</sup> Corresponding author: jes0119@auburn.edu

en from the samples and DNA was extracted using the Zymo Quick-DNA Miniprep Plus Kit, following manufacturer's instructions. Genes were then amplified via PCR using the Promega GoTaq® PCR Core System I. PCR samples were then Sanger sequenced at Azenta Life Sciences (South Plainfield, NJ). Geneious was then used to trim and align all the sequences. The concatenated CytB and CO1 sequences were used to make a haplotype network using Popart [3] and a tree using IQ-TREE [6]. Population differentiation was then estimated with ARLEQUIN [2].

Pinfish were found to have a significant difference in CTmax between populations (p=0.002) (Fig. 2). Alabama's CTmax was on average 0.674oC lower than Florida (p=0.014), 0.695oC lower than Texas (p=0.004), and 0.798oC lower than North Carolina (p=0.001). Florida, Texas, and North Carolina did not significantly differ from one another in CTmax, but there was a higher trend for North Carolina.



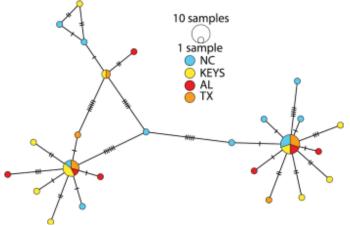
**Fig. 2** Violin plot for the Distribution of Pinfish CTmax data from the different sampling sites.

In terms of genetic difference, Fst values were not significant for any of the comparisons showing no genetic differentiation between populations or biogeographic regions and suggesting dispersal across sites (Table 1). The haplotype network showed two major genetic clusters on both the haplotype network and the tree, but these do not correspond to the individual population, or the biogeographic region (Figs. 3 and 4).

Overall, this study suggests that there can be difference in CTmax among populations, yet these differences do not appear to be associated with the genetic composition of a specific population. We recommend future analyses to evaluate the potential genetic differences between populations and should be conducted at a finer scale using genome sequencing data, as well as evaluating CTmax across different life stages.

**Table 1** Table of pairwise-FST. Below the diagonal the values represent genetic differentiation, above the diagonal line the values are the corrected p-values of each comparison.

	AL	FL	TX	NC
AL	*	0.9	1	0.7
FL	0	*	1	0.3
TX	0	0	*	0.7
NC	0	0	0	*



**Fig. 3** Haplotype Network for the CO1 and CytB genes showing two major haplotype groups.

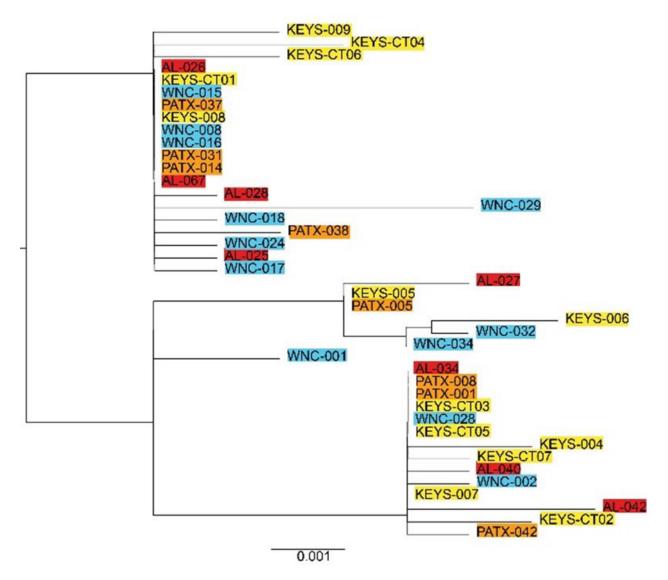


Fig. 4 Maximum-Likelihood Tree showing the genetic composition for the four different populations, using the concatenated mitochondrial markers CO1 and CytB.

#### **Statement of Research Advisor**

During his time in the lab, Jacob completed the research objectives with great enthusiasm, providing essential support for activities in the aquarium and molecular lab. His efforts have provided basic information for an understudied fish species, which will be key for future experiments of thermal tolerance of the pinfish and scientific manuscripts of this relevant fish.

- Moisés A. Bernal, Department of Biological Sciences, COSAM

#### References

[1] Darcy, G.H. (1985). Synopsis of biological data on

the pinfish, Lagodon rhomboides (Pisces: Sparidae). NOAA Technical Report NMFS 23, pp. 23-25.

- [2] Excoffier, L. and Lischer H.E.L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 10: 564-567.
- [3] Leigh, J.W. and Bryant D. (2015). PopART: Full-feature software for haplotype network construction. Methods Ecol Evol. 6(9):1110–1116.
- [4] Oliver, E.C.J., et al. (2018). "Longer and more fre quent marine heatwaves over the past century," Nature

Communications, 9:1324. doi: 10.1038/s41467-018-03732-9.

[5] Randall, Z. (2022). "Lagodon rhomboides (Linnaeus 1766) Family Sparidae," Florida Museum. https://www.floridamuseum.ufl.edu/discover-fish/florida-fishes-gallery/pinfish/

[6] Trifinopoulos, J., Nguyen L.T., von Haeseler A., Minh B.Q. (2016). W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res., 44:W232-W235. https://doi.org/10.1093/nar/gkw256



Moisés A. Bernal, PhD is an Assistant Professor in the Department of Biological Sciences. He is interested in understanding the evolution of marine fishes, with special emphasis on the responses to climate change. At Auburn he teaches classes related with marine biology, evolution, and climate change.

#### **Authors Biography**



Jacob Samenuk is a senior-year student pursuing a B.S. degree in Genetics at Auburn University. He performs research in the Bernal Lab working with Pinfish. He is member of Phi Sigma Pi honors fraternity.



Katie Eaton is a PhD candidate in Biological Sciences at Auburn University. She earned a B.S. in Biological Sciences from the University at Buffalo. Her PhD research focuses on the potential for plasticity and/or adaptation in response to repeated heat stress in the pinfish.

# Sexual Selection in the Wild Inferred using 3-D Printed Decoys and PIT Tags: Male Mate Choice in Yellow-Bellied Slider Turtles (*Trachemys scripta*)

McKae L. Sarkowski<sup>1\*</sup>, Iwo Gross<sup>2</sup>, and Matthew E. Wolak<sup>3</sup>

#### **Abstract**

Mate choice is central to sexual selection, and there is a lack of knowledge regarding male mate choice specifically, despite it being a driver of adaptive evolution and speciation. Mate choice studies have scarcely been conducted in turtles because of the difficulty of observing mating behaviors occurring in water. With developments in technology, observing potential patterns in male mate choice in freshwater turtles has become increasingly feasible. We hypothesize that male mate choice occurs due to female body size being associated with reproductive success. To reflect this, we are testing whether male yellow-bellied slider turtles (*Trachemys scripta*) prefer to interact with females of larger sizes when presented with two 3-D-printed female decoys of differing sizes.

Each decoy is fixed with a passive integrated transponder (PIT) tag reader that records when previously PIT-tagged males approach either decoy within close range (<40cm). We predict that males will approach the larger decoy more frequently than the smaller decoy. By choosing a female of larger size, males should increase their reproductive success due to increased fitness being associated with body size. This novel approach to mate choice study offers a realistic context in which observing mating behaviors in the wild is feasible with conditions that are not traditionally ideal.

Sexual selection is crucial to the reproductive success of an individual, favoring traits that increase an individual's fertilization success when there is reproductive competition[1]. Mate choice is the preferences exhibited by one sex that result in mating biases toward the op-

posite sex[1]. Compared to other taxa, sexual selection is rarely studied in turtles.

Many turtle species with female-biased sexual size dimorphism potentially exhibit male mate choice. Therefore, male mate choice is hypothesized to occur in aquatic turtles because female size is variable, and it is associated with increased reproductive success. Females of larger size can carry more eggs[2] and therefore are able to produce more offspring. In traditional aquatic mating systems, male turtles are likely to encounter multiple females at once and therefore must decide who to mate with to maximize reproductive success.

#### Introduction

Classic mate choice experiments [3] remove individuals from the population to observe an individual choosing between candidate mates. Conversely, observing mate choice in the wild affords a realistic context though potentially sacrificing details that can be observed in artificial experiments. Mate choice studies of either type have scarcely been conducted in turtles because of the difficulty of observing mating behaviors occurring in water. Previous studies[4] have demonstrated the feasibility of mate choice experimentation in the wild using 3-D printed turtle decoys. However, previous studies [4] were done in relatively clear lakes where video recording enabled the observation of mating behavior with decoys. Many turtle habitats throughout the world have turbid water that makes visual observations impossible, requiring the development of a modified method.

Methods Study Site:

<sup>&</sup>lt;sup>1</sup> Undergraduate Student, Department of Biological Sciences, Auburn University

<sup>&</sup>lt;sup>2</sup> Ph.D. Candidate, Department of Biological Sciences, Auburn University

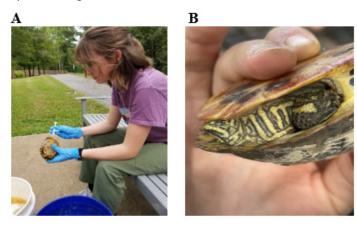
<sup>&</sup>lt;sup>3</sup> Assistant Professor, Department of Biological Sciences, Auburn University

<sup>\*</sup> Corresponding author: mls0169@auburn.edu

This study is being conducted at a pond on the Auburn University campus, off Woodfield Dr. in Auburn, Alabama (GPS coordinates: 32.588887, -85.494796). This location has been part of an ongoing ecological study of aquatic turtles where over 200 turtles have been recorded and individually marked to date (ME Wolak, unpublished data).

#### **PIT-Tagging:**

To determine mate choice behavior in male yellow-bellied slider turtles (*Trachemys scripta*), passive integrated transponder (PIT) tags were inserted into the thigh muscle of male turtles. The PIT tags provide a unique identification associated with each turtle that corresponds with body measurements that have been previously recorded for each tagged individual in the study system (Figure 1).

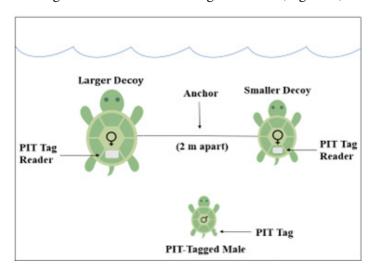


**Figure 1.** PIT-tag (A) Insertion of a PIT-tag that is preloaded into the syringe and (B) a previously PIT-tagged turtle that was recaptured one-week post-injection.

#### 3-D Printing:

Using a preserved female *T. scripta* specimen from the Auburn University Museum of Natural History, a digital scan was developed to create a realistic female decoy. The specimen was scanned and printed in the Auburn University MakerSpace in the Innovation and Research Commons. Two decoys will be 3-D printed and painted to emulate the females that males may encounter in this mating system. The decoys will be identically shaped and painted but scaled to lengths of 81.5 mm or 211.3 mm, corresponding with the 10th and 90th percentiles of female length from previous research at the study site. The decoys will be fully submerged and fitted with PIT tag readers. The readers attached to each decoy will record close interactions (<40 cm) of males that have

PIT tags inserted into their thigh muscles (Figure 2).



**Figure 2.** The PIT-tagged male is given a choice between two female decoys of differing sizes. If the hypothesis is supported, we can expect the reader on the larger female decoy to record more interactions with PIT-tagged males than the reader on the smaller decoy.

#### **Expected Results:**

It is anticipated that the male turtles will interact more frequently with the larger female decoy than the smaller female decoy

#### Acknowledgements

I would like to thank the Undergraduate Research Fellowship and the Department of Biological Sciences at Auburn University for providing funding for this project.

I would also like to thank Gary Hawkins and Yinbo Chen at the MakerSpace in the Innovation and Research Commons at Auburn University for 3-D printing services.

#### Statement of Research Advisor

McKae has introduced a new direction to my research group, by extending our work into sexual selection in turtles. McKae has begun PIT tagging turtles and has overseen the creation of our 3D turtle decoys. Mate choice in turtles is almost completely unstudied and male mate choice is rarely studied in any animal. This work will also test a major evolutionary cause of widespread turtle sexual size dimorphism.

- Matthew Wolak, Department of Biological Sciences,

College of Sciences and Mathematics

#### References

- [1] Westneat, D. & C. W. Fox. Evolutionary Behavioral Ecology. Oxford University Press (2010).
- [2] Edward, D.A. & T. Chapman. The evolution and significance of mate choice. Trends in Ecology & Evolution 26, 647–654 (2011).
- [3] Dougherty, L. R. Designing mate choice experiments. Biological Reviews 95, 759-781 (2020).
- [4] Bulté, G., R.J. Chlebak, J.W. Dawson, & G. Blou-in-Demers. Studying mate choice in the wild using 3D printed decoys and action cameras: a case study of male choice in the northern map turtle. Animal Behaviour 138, 141-143 (2018).





McKae Sarkowski is a junior-year undergraduate student pursuing a degree in Organismal Biology- Ecology, Evolution, and Behavior in the College of Sciences and Mathematics at Auburn University. She is particularly interested in evolutionary and behavioral ecology and conservation.



Iwo Gross is a Ph.D. candidate in the Auburn University Department of Biological Sciences. He is broadly interested in the integration of evolutionary ecological theory and conservation practice. Presently, he is studying the potential conservation implications of mating system dynamics in turtles.



Matthew E. Wolak is an Assistant Professor in the Department of Biological Sciences at Auburn University. Matthew earned a B.Sc. from The College of William & Mary, his Ph.D. from the University of California at Riverside, and completed a postdoctoral research fellowship at the University of Aberdeen in Scotland before moving to Auburn. His research investigates the causes of among individual variation in populations and how they affect evolution by natural selection.

## Representing the Spatial History of Alabama Authors: A Data Visualization Project

Kevien Shelton<sup>1,\*</sup> and Dr. Beverley Park Rilett<sup>2</sup>

Undergraduate Student, Department of Computer Science, Auburn University
 Associate Research Professor and Digital Humanities Coordinator, Special Collections and Archives, Auburn University

Alabama Authors of the 19th and 20th Centuries is a digital archive project developed by Auburn University students and directed by Dr. Beverley Park Rilett. The project was launched in January 2024 and includes an interactive data visualization Kevien helped create, the "Literary Map of Alabama Authors".



**Fig. 1** Kevien presenting the map at the Undergraduate Research Showcase.

The purpose of this literary map is to provide users with accurate information for authors that have some kind of geographical connection to Alabama, whether it be by birthplace, residency, education, death, or burial location. This is primarily what Kevien was explaining to people at the Undergraduate Research Showcase as seen in Fig. 1.The scope of the project includes Alabama-connected authors who published complete books between the years of 1800-1999. In the first iteration of the website, 150 authors are represented, as seen in Figure 2.

As seen in Fig. 2, the map is separated by each county. This allows the user to select any county and view all its associated authors. There is also a list of each county displayed on the right side of the map if users would like to find a county that way. Fig. 2 also contains a heat-like color scheme. The colors are used to represent the number of authors that have a connection to the respective county. A county with a lighter blue indicates fewer authors in the county and a county with a darker blue means there are more authors in the county.

Because the counties are separated into their own units, when clicking on a county the user will be directed to an author list as shown in Fig. 3. Each author list shows the name of the county at the top and every author alongside their specific connection to the county.

After clicking an author's name in any of the author lists (shown in Fig. 3), the user will be directed to the individual author's page such as the one shown in Fig. 4. Here, one can view detailed information about the author selected. This information includes a brief biography, a list of the author's published books, and a brief overview of their writing. This metadata is also completely free to access and download.

This project required Kevien to learn to use a variety of software applications, including Adobe Acrobat, Microsoft Excel, Box, Trello, Omeka, and Neatline, which he'd learned to customize for the particular project's requirements. He also learned another open-source web

This map (Fig. 2) was developed using Neatline, an open-source software package. Its wide range of tools and functionality allowed for the separation of the counties, each with their own selective information about the authors.

<sup>\*</sup> Corresponding author: kbs0067@auburn.edu

#### Aubun94University Journal of Undergraduate Scholarship

application called QGIS, which will support a new version of the literary map in the next phase of the project that will include many more Alabama Authors. He used data science techniques of data collection, data cleaning and disambiguation, analysis, and visualization. In conclusion, being a member of this project-based research team and contributing to the geospatial data visualization has provided an excellent foundation for Kevien's future career in computer science.

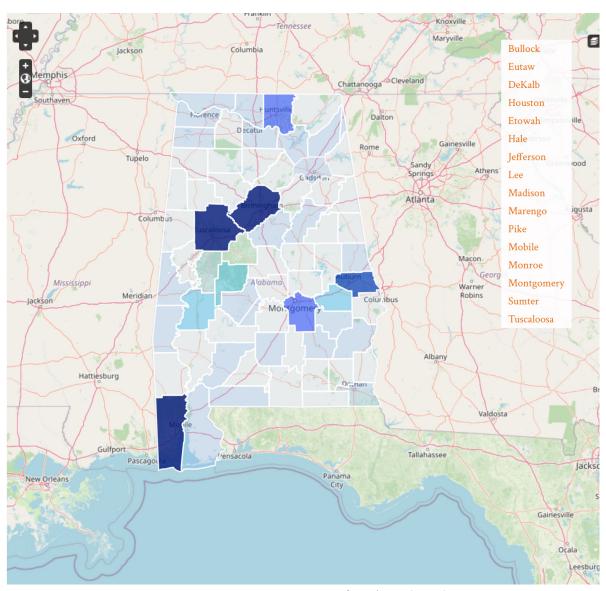


Fig. 2 Current Literary Map of Authors (2023)

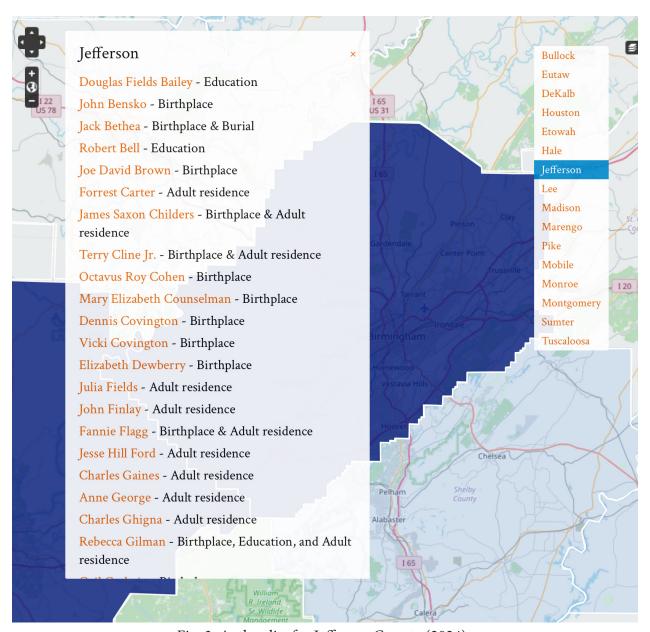


Fig. 3. Author list for Jefferson County (2024)

#### ALABAMA AUTHORS of the 19th & 20th Centuries



#### AL Author Collection (152 total)

Sort by: Author Lifespan

Name/Lifespan Biography Subject **Publications** Agee, James 1909-1955 Permit Me Voyage. James Agee was born James Agee wrote in Knoxville, poetry, short stories, New Haven: Yale Tennessee. He first novels, and University Press, began writing poetry screenplays. He was and short stories interested in both the while attending physical and the Let Us Now Praise Phillips Exeter psychological. Famous Men. Boston: Houghton Mifflin,... Academy, in Exeter, N.H. After graduating from... Andrews, Mary Raymond Shipman Mary Raymond Mary Andrews wrote Vive l'empereur. New Shipman Andrews boys' coming-of-age York; Scribner, 1902. 1860-1936 was born in Mobile, stories and Alabama but grew up sentimental romantic  $A\ Kidnapped\ Colony.$ 

#### Zora Neale Hurston

Image



Brief Zora Neale Hurston was born in Notasulga, Alabama, but was raised in Eatonville, Florida, an
 Biography all-Black town. She experienced racial segregation for the first time when attending boarding school in Jacksonville, Florida. Hurston's passion for writing emerged during her time at

Fig. 4 Sample Author Page (2024)

#### **Statement of Research Advisor**

As an Undergraduate Research Fellow, Kevien Shelton co- developed (with Corey McDaniels) the Literary Map of Alabama Authors, for Alabama Authors of the 19th & 20th Centuries project website. This involved gathering, verifying, analyzing, and coding the geospatial data for 152 authors. After evaluating the feasibility of various open-source mapping applications, including Leaflet, Neatline, ArcGIS, and QGIS, Shelton and McDaniels developed both the Neatline and QGIS maps for the website. Their application was further refined for accessibility (using Colorblindly and other UX assessment tools) and refinements were made (such as changing the heatmap key from red to blue). The visualization was available for the website's official launch in January 2024, and further refined during the Spring 2024 semester. A feature of the map I especially appreciate is the ability to easily incorporate the profiles and geospatial information about other Alabama-connected authors as we add them to the project. I have been very fortunate to work with Kevien Shelton during his very successful undergraduate research fellowship project in 2023-24.

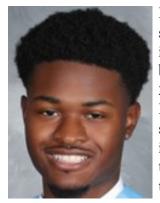
- Beverley Rilett, Special Collections and Archives, Auburn University Libraries

#### References

[1] Alabama Authors of the 19<sup>th</sup> and 20<sup>th</sup> Centuries, edited by Beverley Park Rilett, Auburn University, 2024. https://AlabamaAuthors.org/

[2] Wachter, C. (2022), Representing Spatial History Concepts: Modern East Asian History In A Digital Publication Format, "History and Theory." Vol 61 Issue 4. p178, 13 p.https://historyandtheory.org/61-4/wachter

#### **Authors Biography**



Kevien Shelton is a junior- year student pursuing a B.S. degree in Computer Science at Auburn University. He is a 2023-2024 Undergraduate Research Fellow. Kevien has assisted in sub-projects for the George Eliot Archive and Alabama Authors of the 19<sup>th</sup> and 20<sup>th</sup> Centuries.



Dr. Beverley Park Rilett is a Research Assoc. Professor and Digital Humanities Coordinator at AU Libraries. In addition to Alabama Authors of the 19th & 20th Centuries project, she is the inaugural director of the George Eliot Archive, George Eliot Scholars, and George Eliot Review Online. She also coordinates the AlabamaMosaic project for the Network of Alabama Academic Libraries. "Dr. Bev," as she is known to her students, is working to expand Digital Humanities at AU with internships, course credit, and grant-supported research assistantships.

# Characterization of Acoustic Pressure Waveforms in Rijke Tubes with Spatially Varying Temperature Distributions

Emma T. Signor<sup>1,\*</sup>, Cody M. Shelton<sup>2</sup>, Joseph Majdalani<sup>3</sup>

Undergraduate Student, Department of Aerospace Engineering, Auburn University
 Ph.D. Student, Department of Aerospace Engineering, Auburn University
 Professor and Francis Chair, Department of Aerospace Engineering, Auburn University

The fundamental mechanisms that affect the performance of a Rijke, or open-open ended, tube have frequently been the focus of dedicated investigations by a large group of researchers. These have generally been undertaken through multi-pronged efforts at gaining deeper understanding of the particular thermoacoustic phenomena and criteria that underlie the optimal functionality of combustion devices, especially those incorporating spatially distributed heat sources. The one aspect of Rijke tube operation that has yet to be fully resolved consists of the detailed interactions between the thermal pattern evolving from the presence of a heat source and the resulting acoustic waveform. Although several theoretical models have been devised to explore the nonlinear relations between the heat source and velocity fluctuations, their utility in predicting the complex thermoacoustic interactions observed in various experiments has been somewhat restricted to specific, user-defined temperature jumps, thermal patterns, or boundary conditions [1-3].

In the context of a Rijke tube, the coupling between the unsteady heat and velocity fluctuations extends over two distinct regions that remain separated by a diffuse boundary that is often modeled as a zonal discontinuity at the heat source location. The precise modeling of the temperature profile across the zonal discontinuity and throughout the tube has been shown to exhibit a strong bearing on the accuracy of the ensuing pressure and velocity oscillations. In this vein, recognizing the intricate complexities that accompany unsteady flowheat transfer interactions across different segments of a Rijke tube, this aspect continues to attract attention in the thermoacoustic community.

In the development of industrial, propulsive, and power generation devices, the implementation of favorable thermoacoustic conditions is not merely desirable or advantageous to a variety of combustors, but indeed essential. In fact, maintaining proper thermoacoustic conditions, which is often accomplished using "cut-and-try" methods, has invariably played a crucial role in reducing operational costs, specifically by enhancing combustion efficiency, suppressing instability, and increasing convective heat transfer rates. Moreover, the underpinning mechanisms that promote performance enhancements have been repeatedly shown to be closely tied to the judicious control of the evolving acoustic field. The latter can directly influence the overall character and therefore performance of a combustor.

It should also be noted that, depending on the application at hand, the main objective can be to either promote or suppress thermoacoustic coupling. Although in some instances maintaining a resonant state can be beneficial to thermal efficiency, flame holding, enhanced mixing, and heating or cooling, many cases exist for which thermoacoustic triggering is undesirable, being detrimental to normal operation.

In rocket engines, for example, thermoacoustic oscillations can produce excessive vibrations, noise, and thermal heating, i.e., mechanisms that can adversely affect motor performance and structural integrity to the extent of precipitating outright system failure. It can thus be seen that, in all cases considered, a comprehensive understanding of the criteria that control the onset of thermoacoustic coupling is essential.

<sup>\*</sup> Corresponding author: ets0027@auburn.edu

In this work, we revisited the thermoacoustic field of a one-dimensional tube that contained a heat source element whose position,  $\tilde{x}_f$  length,  $\tilde{l}_c$ , and temperature gain, α, were modified. Since the analytical formulation depended on the temperature distribution, three local profiles that extended over the heat source segment were considered; we also explored a global profile that spanned the entire length of the tube. To avoid a temperature discontinuity, our framework enabled us to provide a detailed description of the temperature variation across the heat source segment using either local or global representations of the thermal field. In the case of the former, three piecewise representations were considered, and these allowed for the juxtaposition of constant-constant temperatures before and after the heat source element with either linear, exponential, or power-law relations across the region of discontinuity, namely, the flame zone.

In the case of a global representation, as conveyed in Figure 1, an S-shaped logistic function was applied uniformly across the entire length of the tube. Overall, we found the temperature gain,  $\alpha$ , to have the most appreciable bearing on the pressure mode shapes. Furthermore, growths in  $\alpha$  led to flatter mode shape patterns in global representations. A similar effect was associated with successive increases in  $\tilde{l}_c$  As for the flame zone location,  $\tilde{x}_f$ , its rearward displacement to the tube's downstream end led to a gradual shift of the pressure antinode to the tube's midpoint location.

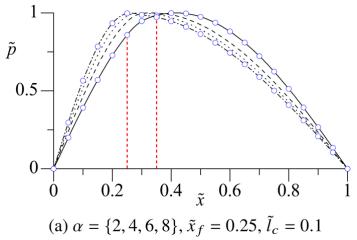


Fig. 1 Comparison of acoustic pressure mode shapes for variations in  $\alpha$  for a logistic profile, where  $\tilde{x}_f$  and  $\tilde{l}_e$  are held constant. Individual variations defined in the subcaption are denoted sequentially by solid, dashed, dotted, and dash-dotted lines. Hollow circles represent

predictions obtained from an open-source solver. Here, the sigmoidal steepness  $\beta$  values correspond to: {84.9, 100, 106, 110}.

These findings demonstrated that the proper choice of a thermal profile, be it locally or globally applied, could substantially influence the overall pressure mode shapes and frequencies. In future analysis, we hope to extend this framework to the analysis of acoustic velocity modes.

#### **Statement of Research Advisor**

Emma is to be commended for volunteering to undertake this challenging project on Rijke tube analysis that ended up exceeding all expectations. It all started with a longstanding project that Cody Shelton and I decided to revisit, when Emma happened to join our group. Working closely with Cody, not only did Emma present some of her Rijke tube analysis at SciTech'24, where she captivated numerous members of the propulsion community, she proceeded to publish her analysis with Cody in the prestigious Physics of Fluids as a "Feature" article (https://doi.org/10.1063/5.0194337). Shortly thereafter, Emma competed and placed First in the AIAA Southeast Regional Student Competition, thus winning the highly coveted Stan Powell Prize! Specifically, Emma competed in the highly-disputed Undergraduate Student Division of Region II, which covers 8 southern states and countless universities. According to the organizers, this particular year attracted a record number of papers exceeding thirty-five. Despite this compelling competition, Emma presented superbly well and received a nearly perfect score from all attending judges. As a result, Emma's name has been inscribed on the Stan Powell Plaque, a tremendous recognition that will grace Auburn's main hallway in Davis for the next calendar year! We are supremely proud of Emma and her team mentor, Cody Shelton.

-Joseph Majdalani, Aerospace Engineering, Samuel Ginn College of Engineering

#### References

[1] A. Bigongiari and M. A. Heckl, "A Green's function approach to the rapid prediction of thermoacoustic instabilities in combustors," J. Fluid Mech. 798, 970–996 (2016).

[2] J. Li and A. S. Morgans, "The one-dimensional acoustic field in a duct with arbitrary mean axial temperature gradient and mean flow," J. Sound Vib. 400, 248–269 (2017).

[3] T. C. Lieuwen, Unsteady Combustor Physics (Cambridge University Press, 2012).

#### **Authors Biography**



Emma T. Signor is a senior pursuing a B.S. degree in Aerospace Engineering at Auburn University, where she conducts undergraduate research an active member of the Advanced Propulsion Research Lab (APRL). She has presented related articles at the AIAA SciTech Forum in Orlando, FL, as well as the AIAA Region II Conference in Cape Canaveral, FL. On April 5, 2024, Emma became Auburn's fifth recipient of the highly-coveted Stan Powell Prize. On April 19, Emma received the Mark A. Spencer Creative Mentorship Award alongside her faculty advisor, Professor Joe Majdalani. Following graduation, Emma will intern at Dynetics and then continue her education with a Master of Science degree in Aerospace Engineering.



Cody M. Shelton is a doctoral candidate and Department of Defense (DoD) SMART Scholar. As a leading member of APRL, he is pursuing a Ph.D. degree in Aerospace Engineering. Cody has completed both an M.S. in Aerospace Engineering (2020) as well as an M.S. in Applied Mathematics (2023). After graduation, Cody

will join the Coastal Hydraulics Laboratory at ERDC, the U.S. Army Engineer Research and Development Center, as a Research Mechanical Engineer. Cody has received several awards and recognitions that include: (1.) the AIAA Outstanding Graduate Student of the Year (2023) within the Greater Huntsville Section, (2.) the DoD SMART VKI International Internship at the von Kármán Institute in Belgium (Summer 2023), (3.) the Auburn University Outstanding Doctoral Student of the Year (2023), (4.) the DoD SMART Fellowship (2022), (5.) the Merriwether Fellowship (2022), (6.) the First Year Experience (FYE) Mentorship Award (2021), (7.) the Naval Horizons Award (2021), and (8.) the Walter and Virginia Woltosz Fellowship (2019-2022) for incoming graduate students.



Najdalani serves as Francis Chair of Excellence and Professor of Aerospace Engineering at Auburn University. He directs the APRL group with a focus on improving the overall performance and acoustic instability of swirl-driven vortex rocket engines. His group has coauthored over 330 publications that have attracted nearly 20,000 citations and placed him in the top 0.8% of Stanford's list of the most cited aerospace engineering researchers worldwide. As one of twelve AIAA Fellows in the Greater Huntsville Section

(GHS), Dr. Majdalani serves as the Southeast's Deputy Director for Technical Activities as well as the GHS Council. Throughout his academic career, Dr. Majdalani has received several professional recognitions and accolades that include, but are not limited to, the (1.) Mark A. Spencer Creative Mentorship Award (2024), (2.) Wyld Propulsion Award (2024), (3.) AIAA Von Kármán Lecture in Astronautics (2023), (4.) Senior Research Award for Excellence (2023), (5.) Auburn University Book Author Award (2022), (6.) Abe M. Zarem Educator Awards (2022, 2017, 2012), (7.) Five AIAA Best Papers (AIAA Paper № 2022-1898, 2017-4779, 2014-4016, 2014-0006, 2004-4054), (8.) Auburn University Outstanding Graduate Mentor Award (2021), (9.) ASEE/AIAA/Rockwell Leland Atwood Award (2018), (10.) AIAA Foundation Sustained Service Award (2016), (11.) AIAA Foundation Faculty Advisor of the Year Award (2015), (12.) Auburn University Aerospace Engineering Outstanding Faculty Member Award (2015), (13.) AIAA Konrad Dannenberg Educator of the Year Award (2014), (14.) AIAA Special Award (2013), (15.) AIAA General Hap Arnold Award (2007), (16.) SAE Ralph Teetor Educational Award (2007), (17.) NASA Faculty Research Infrastructure Awards (2002-2004), (18.) NASA Higher Education Incentive Award (2002–2003), (19.) NSF CAREER Award (2003), (20.) Marquette University Outstanding Teaching Awards (1998–2000), and (21.) Marquette University College of Engineering Research Award (1997–1998).

### Pecan Shells as an Alternative to Pine Bark as Substrate for Rabbiteye and Southern Highbush Blueberry Cultivation

Lucas K. Speer<sup>1,\*</sup> and James D. Spiers<sup>2</sup>

#### Introduction

Growers throughout the southeast generally use milled pine bark as a primary component in soilless substrates for growing blueberry cuttings and other nursery crops due to its favorable physical properties such as acidic nature (Altland et al., 2018). Cheaper and more available alternatives are being sought; one alternative is pecan shells (*Carya illinoinensis*) (Hoppers et al., 2018). Pecan orchards are very common throughout Georgia and Alabama, and when harvest comes around, the processing of the pecans leaves heaps of shells, which are typically discarded and treated as waste.

Utilizing the availability of the shells coupled with a low demand means growers would be able to acquire these shells at a lower cost than pine bark. The purpose of this study was to determine if milled pecan shells could be used as a substrate component for blueberry cultivation to achieve similar growth to the commercial standard, milled pine bark.

#### **Materials and Methods**

An experiment was conducted growing 1-year-old blueberry cuttings in different substrate treatments. 1 rabbiteye cultivar and 1 southern highbush cultivar were chosen for the study, *Vaccinium virgatum* 'Brightwell' and *V. corymbosum* hybrid 'Legacy', due to their popularity among growers in the southeastern United States.

One-year-old blueberry cuttings were purchased online from Finch Blueberry Nursery in North Carolina. Pecan shells were picked up from Whaley's Pecan Co. in Troy, Alabama, and were mostly ground up and dried. Milled pine bark was collected from Paterson Greenhouse Complex on the Auburn University campus.

There were 6 blocks of each culitvar, each block containing one of each of the substrate types. Substrates were labeled Treatment 1-5 with different ratios of pine bark (PB) to pecan shells (PS); 1PB:0PS (Treatment 1), 3PB:1PS (Treatment 2, 1PB:1PS (Treatment 3), 1PB:3PS (Treatment 4), 0PB:1PS (Treatment 5). Order of the treatments within the blocks was randomized (Fig. 1). Plants were irrigated by hand twice daily for the first two weeks, and plants that did not establish were taken out and replaced with a healthy plant. Each plant was trimmed down to just one branch off of the central leader, the branch was trimmed down to approximately 2.5 cm.

Block 1	<b>▼</b> Block 2	<b>■</b> Block 3	<b>■</b> Block 4	<b>▼</b> Block 5	<b>■</b> Block 6	▼
T2	T4	T2	T5	T1	T3	
T1	T5	T1	T3	T5	T4	
T3	T2	T5	T2	T2	T1	
T5	T1	T3	T1	T4	T2	
T4	T3	T4	T4	T3	T5	

**Fig. 1.** Example plot map with randomized order of treament groups

The total shoot length was measured with a ruler and recorded once weekly for a month to monitor growth. After a month, SPAD (Soil Plant Analysis Development) measurements, which measure leaf greenness

<sup>&</sup>lt;sup>1</sup> Undergraduate Student, Department of Horticulture, Auburn University

<sup>&</sup>lt;sup>2</sup> Associate Professor, Department of Horticulture, Auburn University

One gallon (3.79 L) pots were purchased from Cassco in Montgomery, AL. The substrate was mixed with ~91 grams of Osmocote 15-9-12 fertilizer and ~9 grams of Micromax fertilizer per container. The one-year-old cuttings were planted in the containers and placed in the Propagation Greenhouse in Paterson Greenhouse Complex.

<sup>\*</sup> Corresponding author: lks0053@auburn.edu

(indicator of chlorophyll content), were taken with the Minolta Chlorophyll Meter SPAD-502 on the three most physiologically mature leaves of each plant, and an average was recorded. Plants were then carefully removed from the containers with roots in tact and gently but thoroughly rinsed to unbind as much substrate as possible without damaging the roots. They were subsequently placed in paper bags and dried at 71.1 °C for ~48 hours, and then taken to the lab for weighing. A total dry weight, shoot weight, and root weight were taken.

#### Results

When results were analyzed, not much of a difference was seen between the treatments. In almost all cases, each cultivar did not show much of a preference to any one of the substrates (as seen in Fig. 2 and Fig.3). This possibly indicates that pecan shells could compete with pine bark in the short term. This study was conducted over just one month, so long term results could differ highly. Future tests over a longer period of time should be conducted.

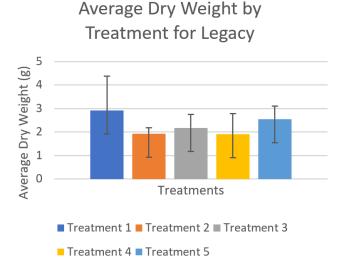


Fig. 2. Average Dry Weight by Treatment for Legacy

However, a slight difference between the cultivars was seen in the total growth of the shoot as shown in Fig. 4 and Fig. 5. 'Brightwell' had a higher total growth across all treatments, possibly suggesting more resistance to transplant stress and higher adaptability to soil composition. 'Legacy' did appear to have greater shoot growth in 100% pine bark compared to substrates with pecan shells, but not very different over this short duration. There were no differences for SPAD measurements (data not shown).

### Average Dry Weight by Treatment for Brightwell

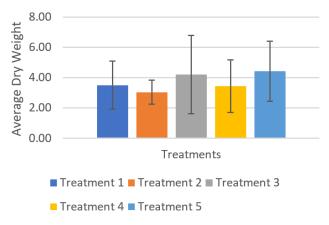


Fig. 3. Average Dry Weight by Treatment for Brightwell

#### Average Total Shoot Growth of Brightwell

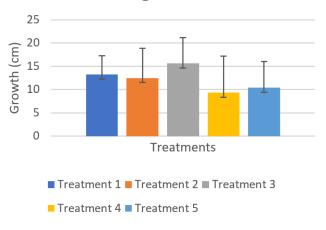
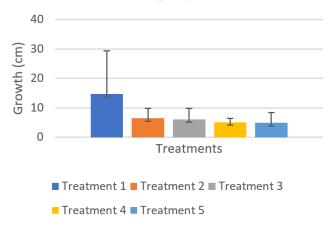


Fig. 4. Average Total Shoot Growth of Brightwell

#### Average Total Shoot Growth of Legacy



**Fig. 5.** Average Total Shoot Length of Legacy



Fig. 6. Comparison of Brightwell at End of Study

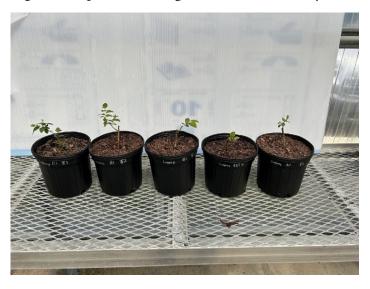


Fig. 7. Comparison of Legacy at End of Study

#### Conclusions

Although no definite conclusions can be drawn from this data, the pecan shells performed similarly to the pine bark (Fig. 6 and Fig. 7). It is likely that pecan shells could be a substrate component when combined with pine bark. With pecan orchards in such close proximity to blueberry growers, proving pecan shells a viable alternative could mean a shift in the industry and less costs for the grower. More research needs to be done to determine if pecan shells are a reliable substrate component for long-term cultivation.

#### **Statement of Research Advisor**

Pine bark is a major component of blueberry cultivation in the southeastern U.S. It is the primary substrate used for blueberry propagation, container production, and for soil amendments and mulch for field production. Due to the high demand for utilization, pine bark has become more costly. Pecan shells are not near as plentiful, but are available in the southeastern U.S. for minimal costs. Lucas's project aimed to determine if pecan shells could be utilized similarly to pine bark, or as a component along with pine bark for blueberry cultivation. His research indicated that pecan shells have potential as a substrate component for blueberries and additional research for a longer duration may be warranted.

- James Spiers, Department of Horticulture, College of Agriculture

#### References

Hoppers, A.A., Spiers, J.D., Kessler, R.J., Wright, A.N., Powell, A.A., Root distribution of 'Brightwell' and 'Premier' rabbiteye blueberries as influenced by pecan shell mulch. Journal of the American Pomological Society, 72(2):101-112 (2018).

Altland, J. E., Owen, J. S., Jr., Jackson, B. E., & Fields, J. S. (2018). Physical and Hydraulic Properties of Commercial Pine-bark Substrate Products Used in Production of Containerized Crops. HortScience horts, 53(12), 1883-1890. Retrieved Oct 2, 2024, from https://doi.org/10.21273/HORTSCI13497-18

#### **Authors Biography**



Lucas K Speer is an undergraduate Senior at Auburn University pursiung a B.S. Degree in Agriculture Science. He has been a student worker under Dr. Spiers, aiding in blueberry, blackberry, and raspberry research.

#### Aubung5University Journal of Undergraduate Scholarship



Jay Spiers is an Associate Professor in the Department of Horticulture in the College of Agriculture at Auburn University. His responsibilities include research and teaching fruit cultivation. Spiers' research has been focused on blueberry, blackberry, kiwifruit, and satsuma best management practices.

## Ideal Gas Law Model for Presynapses and Presynaptic Vesicles

Henry Stephens<sup>1,\*</sup>, Noah Kim<sup>2</sup>, Luca Cmelak<sup>3</sup>, Mason Parkes<sup>4</sup>, Paxton Wilson<sup>4</sup>, Michael Gramlich<sup>5</sup>

Undergraduate Student, Department of Physics and Department of Biological Sciences, Auburn University
 Graduate Student, Department of Mechanical Engineering, Rice University
 Undergraduate Student, Department of Psychological Sciences, Auburn University
 Graduate Student, Department of Physics, Auburn University
 Assistant Professor, Department of Physics, Auburn University

#### **Abstract**

The brain is made up of billions of interconnected cells called neurons. Neurons connect to other neurons by a synaptic connection, where a chemical signal travels from a presynapse to a postsynapse. The strength of the synaptic connection can change over time in a process called plasticity. There are two different kinds of plasticity where synaptic strength either increases or decreases. One kind of increasing plasticity is called longterm potentiation (LTP), which is typically studied by looking at changes that occur on the postsynaptic side because many studies have shown postsynaptic changes. However, the role of the presynapse during LTP has been implicated but is not well understood. This project seeks to create an experimental and model framework for how molecular changes in the presynapse mediate LTP. We used fluorescent microscopy to observe and record the mobility and mechanics of neurotransmitter-carrying vesicles, which are used in synaptic communication, and their role in supporting LTP. We observed vesicle movement during LTP in combination with computational analysis to quantify changes in vesicle dynamics. We then used experimentally observed changes in vesicle mechanics to develop a model to predict how changes in presynaptic function support LTP.

#### Methods

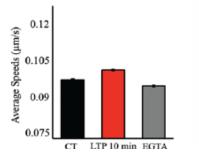
We studied live mouse hippocampal neurons because the hippocampus is essential for memory formation and cognition. We grew the neurons on glass cover slides to label and image at the molecular level. LTP was induced in samples using an established sequence of electrical stimulations. Ten minutes after LTP induction, single vesicles were labeled with a fluorescent reporter and their movements were recorded.

\*Corresponding author: hls0053@auburn.edu

Advanced MATLAB algorithms were used to: (i) identify vesicles, (ii) extract different kinds of motion, and (iii) model the relationship between vesicle speeds and positions across different experimental conditions

To test our hypothesis that activity mediates changes in vesicle dynamics we inhibited calcium, which is essential for synaptic communication and vesicle dynamics. We blocked calcium influx during LTP induction using EGTA. (1)

#### Results



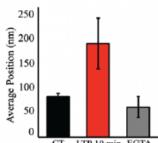
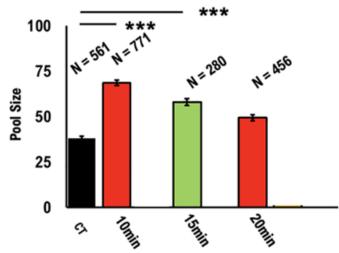


Fig. 1 Average speeds and positions of vesicles under different conditions

We observed average vesicle speed and displacement within presynapses increases after LTP induction (Red, Fig. 1), compared to neurons without LTP (Black, Fig. 1). We also observed that EGTA inhibited LTP-mediated vesicle speed and displacement changes (Grey, Fig. 1). These results show that vesicle dynamics change during LTP.

We studied the number of vesicles within the presynapse by labeling every vesicle using our previously es-

tablished approach. (2) We induced LTP using the same protocol and observed an increase in the number of vesicles after 10 min LTP (Fig. 2). The vesicle number then decreases over time (Fig. 2). This suggests that the number of vesicles in a presynapse influences LTP.



**Fig. 2** Vesicle pool sizes at different time intervals after LTP induction

#### Model

We modeled changes in the presynapse using the Ideal Gas Law (eqn. 1). We then related vesicle energy to its speed (eqn. 2). Finally, we related synaptic communication (f) to the total pressure inside the presynapse with a correction factor (eqn. 3).

$$PV=NK_BT$$
 (eqn. 1)

$$K_BT \sim v^2$$
 (eqn. 2)

$$f{\sim}\Psi*P = \sum_{i=1}^{N_{pools}} \psi_j \sum_{i=1}^{N_j} v_{ij}^2 \tag{eqn. 3} \label{eq:pools}$$

This model predicts that the increase in vesicle number and mobility leads to an increase in synaptic communication. We hypothesize that the increase in synaptic communication in turn, supports LTP-induced changes.

We used our experimentally measured vesicle mobility and number results (Fig. 1 and 2) as parameters for our model (eqn. 3). We were then able to reproduce observed changes in presynaptic communication during LTP (data not shown).

stand vesicle mobility during LTP. Henry and Noah Kim performed original experiments under the advisement of Mason Parkes and Paxton Wilson. Henry then led the effort, in collaboration with Luca Cmelak, to analyze and model vesicle mobility and number during LTP. This work has resulted in novel and important results that will advance the field of synaptic neuroscience.

- Michael Gramlich, Department of Physics, College of Sciences and Mathematics

#### References

[1] EGTA Can Inhibit Vesicular Release in the Nanodomain of Single Ca2+Channels. Nakamura Y. Front Syn. Neurosci. 11 (2019) doi: 10.3389/fnsyn.2019.00026. PMID: 31632263

[2] rTg (TauP301L) 4510 mice exhibit increased VGLUT1 in hippocampal presynaptic glutamatergic vesicles and increased extracellular glutamate release. E Taipala et al., Front. Syn. Neurosci. 14 (2022) https://doi.org/10.3389/fnsyn.2022.925546

#### **Authors Biography**



Henry Stephens is a junior-year student pursuing concurrent B.S. degrees in Physics and Biomedical Sciences at Auburn University. He and Dr. Gramlich carried out the study and collected and analyzed the vesicle mobility data under the control, LTP, and EGTA conditions.



Noah Kim is a graduate student in the George R. Brown School of Engineering at Rice University. He helped collect and analyze the vesicle mobility data for the control and LTP conditions. He also coded MATLAB algorithms used in the analysis steps.

#### Statement of Research Advisor

Henry Stephens proposed and led this effort to under-



Luca Cmelak is a senior-year student pursuing a B.S. degree in neuroscience at Auburn University. He helped by analyzing the vesicle pool size data.



Mason Parkes is graduate student in the College of Sciences and Mathematics at Auburn University. He helped by collecting the vesicle pool data. He also helped code MATLAB algorithms used in the analysis steps.



Paxton Wilson is graduate student in the College of Sciences and Mathematics at Auburn University. He helped by collecting and analyzing the vesicle pool data. He also helped code MATLAB algorithms used in the analysis steps.



Michael Gramlich is an Assistant Professor in the College of Sciences and Mathematics at Auburn University. Dr. Gramlich leads the Neurophysics lab that seeks to understand the physics of cognition and memory formation from the molecular to circuit level.

### Nine-banded Armadillo (*Dasypus novemcinctus*) Burrow Use by Commensal Species

Lauren R. Stevens<sup>1,\*</sup>, Wesley M. Anderson<sup>2</sup>, and Olivia F. Sciandra<sup>3</sup>

Undergraduate Student, Wildlife Ecology and Management College of Forestry, Wildlife and Environment
 Assistant Professor/Extension Specialist, Forestry and Wildlife Sciences, College of Forestry, Wildlife and Environment
 Graduate Student, Wildlife Sciences, College of Forestry, Wildlife, and Environment

Wildlife burrows offer important habitat and refugia for numerous commensal species. This phenomenon is particularly well-studied with gopher tortoises in the southeastern United States with over 360 commensal species documented from their burrows, including federally-listed species like the eastern indigo snake. However, gopher tortoise populations west of the Tombigbee River in Alabama are also listed as threatened under the Endangered Species Act, which could have negative impacts on commensal wildlife dependent on their burrows. Conversely, the nine-banded armadillo, another wildlife species that creates burrows, is expanding its range nationwide. Prior to the 1900s, no armadillos existed in Alabama. Today they are established statewide with their presence the longest in the southern part of the state.

Within the last decade, more attention has been paid to commensal species using armadillo burrows. Studies have documented other wildlife in and around armadillo burrows both domestically[2,3] and abroad.[4] To further examine if nine-banded armadillo burrows help mitigate the loss of gopher tortoises, we sought to investigate commensal use in southwest Alabama – an area where armadillo populations are dense and gopher tortoises have declined. We hypothesized that while we would document commensal use it would be lower than in gopher tortoise burrows because armadillos may consume some commensal species, particularly invertebrates.

We conducted this study in Mobile Co., Alabama from May-August 2023. We surveyed for burrows on private property across the county. Once a burrow was located, we took a photograph and recorded burrow location (rural or urban), time, weather conditions, GPS coordinates, and any signs of armadillo activity. Using

a gopher tortoise camera system, we recorded footage of the burrow to document commensals. We then recorded burrow height, width, and length, and noted if length was measured to the end of the burrow. All burrow footage was reviewed twice. Commensal identity and abundance were recorded for each burrow. We verified commensal identification through consultation with Dr. Meredith Shrader, an insect diagnostician with Alabama Extension, and by uploading all screenshots to iNaturalist. Each commensal taxon was recorded to the lowest taxonomic level possible (Table 1).

**Table 1.** Count summary by species for commensals found in rural and urban burrows.

Taxonomy	Common Name	Rural	Urban
Rhaphidophoridae (Family)	Camel cricket	39	8
Pholcus sp.	Cellar spider	2	0
Polydesmida (Order)	Flat-backed millipedes	21	1
Gryllus sp.	Field cricket	4	0
Didelphis virginiana	Virginia Opossum	1	1
Anura (Order)	Frogs and Toads	5	4
Araneae (Order)	Spiders	10	6
Anaxyrus sp.	North American Toad	2	1
Orthoptera (Order)	Grasshoppers, crickets	1	10
Diplopoda (Class)	Millipedes	1	0
Opiliones (Order)	Harvestmen	1	1
Formicidae (Family)	Wasps and bees	0	3
Tipuloidea (Superfamily)	Crane fly	0	1

We analyzed burrow dimensions using a Wilcoxon sum rank test. We used a two-sample test for equality of proportions to compare species richness and presence of individual taxa between urban and rural burrows. To visualize the level of similarity between burrows that contained at least one commensal and to determine significant environmental effects and species associations, we conducted non-metric multidimensional scaling (NMDS) analyses.

We found that height was significantly greater for rural burrows (19.3 cm  $\pm$  0.7 vs. 17.5 cm  $\pm$  0.6, p=0.02). Length (141.2 cm  $\pm$  9.0 vs 127.2 cm  $\pm$  12.0, p=0.35) and

<sup>\*</sup> Corresponding author: lrs0073@auburn.edu

width (24.5 cm  $\pm$  0.9 vs 24.3 cm  $\pm$  1.0, p=0.90) showed no significant difference between rural and urban burrows, respectively. Species richness between urban and rural burrows was not significantly different (0.8  $\pm$  0.1 vs 0.7  $\pm$  0.1, p=0.45, W=586). There were no significant differences detected for individual taxa between habitat

Species richness, camel crickets, spiders, and millipedes had significant associations with NMDS axes 1 and 2 whereas no environmental variables did. Visual interpretations of the plot showed almost complete overlap between urban and rural burrows; however, rural burrows exhibited more variation.

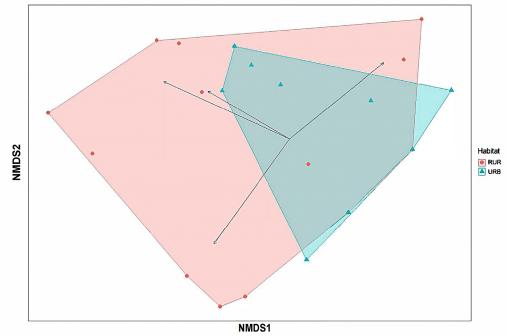


Fig 1. Non-Metric Multidimensional Scaling (NMDS) plot showing overlap between commensal species in urban and rural burrows.



Fig. 2. Representative photographs of commensal species observed in armadillo burrows in Mobile Co. AL.

Species richness was lower than in other studies, but armadillo burrows seem to be important refugia for certain taxa such as camel crickets, anurans, and millipedes (Fig. 2.). Other studies were conducted over longer time periods, which may have increased the number of species detected or exposed differences in species occurrence between habitat types.

Overall, our research suggests and supports previous studies that armadillo burrows are not replacements for gopher tortoise burrows, but still hold some conservation value.[5] Although no difference in taxa was noted between urban and rural burrows, all burrows were on residential properties and further research in more natural areas is warranted. Federally-listed spec-

ies that may utilize armadillo burrows, such as the eastern indigo snake, are less likely to be found in residential areas. By expanding future studies to these areas, the conservation value of armadillo burrows for commensal species can be more thoroughly examined.

#### Statement of Research Advisor

Ms. Stevens has done a fantastic job conducting independent research, both in the field and lab, on a timely issue. Since initiating this study additional manuscripts have been published on the topic from other parts of the country indicating that this is an important research question. Others are recognizing her work, as well, and her efforts were rewarded by placing 2nd in the research poster competition at The Wildlife Society's 2024 Southeastern Student Conclave in March 2024.

- Wesley M. Anderson, Forestry and Wildlife Sciences, College of Forestry, Wildlife and Environment

#### References

- [1] Smith, R.B., Tuberville, T.D., Chambers, A.L. Herpich, K.M. and Berish, J.E., 2005. Gopher tortoise burrow surveys: External characteristics, burrow cameras, and truth. Applied Herpetology, 2(2): 161–170.
- [2] Butler, Z.P., 2020, Assessing the Ecological Roles of Nine-Banded Armadillos on Georgia's Barrier Islands (Master's thesis, University of Georgia).
- [3] DeGregorio, B.A., Veon, J.T., and Massey, A., 2022. Wildlife associates of nine-banded armadillo (Dasypus novemcinctus) burrows in Arkansas. Ecology and Evolution, 12(5): e8858.
- [4] Di Blanco, Y.E., Desbiez, A L. J., Di Francescantonio, D., and Di Bitetti, M. S., 2020. Excavations of giant armadillos alter environmental conditions and provide new resources for a range of animals. Journal of Zoology, 311(4): 227–238.
- [5] Lamb, B.D., Anderson, C.D., Mcdonough, C.M., Lockhart, J M., and Butler, Z. P., 2023. A Comparison of Vertebrate Associates of Gopher Tortoise and Nine-Banded Armadillo Burrows in South Georgia. Chelonian Conservation & Biology, 22(2): 184–196.

#### **Authors Biography**



Lauren R. Stevens is a senior-year student pursuing a B.S. degree in Wildlife Ecology and Management at Auburn University. She has played key research roles in project development, data collection, organization, and writing of the highlight



Wesley M. Anderson is an assistant professor in the College of Forestry, Wildlife and Environment as well as a wildlife specialist with Alabama Extension. Much of his work relates to human-wildlife interactions and mitigating wildlife conflicts. He also is the program coordinator for Alabama Master Naturalist and leads citizen science initiatives through his Alabama Extension programming.



Olivia F. Sciandra is in the second year of her Wildlife Sciences master's program at Auburn University. Originally from Nevada and then Florida, she attended the University of South Florida, where she was awarded a B.S. in Environmental Biology and a B.A. in Anthropology. Her research investigated leprosy prevalence in nine-banded armadillos of Alabama and evaluated homeowner perceptions and management needs of Alabama residents.

## Living in the Tensions: Investigations of Gender Identity and Performativity in STEM

R. Smith Strain<sup>1</sup>, Noah Leibnitz<sup>2</sup>, Reagan Ruben<sup>3</sup>, Doris Li<sup>4</sup>, and Eric Burkholder<sup>5,\*</sup>

<sup>1</sup> Undergraduate Student, Department of Civil Engineering, Auburn University
 <sup>2</sup> Graduate Student, Department of Physics, Oregon State University
 <sup>3</sup> Undergraduate Student, Department of Biomedical Engineering, Georgia Institute of Technology
 <sup>4</sup> Assistant Professor, Department of Physics, Oregon State University
 <sup>5</sup> Assistant Professor, Departments of Physics & Department of Chemical Engineering, Auburn University

Judith Butler's "Theory of Gender Performativity" suggests that gender is directly impacted by the environment in which a person is situated, implying that gender is not inherent but also dependent on social influences [1]. This theory guided the research conducted, which sought to question whether the male-dominated STEM environment directly influenced how a student saw his/her/themself in a way that led to "over-performing" a gender stereotype associated with STEM (i.e., women associating themselves more with masculinity than femininity). Prior research suggests that feminine and masculine identities are not mutually exclusive and opposite, so it was also recognized that a student could feel both masculine and feminine at the same time (or neither), regardless of self-identification [2].

This research was conducted via a Qualtrics survey and semi-structured interviews. The survey was administered to students in all introductory physics courses, including calculus-based, algebra-based, and specialty courses (e.g., physics for aviation majors). We identified students who (1) consented to participate in the study, (2) self-identified as a woman or nonbinary or a student of color, and (3) indicated discrepancies between their gender identity and their reflected appraisal of gender. Data for (3) were collected using student responses to the following questions, which were Likert-scale questions ranging from 0 (not at all) to 6 (Very).

- 1. In general, how do you see yourself (Please answer all three scales.)
- (a) Feminine (b) Masculine (c) Androgynous (Neither feminine nor masculine)
- 2. In general, how do most people see you? (Please

answer all three scales.)

(a) Feminine (b) Masculine (c) Androgynous (Neither feminine nor masculine)

These students were then invited to participate in a post-survey interview to gain further insight into their answers. As compensation, each participant (then total) was given a \$25 Amazon gift card. The interview process for each interviewee consisted of the same protocol: each student was asked the same questions from the original survey, and changes in answers were noted. If there was a substantial difference between survey answers and interview answers, students were asked to explain why there may be such a distinct change. We also probed answers which showed misalignment between self-identified gender and gender performativity. For example, if a student identified as a female but displayed high scores associated with masculinity and/or androgyny, they were also asked to explain further.

A codebook was generated by the first and second authors of this study after analyzing interviews for similarities between each one (see Figure 1). The white cells with bolded text represent categories of themes, and the color-coded cells below those are the sub-themes within that category.

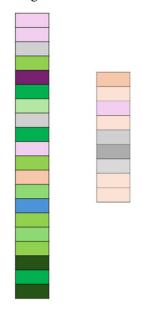
After compiling the codebook, R. S. S. and N. L. listened to the interviews again and noted each time a specific code was mentioned by a participant. These data were compiled in Excel, utilizing the highlighter tool to fill cells with the color associated with the corresponding code. By doing this, we could see patterns in interviews, such as an interviewee mentioning gender ratios significantly more than others did or an interviewee offer-

<sup>\*</sup> Corresponding author: ewb0026@auburn.edu

ing no reasoning at all for their answers. An example of two (out of 10) vastly different interviews is shown below in Figure 2.

Gender Consciousness	Gender ID RationaleAspecific	
Behavior Change	Feeling	
Other	No Reasoning	
STEM Culture	Unable to Relate	
Gender Ratios	Unimportance	
Overt Stigmas	Gender ID RationaleBehaviors	
Pressure of Expectation	Other Behaviors	
Institutional Culture	STEM Behaviors	
Negative Influence	Gender ID RationaleTraits and Characteristics	
Positive Influence	Other Traits	
Neutral	STEM Traits	

**Figure 1:** Codebook generated from interviews.



**Figure 2:** Ginny (left) and Hannah's (right) interviews.

These two interviews reflect the substantial variations in how self-identified women perceive and perform their gender (Figure 2). The first woman, "Ginny" (pseudonym), touches on numerous themes during her interview. Her answers to questions asking her to re-evaluate herself on the gradational scale of gender identity were based on a "feeling" and no specific explanation. However, Ginny also displayed a high gender stigma consciousness in her other answers through her descriptions of her own behavioral changes with respect to both STEM and non-STEM cultures at Auburn. She mentioned gender ratios and overt discrimination she has heard of or faced within her major. She mentions

a time when no male students in the lab wanted to be involved in a lab group that was comprised of women, even though, for the most part, her group scored higher on assignments than the male groups. Ginny also mentioned the pressure of expectation at the end of her interview, which she described as the feeling of needing to over-perform to compensate for her simply being a woman in STEM as if she needed to prove something more than her male counterparts. We noted similar themes in a prior investigation of reasons why women decided to leave physics [3]. This theme did not appear as frequently as expected across the other interviews, perhaps because interviewees were unsure how to convey this idea or it was not specifically elicited by the protocol.

Hannah's interview (on the right in Figure 2) is significantly more concise and rarely mentioned the STEM classroom or environment. She described her identity using traits stereotypically associated with femininity, such as being caring or compassionate, suggesting the presence of internalized stereotypes about what a woman is or should be.

These two cases illustrate two very different ways that women in STEM perform and feel their gender and suggest that, while STEM classrooms do have a substantial influence on how some women perform their gender, there are other factors that influence how women feel and perform their gender. Due to the qualitative nature of this research, we cannot make claims about the generalizability of our findings. However, we believe that this research offers insights for future studies of how women in STEM perform their gender in other contexts. For example, another institution may find different conclusions using the same methodology. We are hopeful about the implications this and further research will have on the future of women in STEM fields, whether it be focusing on recruitment or retention within the subject.

#### **Statement of Research Advisor**

The research being conducted by Smith is a ground-breaking study of the nuanced ways gender is felt and performed by undergraduate engineering students. Smith conducted the interviews and transcribed and analyzed the data in conjunction with researchers at Oregon State University. This will lay the groundwork

for future studies about how gender performance influences academic and psychological outcomes for engineering students in physics courses.

-Eric Burkholder, Department of Physics and Department of Chemical Engineering, College of Science and Mathematics

#### References

[1] Butler, Judith "Performative Acts and Gender Constitution: An Essay in Phenomenology and Feminist Theory", March 19, 2024, (1990).

[2] Li, Yangqiuting & Burkholder, Eric, "Investigating students' self-identified and reflected appraisal of femininity, masculinity, and androgyny in introductory physics courses" Physical Review Phys. Educ. Res. (2024).

[3] Strain, R. Smith, Shepherd, Matthew S. & Burkholder, Eric, "Defiance in the face of adversity: a qualitative study of women's attrition from and persistence in physics," PERC Proceedings (2023).

#### **Authors Biography**



Smith Strain is a junior student pursuing a B.S. degree in Civil Engineering at Auburn University. She has been conducting research under her mentor and advisor, Dr. Eric Burkholder, for the past two years, studying physics education research (PER) and gender issues within physics and STEM more broadly.



Noah Leibnitz is a graduate student in the Physics Department at Oregon State University (OSU). He plans to continue being involved with PER and gender research in the future as he pursues higher education.



Reagan Ruben is an undergraduate student pursuing a B.S. degree in Biomedical Engineering at the Georgia Institute of Technology. She has a strong interest in continuing research in PER and gender and STEM-related topics in the future.



Yangqiuting (Doris) Li is an Assistant Professor of Physics at OSU specializing in PER. She holds a B.Sc. in physics from Nanjing University and a Ph.D. in PER from the University of Pittsburgh. Before joining OSU, she was a postdoctoral researcher at Auburn University. Her research centers on improving students' learning and motivational beliefs in introductory and advanced physics courses.



Eric Burkholder is an Assistant Professor of Physics at Auburn University, specializing in physics education research. He has a B.S. in Chemical Engineering from Cornell University and a Ph.D. in Chemical Engineering from the California Institute of Technology. Prior to working at Auburn, he was postdoctoral research at Stanford University. His research focuses on understanding the teaching and culture of physics within broader educational systems.

## **Modelling Interorgan Transfer of Ionizing Radiation Amongst In Vivo Species**

Jack Terry 1,\*, Isaac Meyer2, and Minseo Park3

<sup>1</sup> Undergraduate Student, Dept. of Physics, Auburn University
 <sup>2</sup> Postdoctoral Researcher, Dept. of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School
 <sup>3</sup> Professor, Dept. of Physics, Auburn University

In a world where cancer treatment is on the forefront of medical research, radiation therapy has become extremely popular. Radiation therapy is the use of ionizing radiation to target and destroy cancer cells while minimizing damage to surrounding healthy tissues. Radiation therapy can be administered externally, where radiation is delivered from outside the body using a machine, or internally, where radioactive materials are placed directly into or near the tumor site. Maximizing the tumor-to-normal ratio of radiation is the subject of modern radiotherapy research, and one area of focus is on the behavior of ionizing energy inside the body.

To model radiotherapy, our lab uses TOPAS (Tool for Particle Simulations), which is a Monte-Carlo simulation program for radiation onto phantoms (Perl et al., 2012) Therefore, our project is focused on using TOPAS to model the interorgan transfer of ionizing radiation inside the body of a male mouse and bolster the functions of TOPAS to enable us to model the change in radioactivity over time in different areas, organs, of the body. In doing so, we will be able to unite in vivo data with a visualization tool, MOBY/ROBY, which is a phantom created by Duke University and shown in Figure 1 (Duke).

Although we had a specific data set, we wanted to make the program general so that it could be widely used. This started with the creation of classes and functions that allowed us to model the movement of radiation through position space in the body over time (Perl et al.) We then coupled those with existing code that was used to model the effects of radiation on a mass, as shown in Figure 2, and are working to put it all together into a neatly packed, easy to use system.

**Figure 1:** Duke University MOBY phantom used for the visual modeling of interorgan transfer (Duke).

```
GAThreeVector 'matMinVec, 'matMaxVec;
GAThreeVector 'matMinVec, 'matMaxVec;
GAVisExtent 'scurExtent;
std::pair<GAThreeVector, GAThreeVector> extent;
for (auto org: forganToMaterialMap){
    fOrganExtentMap[org.first] = GAVisExtent(DBL_MAX, DBL_MIN, DBL_MAX, DBL_MA
```

**Figure 2:** Code example for uniting our material activity distribution to phantom.

In specific, my emphasis has been on the creation of a program that can match and map different organs if data does not match the prespecified MOBY organs. This catalog of organ-to-organ maps will allow for a wide range of data to be used and allow researchers to use data that was not necessarily designed for MOBY to still be incorporated and visually modeled.

LA LV RV LV

<sup>\*</sup> Corresponding author: jft0010@auburn.edu

Although our code is not yet finished, the importance of it will be paramount in allowing many researchers to simulation and test concepts in the time effects of radiation and specifically the movement and migration of radiation from the tumor locale. This will allow doctors to better allocate radiation doses and understand more of the adverse effects that plague cancer patients.

#### **Statement of Research Advisor**

This undergraduate research project that Mr. Jack Terry is carrying out is about simulating the process of transfer of ionizing radiation amongst organs in a living species using a Monte Carlo type simulation package named TOPAS for the modeling of the post-treatment efficacy of radiation damage in a living species. This work was performed by Jack Terry in collaboration with Dr. Isaac Meyer at Harvard medical school, with my assistance in their research activities.

-Minseo Park, Department of Physics, College of Sciences and Mathematics

#### References

MOBY/ROBY Phantom, Duke University CVIT. https://cvit.duke.edu/resource/moby-roby-phantoms/

Perl J, Shin J, Schumann J, Faddegon B, Paganetti H. TOPAS: an innovative proton Monte Carlo platform for research and clinical applications. Med Phys. 2012 Nov; 39(11):6818-37

Perl J, Shin J, Schumann J, Faddegon B, Paganetti H. Open TOPAS. https://github.com/OpenTOPAS/OpenTOPAS.

#### **Authors Biography**



Jack Terry is a senior-year student pursuing concurrent B.S. degrees in Physics and Economics at Auburn University. He has participated in medical physics research through the American Association of Medical Physicists as a summer undergraduate fellow. Additionally, he is a member of FarmHouse Fraternity where he served as Executive VP. He will be pursuing his MSc Economics next year at London School of Economics and Political Science.



Isaac Meyer is a Postdoctoral Fellow in Radiation Oncology Reasearch at the Massachusetts General Hospital and Harvard Medical School. He received his B.S. (2017) in Nuclear Engineering at the University of California, Berkeley. He obtained his Ph.D. (2023) at the Massachusetts Institute Technology where he worked on uncertainty propagation of nuclear cross section data and was sponsored by the Nuclear Critical Safety Program at Oak Ridge National Laboratory. His postdoctoral work has focused on Monte Carlo transport in radiation therapy: including secondary cancer risk estimation and novel DNA geometries. He is an active collaborator with the OpenTOPAS code and organization which wraps and extends Geant4 for use in medical physics.



Dr. Minseo Park is a Professor in the Department of Physics at Auburn University. He received his B.S., M.S., and Ph.D. from Yonsei University, KO-REA (1991), Iowa State University (1994), and North Carolina State University (1998), respectively. Since he joined Auburn University as an Assistant Professor in 2003, he has produced 14 Ph.D. under his guidance. Dr. Park has taught classes such as general physics, engineering physics, introduction to solid state physics, physics laboratory skills, fundamental experiments in physics, introduction to nanotechnology, physics of the world around us (physics of music), etc. His research program focuses on wide band gap semiconductors, nanotechnology, optical spectroscopy, and biosensors. Park's research has been funded by the National Science Foundation (NSF), United State Department of Agriculture (USDA), sile Defense Agency (MDA), Korea Institute of Industrial Technology (KITECH), Army Research Office (ARO), etc. He was a holding a position of Scharnagel Endowed Professorship (10/2010 - 10/2012) and Walter Endowed Professorship (10/2012 - 05/2017). He was a recipient of the following awards; Eugene J. Clothiaux Faculty Award, COS-AM, Auburn University (2022), Outstanding Faculty Member Award, Graduate School, Auburn University (2016). Mer-Award, itorious KSEA-AL (2010), and Young Scientist Award, IVMC'98, Asheville,

NC, USA (1998). He has published about 85 peer-reviewed research articles in internationally reputable journals. He is holding the following 2 US Patents: "Radiation Hardened Thin-Film Transistors", U.S. Patent 11,682,734 B2 and 11,069,815, issued in 2023 and 2021, respectively.

### A Landmark-Based Assessment of Humeri and Femora from Highly Fragmentary and Commingled Archaeological Skeletal Remains

Cameron G. Troyer<sup>1,\*</sup>, Anna M. Buckingham<sup>2</sup>, Toni M. Lee<sup>2</sup>, and Kristrina A. Shuler<sup>3</sup>

Auburn Alumni, Department of Sociology, Anthropology, and Social Work, Auburn University
 Undergraduate Student, Department of Sociology, Anthropology, and Social Work, Auburn University
 Associate Professor, Department of Sociology, Anthropology, and Social Work, Auburn University

#### **Abstract**

Large samples of fragmentary and commingled skeletal remains present unique challenges in bioarchaeology. We examined non-repeating osteological landmarks (Mack et al. 2016) to assess Minimum Number of Elements (MNE) for humeri and femora from a highly disturbed area from the Newton Plantation archaeological site in Barbados. After sorting and refitting adult humeri and femora from the site, we assessed 15 and 13 possible landmarks on each element, respectively. Only bone fragments that could be sided with at least one (>50%) observable landmark present were counted to avoid recounting the same bone. The Number of Identified Specimens (NISP or fragments) for humeri included 100 elements with 315 non-repeating landmarks. The NISP for femora was 93 elements with 239 non-repeating landmarks. Completeness (preservation) scores were low for humeri (left 33%; right 17%) and femora (left 19%; right 20%). Fragmentation scores further showed discrepancies in left (1.38) and right (2.03) humeri and for left (2.09) and right (1.68) femora. While the highest MNE was seen in the distal shaft of humeri, the left side (n=18) fits observations from other landmarks more closely than the right (n=37). Conversely, femora showed more similarity between sides than humeri with (n= 21) left and (n=29) right, respectively. The traditional approach to counting commingled remains from archaeological contexts (Buikstra and Ubelaker 1994) was not possible for this assemblage due to fragmentation and inability to accurately differentiate overlapping segments. Grouping sides of each element together exaggerated the calculated MNI, portions preserved, NISP, portions defined, and fragmentation index. The specimen-by-specimen evaluation using the landmark method is more practical and

approachable in a highly fragmented commingled and taphonomic context.

Keywords: Archaeology, Commingled remains, Landmarks, Fragmentation, Taphonomy

#### Introduction

Bioarchaeology is the reconstruction of life, health, and nutrition from human skeletal remains in archaeological contexts (Larsen 2015). Large samples of fragmentary and commingled skeletal remains can present unique challenges in bioarchaeology, including the ability to accurately assess the number of individuals and bony elements interred within a site. In this study, we attempt to use a recently published method for assessing bony landmarks to evaluate a large series of highly fragmentary and commingled skeletal remains from the Newton Plantation archaeological site in Barbados (Shuler 2005, Shuler et al. 2008, 2019).

Newton Plantation in Barbados was an active British sugar plantation from ca. 1660 to 1820 (Handler and Lange 1978). An archaeological site at the Newton Plantation is one of the largest archaeological projects to explore the lives of enslaved Africans in the Atlantic world. Bioarcheological research on Newton has focused on skeletal remains found during excavations in 1971-1973 by J. Handler (N=104, subsequently reinterred) and 49 articulated individuals excavated in 1997-1998 by K. Shuler and R. Pasquariello. This project includes ongoing international collaborations and has resulted in numerous publications and Ph.D. and M.A. theses on health, demography, biogeography, and early childhood growth and development. Research on Newton Plantation has been conducted at Auburn Uni-

<sup>\*</sup> Corresponding author: cgt0021@auburn.edu

versity since 2010 with permission from the Barbados Museum (Shuler et al. 2019).

The site was first excavated from 1971-1973, uncovering the remains of 104 individuals who were subsequently reinterred by the original excavators (Handler et al. 1989). Excavations by K. Shuler and R. Pasquariello in 1997 and 1998 revealed remains of 49 articulated individuals, some of whom had been reinterred from previous excavations at the site. During the 1997 excavation, a large mass of highly commingled and fragmented remains was encountered in Mound 2 along with modern objects, including candy wrappers and a 1965 coin, suggesting that these are reinterred elements from the original excavations (Shuler 2005, Shuler et al. 2008). Previous study of the Mound 2 remains using traditional methods for assessing commingled remains (Buikstra and Ubelaker 1994) indicated a MNI of approximately 24 individuals (Shuler et al. 2008, Herndon and Shuler 2012).

Commingled remains necessitate unique methods of analyses (used in forensic anthropology) and ample lab time. For this project, preliminary sorting of the highly commingled and fragmentary bones and dentition from the site was conducted with the help of numerous osteology students since 2010 under the direction of Dr. Shuler in the Bioanthropology Laboratory at Auburn University. Fine sorting of humeri and femora (along with other elements) was conducted again over two semesters in 2022-2023, led by the first two authors. We focused on identifying the Minimum Number of Individuals (MNI) and Minimum Number of Elements (MNE), which will contribute to reuniting elements from individuals to facilitate respectful interment of the remains.

#### Methods/Materials

The process began with sorting, siding, and refitting fragments of adult humeri and femora. Fragments that could be confidently sided with at least one observable (>50%) landmark were then analyzed using the landmark method detailed by Mack et al. (2016). The lead author created forms to capture the landmark data from each element consisting of a blank table to be filled out by the assessor. Each form included equations for the NISP, PP, PD, FI and MNE, along with each landmark for that specific element. Following fine sorting of the

two elements, counts of each element were conducted by the first author, confirmed by Dr. Shuler, and recorded on the relevant form.

#### Results

The humeri NISP is 100 with a total of 315 non-repeating landmarks observed. The femora included NISP of 93 with 239 non-repeating landmarks observed. The completeness (preservation) scores were low for the humeri (left 33%; right 17%) and the femora (left 19%; right 20%). Fragmentation index scores further showed discrepancies in left (1.38) and right (2.03) humeri and for left (2.09) and right (1.68) femora. The highest MNE for the humeri was observed in the distal shaft landmark. The MNE for the left side (n=18) fits observations from other landmarks more closely than the right (n=37). Conversely, the linea aspera of the femora showed more similarity between sides with left (n=21) and right (n=29).

#### Discussion

The MNI determined by this study is 37 individuals. This is higher than the MNI of 24 individuals previously determined from the Max (L, R) method (Herndon and Shuler 2012), but is closer to the estimate that 40 - 50 individuals were recovered from the Mound 2 area by excavations in 1997 (Shuler 2005). Mound 2 had been partially bisected during the first excavations, potentially leading to disparities in elements represented from each side (Shuler 2005, Shuler et al. 2008). The landmark method by Mack et al. (2016) proved beneficial for this study due to ease of application and less reliance on subjective determinations. Our assessment of MNE and MNI using the landmark method is limited and likely undercounted the MNI in that we did not consider elements that could not be confidently sided and excluded subadult remains from assessment. However, this method suggests greater accuracy than traditional methods and, with the use of published reports and unpublished documentation, future analyses of the remaining commingled elements may offer the best potential for approximating the number of individuals who were reinterred at this site.

#### Conclusion

Large samples of fragmentary and commingled skeletal remains present unique challenges in bioarchaeology. The traditional approach (Buikstra and Ubelaker 1994) was attempted but was problematic due to fragmentation and inability to accurately differentiate overlapping segments (Shuler et al. 2008). Grouping sides of each element together exaggerated the calculated MNI, portions preserved, NISP, portions defined, and fragmentation index. The specimen-by-specimen entry from the Landmark method is more practical and approachable in a highly fragmented commingled and taphonomic context.

#### Acknowledgements

The authors would like to thank the Barbados Museum and Historical Society and the nation of Barbados for their continued support of the Newton Plantation bioarchaeological research project. We would also like to thank the faculty and students from the Department of Sociology, Anthropology, and Social Work at Auburn University for their support and assistance on this project, including initial sorting of elements. We appreciate the efforts of Jett Kasch (Auburn University) who assisted with graphics.

#### Statement of Research Advisor

Cameron, Anna, and Toni did an excellent job in their collaboration, organization, and respective contributions to this project. After serving as team leaders on a class osteology project in the Fall of 2022, Cameron and Anna spent extensive time in the lab over the next year honing their knowledge of human skeletal anatomy and assisting with the identification and sorting of highly commingled and fragmentary bones from individuals whose remains had been reburied in mass pits following excavations at the Newton Plantation archaeological site in the 1970s. Toni aided the team with communication of the results by designing an effective poster for a national conference, which Cameron and Anna presented, and by organizing the highlights article for this journal. Based on documentation from the original excavations in the 1970s, this current assessment of humeri and femora suggests that the new landmark method may provide a more accurate representation of the minimum number of individuals who were reburied in the mass context than traditional counts that my team previously attempted. Results of this preliminary study will be incorporated with ongoing work on other elements from the site and will be

vital in reuniting the remains of individuals to facilitate their respectful reinterment in Barbados.

- Kristrina Shuler, Anthropology, College of Liberal Arts

#### References

Buikstra, J. E. and D. H. Ubelaker. 1994. Standards for data collection from human skeletal remains. Arkansas archaeological survey research series.

Handler, J.S. and Lange, F.W., 1978. Plantation slavery in Barbados: an archaeological and historical investigation. Harvard University Press.

Handler, J.S., M.D. Conner, and K.P. Jacobi. 1989. Searching for a slave cemetery in Barbados, West Indies: a bioarchaeological and ethnohistorical investigation. Carbondale: Southern Illinois University Center for Archaeological Investigation.

Herndon, K.E., and K.A. Shuler. 2012. Analyses of pathological lesions in commingled remains from a slave cemetery at Newton Plantation, Barbados. The 39th Annual Meeting of the Paleopathology Association, Portland, OR.

Larsen, C.S. 2015. Bioarchaeology: interpreting behavior from the human skeleton (Vol. 69). Cambridge University Press.

Mack, J. E., Waterman, A. J., Racila, A. M., Artz, J. A., & Lillios, K. T. 2016. Applying zooarchaeological methods to interpret mortuary behaviour and taphonomy in commingled burials: the case study of the Late Neolithic site of Bolores, Portugal. International Journal of Osteoarchaeology.

Shuler, K.A., 2005. Health, history, and sugar: a bioarchaeological study of enslaved Africans from Newton Plantation, Barbados, West Indies. Southern Illinois University at Carbondale.

Shuler, K.A., R.D. Pasquariello, R.D., R.S. Corruccini, and H. Schroeder. 2008 Newton revisited: The 1990s excavation, reconstruction, and interpretation of a 1970s-excavated mortuary complex. The 107th Annual Meeting of the Association of American Anthropologists, San Francisco.

Shuler, Kristrina A., Hannes Schroeder, and William D. Stevens. 2019. "Health and life histories of enslaved sugar producers. Bioarchaeology of the Newton Plantation, Barbados." In Pre-Colonial and Post-Contact Archaeology in Barbados: Past, Present and Future Research Directions, edited by de Waal, Maaike S., Niall Finneran, and Matthew C. Reilly with Douglass Armstrong and Kevin Farmer, 269-286. Netherlands: Sidestone Press. ISBN 978-9088908460.

#### **Authors Biography**



Cameron Troyer is a 2024 Auburn University graduate with a B.A. in Anthropology a minor in history. She will be pursuing an M.A in Anthropology with a focus in Bioarchaeology at the University of Southern Mississippi as a graduate assistant beginning in Fall 2024. Her research interests include paleopathology, taphonomy, and reconstructing past lifethrough osteological ways analysis.



Anna M. Buckingham is a senior-year student pursuing a B.A. in Anthropology with a minor in Biological Sciences at Auburn University. Her research interests include bioarchaeology and evolutionary skeletal development of hominids and non-human primates.



Toni M. Lee is a senior student at Auburn University. She is currently working towards a B.A in Anthropology with a minor in Public Health. She is an Undergraduate Research Fellow at Auburn University. Her research interests lie in reconstructing the health of past populations, paleopathology, and bioarchaeology.



Kristrina A. Shuler is an associate professor in the department of Sociology, Anthropology, and Social Work at Auburn's College of Liberal Arts. She is a biological anthropologist whose primary focus is upon health and nutrition in early colonial populations from the Caribbean, Southeaster U.S., and Latin America.

# Functional Analysis of Subpopulation-Specific DNA Ligase 1 Gene Polymorphisms in Human Cancers

Abigail Weir<sup>1,\*</sup>, Katherine Marlow<sup>2</sup>, Razan Waliagha<sup>3</sup>, and Amit Kumar Mitra<sup>3,4</sup>

Department of Chemistry and Biochemistry, COSAM, Auburn University
 Department of Curriculum and Teaching, College of Education, Auburn University
 Department of Drug Discovery and Development Harrison, College of Pharmacy, Auburn University
 Center for Pharmacogenomics and Single-Cell Omics initiative (AUPharmGx), Auburn University

Pharmacogenomics leverages the relationship between a person's genome and its specific effect on therapeutic treatments [8]. The use of pharmacogenomics has been a growing topic in the pharmaceutical industry because it determines how the unique genetic makeup of each person determines the best course of therapeutic treatment. The link between the genome and treatments can be seen in the use of single nucleotide polymorphisms (SNPs). SNPs are single base pair changes of DNA, or point mutations, and are one of the sources of variations between human genomes. These variations can act as genetic markers of how an individual's genome responds to a therapeutic treatment [7].

Global projects, like the Human Genome Project, looked to understand the implication of genetic variations on human health. The Human Genome Project has come to understand much about common and rare diseases using a full human genome sequence [1]. However, this project had a broad focus on global populations. SNPs can vary by region, and projects like the Human Genome Project, do not consider the higher frequency of SNPs and genetic heterogeneity in certain areas.

India is a very diverse region. It takes up 2.4 % of the world's land area and has over 17.5 % of the world's population. India also contains a wide range of subpopulations divided by factors such as language, religion, geographic region, etc. The goal of the Indian Genome Variation Consortium was to perform an in-depth study of SNPs and repeats specific to Indian subpopulations [2]. Our primary focus was to identify functionally relevant SNPs involved in DNA repair and replication, such as DNA Ligase 1 [4].

This project was planned in 3 phases. Phase 1: SNPs in LIG1 were evaluated based on the predicted functional effect. The collected data was used to determine the SNP predicted to be the most damaging. Phase 2: Determine the difference in expression of LIG1 and verify the presence of the SNP in different prostate cancer cell lines. Phase 3: Investigate the role of the top functional SNPs on the DNA repair and replication system by introducing the SNP into human cancer cell lines.

Successful completion of this project will determine deleterious SNPs in LIG1 associated with DNA repair and replication defects and eventually resistance to anti-cancer drugs. Ultimately, this project will assist with a personalized medicine approach to DNA-repair-deficiency-associated cancers specific to world subpopulations like India.

The SNP discovery and validation were based on determination of minor allele frequencies (MAF) in 55 different subpopulations of India representing all major linguistic subgroups and ethnicities. Further SNP evaluations were conducted using the National Center for Biotechnology Information (NCBI). NCBI data provides information about the SNP's chromosome position, alleles, clinical significance, gene consequence,

DNA Ligase 1 (LIG1) is a replicative ligase involved in multiple mechanisms of DNA repair and replication. LIG1 functions by reforming the phosphodiester bond of a nicked DNA strand. This enzyme works to join Okazaki fragments in DNA replication as well as functions in DNA repair pathways such as base excision repair and nucleotide excision repair [9].

<sup>\*</sup> Corresponding author: aew0106@auburn

and aggregate allele frequency. Criteria for SNP selection involved gene importance in cancer mechanisms, consequence of the variation, and frequency of the SNP.

For pharmacogenomic evaluation, we selected five genes. These genes are involved in DNA repair and replication, LIG1 APEX1, and drug metabolism, CYP2E1, CYP2B6, and PTGIS. The reference sequence for each gene was collected and annotated from the NCBI gene database (https://www.ncbi.nlm.nih.gov/gene/). Established relationships between SNPs in certain genes and drug treatments were explored using drug label annotations and clinical annotations from PharmGKB (https://www.pharmgkb.org/) as well as GeneCards (https://www.genecards.org/). Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for gene-drug relationships were noted if available [6]. LIG1 was selected for further study due to the number of DNA repair and replication mechanisms it is involved in and its unexplored link to current chemotherapy treatments.

The gene consequence of the SNPs was evaluated to determine the predicted damaging effect of each variation. SNPs which were predicted to be missense mutations and, therefore, change the structure and function of the produced enzyme, were selected for further study. The predicted gene consequence was determined using NCBI SNP database (https://www.ncbi.nlm.nih.gov/snp/), SIFT prediction (https://sift.bii.a-star.edu.sg/www/SIFT\_dbSNP.html), and the HumDiv and HumVar models from PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) (Fig. 1). Within LIG1 there was predicted to be five deleterious mutations: rs3731003, rs4987070, rs4987181, rs11666150, and rs12981963.

In comparison to allele frequencies from Indian subpopulation data, the SNPs of interest are the ones in which the global NCBI frequency is much lower than that of the subpopulations (Fig. 2). These SNPs are likely overlooked by global genome studies and require a more specific study. There were three SNPs within LIG1 which had a higher MAF in the Indian subpopulation as compared to the global population: rs4987070, rs4987181, and rs11666150. Based on the criteria, the SNP list was narrowed down to rs4987181 from LIG1.

Primers were designed and ordered for the SNP using

Primer3 (https://bioinfo.ut.ee/primer3-0.4.0/). The section of the gene chosen was ensured to be adequate for testing through PCR and DNA Sanger sequencing. The gene of interest was amplified by PCR in six DNA samples isolated from human cancer (multiple myeloma) cell lines using an optimized annealing temperature of 57.9 °C. The samples used included U266P, JIM3, U266VR, RPMI8226VR, MMISVR, and UTMC2. There was successful amplification of LIG1 in all the samples except UTMC2.

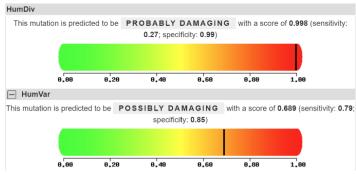
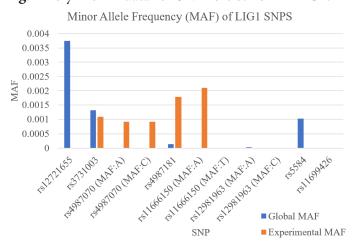


Fig. 1 PolyPhen-2 data for SNP rs4987181 in LIG1.



**Fig 2.** Minor allele frequency of SNPs in LIG1 in global population data (blue) and Indian subpopulation data (orange).

Bidirectional sanger sequencing was performed on the five successful samples using the SeqStudio Genetic Analyzer System with SmartStart (Applied Biosystems, Foster City, USA). Results from sequencing were analyzed using DNASTAR software for sequence alignment (DNASTAR, Madison, WI, USA). The sequence alignment confirmed the primers accurately targeted the region of LIG1 surrounding the SNP of interest. Although, the SNP of interest was not present in any of the multiple myeloma samples tested. This is because all the cancer cell lines belonged to non-Indian ances-

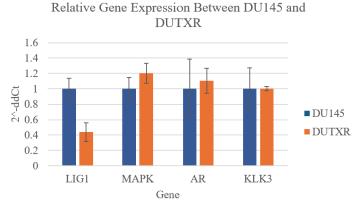
tries (non-Hispanic whites, African Americans, and East Asians), further emphasizing the importance of subpopulation-specific functional analysis. Within the targeted region, two other SNPs were present in several of the samples: rs3730861 and rs3730862. These SNPs are both in intronic regions and have no linked clinical significance. This indicates that they should not interfere with further testing in this region of LIG1.

Throughout global populations, prostate cancer (PC) remains one of the leading causes of cancer related death in males [3,5]. Its growing prevalence in the world necessitates the need for better and more personalized treatments. Recently approved PC therapies have focused on targeting the DNA repair pathways. Therefore, in our SNP evaluation, established prostate cancer cell lines were used for differential gene expression analysis. First, we compared the Castration-resistant prostate cancer cell line DU145 and the clonally derived taxane resistant line DU145-TxR (DUTXR). Thus, DUTXR differs from DU145 in its resistance to taxanes, a type of chemotherapy. Since both cell lines are androgen receptor negative, they will continue to grow in the absence of male hormones [3,5].

Relative gene expression in prostate cancer cell lines were determined using quantitative PCR (qPCR) through the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). LIG1 was measured in both cell lines in comparison to the housekeeping gene Beta-Actin (ACTB) using the ΔΔCt method (Fig. 3). It was determined that there was a higher relative LIG1 expression in the DU145 cell line compared to the DUTXR cell line. Other genes measured to confirm the AR status of the cell lines were Androgen Receptor (AR), Kallikrein Related Peptidase 3 (KLK3) or Prostate-Specific Antigen, and Mitogen-Activated Protein Kinase 14 (MAPK14). These genes all had similar expression between the two cell lines indicating they were both AR negative (Fig. 3).

In conclusion, the evaluation of the SNPs revealed multiple SNPs that were uniquely significant to India and SNPs that were unique to specific subpopulations, such as rs4987181. The SNP evaluation also revealed that multiple SNPs are uniquely prevalent in these subpopulations and have deleterious effects. This alludes to the high diversity of subpopulations in India and the need

for subpopulation-specific studies in relation to genetic variations. Furthermore, we validated the differential expression of LIG1 between taxane-sensitive and taxane-resistant AR negative cell lines. Comparison of established prostate cancer lines suggests that DUTXR could be more affected by a mutation in LIG1. This might be of significance for chemotherapies targeting DNA repair in these aggressive forms of cancer.



**Fig. 3** Relative gene expression of LIG1, MAPK, AR, and KLK3 between DU145 and DUTXR cell lines.

Future work in this project will measure other relative gene levels in other established prostate cancer cell lines. These cell lines include an AR-positive line, Ln-CAP, and an AR-negative cell line with high metastatic potential, PC3M. The results of the SNP evaluation also motivated the exploration of how rs4987181 affects cancer cell lines. This will be studied using an in vitro model. Therefore, once all cell lines have been assessed for LIG1 levels, the SNP rs4987181 will be introduced to the cell line using site-directed mutagenesis (recombinant NA technologies). A cytotoxicity study for both the wild-type cell line and mutated cell line will be conducted using standard and novel chemotherapeutic agents to investigate the effect of LIG1 SNPs on drug efficacy.

#### **Statement of Research Advisor**

Abigail's research involves the functional analysis of the DNA repair gene LIG1 mutations. Her work incorporates bioinformatics and molecular biology approaches, i) to identify novel and reported SNPs in LIG1, ii) to predict the functional relevance of top LIG1 SNPs, iii) to investigate the effect of LIG1 SNPs on differential gene regulation in cancers, iv) to perform functional genomics studies to study the impact of differential LIG1 expression on DNA repair mechanisms vis-à-vis

cancer chemotherapy. We are planning to further this work with a particular focus on world populations and underserved subpopulations.

- Amit K. Mitra, Department of Drug Discovery and Development, Harrison College of Pharmacy

#### References

- [1] Birney, E. The International Human Genome Project. Hum Mol Genet 30, R161-R163 (2021). https://doi.org/10.1093/hmg/ddab198
- [2] Indian Genome Variation, C. The Indian Genome Variation database (IGVdb): a project overview. Hum Genet 118, 1-11 (2005). https://doi.org/10.1007/s00439-005-0009-9
- [3] Mazumder, S. et al. Integrating Pharmacogenomics Data-Driven Computational Drug Prediction with Single-Cell RNAseq to Demonstrate the Efficacy of a NAMPT Inhibitor against Aggressive, Taxane-Resistant, and Stem-like Cells in Lethal Prostate Cancer. Cancers (Basel) 14 (2022). https://doi.org/10.3390/cancers14236009
- [4] Mitra, A. K., Singh, A., Indian Genome Variation, C. & Rath, S. K. LIG1 polymorphisms: the Indian scenario. J Genet 93, 459-469 (2014). https://doi.org/10.1007/s12041-014-0415-7
- [5] Mitra Ghosh, T. et al. Metronomic Administration of Topotecan Alone and in Combination with Docetaxel Inhibits Epithelial-mesenchymal Transition in Aggressive Variant Prostate Cancers. Cancer Res Commun 3, 1286-1311 (2023). https://doi.org/10.1158/2767-9764. CRC-22-0427
- [6] Relling, M. V. et al. The Clinical Pharmacogenetics Implementation Consortium: 10 Years Later. Clin Pharmacol Ther 107, 171-175 (2020). https://doi.org/10.1002/cpt.1651
- [7] Relling, M. V. & Evans, W. E. Pharmacogenomics in the clinic. Nature 526, 343-350 (2015). https://doi.org/10.1038/nature15817
- [8] Roden, D. M. et al. Pharmacogenomics. Lancet 394, 521-532 (2019). https://doi.org/10.1016/S0140-6736(19)31276-0

[9] Sallmyr, A., Rashid, I., Bhandari, S. K., Naila, T. & Tomkinson, A. E. Human DNA ligases in replication and repair. DNA Repair (Amst) 93, 102908 (2020). https://doi.org/10.1016/j.dnarep.2020.102908

#### **Authors Biography**



Abigail Weir is a senior-year undergraduate pursuing a BS degree in Biochemistry at Auburn University. As an undergraduate research trainee in Dr. Mitra's lab (DDD), she has developed the expertise molecular biology tools (cloning, Sanger DNA sequencing, Real-time qPCR, etc.) to perform mutational analysis in DNA repair genes such as LIG1 and MSH2.



Katherine G. Marlow is a Masters student pursuing an M.S. degree in General Science Education at Auburn University. She holds a B.S. in Genetics from Auburn University. She has played key research roles in understanding pharmacogenomic biomarkers in drug metabolism genes and DNA repair genes.



Razan Waliagha, MS is a current third year dual degree PharmD./PhD. student at Auburn University Harrison College of Pharmacy. Her current work involves understanding the novel mechanisms of resistance to anti-cancer therapies in solid tumors

#### Auburad University Journal of Undergraduate Scholarship



Dr. Amit Kumar Mitra is an Assistant Professor at the Harrison College of Pharmacy at Auburn University. He is also the founding Director of the Center for Pharmacogenomics and Single-Cell Omics (AU-PharmGx). Dr. Mitra's current research involves integrating in vitro and ex vivo drug response modeling, single-cell multi-omics, and functional genomics with translational bioinformatics to investigate inter-tumor and intra-tumor (subclonal) heterogeneity in human cancers.



Undergraduate Research Senior Vice President for Research and Economic Development 206 Cater Hall Auburn, AL 36849