Undergraduate Journal of Scientific Research

scientia

Vol. 11
Spring 2020

scientia.nd.edu

Understanding the Surplus of Gas in the Milky Way

Astrophysics research works to understand the gaseous complexities of our galaxy

PAGE 15

Research lab uses nuclear physics to detect toxic PFAS chemical class

Using ion beam analysis to efficiently detect PFAS in everyday items

PAGES 17-18

Advancing Ecology Research

Research conducted at UNDERC property accelerates our knowledge of fresh-water ecosystems

PAGES 27-38

UNIVERSITY OF NOTRE DAME | SCIENCE

CHARLES EDISON FUND

Letter from Dean Galvin

Dear College of Science Undergraduates,

As I write this letter, during an unprecedented time in our history, I am struck by the resilience you have demonstrated this semester. You have adapted quickly to an e-learning environment. You have utilized technology to its fullest potential to stay connected with your classmates, friends, and professors. At a time when we can't physically be together on campus, you have worked hard to keep the spirit of the Notre Dame community alive. Thank you for all you are doing to make the most of this situation in which the COVID-19 pandemic has placed us.

During this time in particular, research has become vital to solving the world's most pressing problems. Undergraduates who perform scientific research are well positioned for the next step in their careers, whether that path may lead to attendance in graduate or medical school, or to work in the private sector. *Scientia*, our student-led scientific journal, provides Notre Dame undergraduates with the opportunity to publish their research, and further illustrates how scientific inquiry in the laboratory eventually may become published research.

I am grateful to our many generous donors who have made it possible for Notre Dame undergraduates to undertake scientific research opportunities. These opportunities, whether on or off campus, during the summer or academic year, give students significantly important exposure to working in a laboratory setting or field environment with professional scientists and other experts in their fields. These experiences allow for a deeper dive into a specific area of interest for each student. This process, along with publishing their work in *Scientia*, helps prepare them for their future careers.

This issue marks the first time that *Scientia* is being published and distributed primarily online. This is perhaps a fitting example of how our students have adapted to our new reality of being physically separated but united in spirit and goals. Now, more than ever, the scientific inquiry of you, our students, is vitally important to the challenges our world faces. I'm proud of you and the Notre Dame community we share, and I look forward to when we as a community can reconvene on campus.

In Notre Dame,

Mary E. Galvin

Mary E. Galvin, Sc.D.

William K. Warren Foundation Dean of the College of Science
Professor of Chemistry

2019-2020 Editorial Board

Editors-in-Chief
Rosie Crisman
Vaishali Nayak

Managing Editors
Lauren English (F)
Aidan Crowley (S)

Outreach Coordinator (new position)
Megan McCabe

Biology
Ryan Middleton, SE
Gretchen Andreasen, JSE (S)
Olivia Jazbutis, JSE (S)
Kara Miecznikowski, Fall Part-Time

Health
Michael Sokolowski, SE
Daniel Guobadia, JSE
Emilia Wang, JSE

Physics
Kevin Greif, SE
Michael Kurkowski, SE
Nicole Lee, JSE

Mathematics
Alex Kokot, SE
Ting Gong, JSE

Chemistry and Biochemistry
Tim Mikulski, SE
Paul Wasuwanich, SE
Kimberly Riordan, JSE (S)

Layout, Design, and Publishing
Lindsey Mortensen, SE

News
Kara Miecznikowski, SE
Mady Andreas, SE
Abigail Abikoye, JSE
Ryan Middleton, JSE

Letter from the Editors

Since its inception in 2009, *Scientia* has been dedicated to promoting the research of undergraduates. This dedication is reflected through the production of this journal, platforms such as 'Talk Science' seminars, financial support for undergraduate research through the Charles Edison Foundation Fellowship, and a renewed social media effort to strengthen both reach and involvement. The goal of our endeavors throughout the academic year has been to highlight the accomplished students and faculty who make up our College of Science community at Notre Dame. We are especially honored and grateful to have included our Charles Edison Fellows, Rachel Hughes and Andrea Lebron, in our 2020 publication and February Talk Science seminar respectively. With the support of both the Foundation and Notre Dame's College of Science, *Scientia* is able to offer this award in its fourth year.

In addition to continuing the Student Spotlight section to highlight talented undergraduates and their research endeavors, we have introduced an Alumni Spotlight to feature the groundbreaking research of Dr. Elizabeth Berry-Kravis '79. One of the first women to attend Notre Dame, Dr. Berry-Kravis has advanced the study of Fragile X syndrome and other rare diseases.

With Notre Dame switching to online classes on March 23, 2020, *Scientia* entered an unprecedented period and relied on the innovative thinking of its board members and advisory staff. Given the unique challenges that this presented, the decision was made to focus on an online publication for this journal and consider physical printing later in the fall. We are grateful for the access, provided by the University, to graphic design software that made remote coordination possible, and to our layout team who worked hard to make the online copy available despite these challenges.

We are excited to pass *Scientia*'s leadership in the 2020-2021 academic year to Aidan Crowley and Lauren English, two talented rising seniors with backgrounds in neuroscience, bioethics, and interdisciplinary research. They both have dedicated time and effort to promote Scientia and scientific literacy at our University, and we are thrilled to see where this journal will go in the coming years.

In Notre Dame,

Rosie Crisman & Vaishali Nayak

Editors-in-Chief

Table of Contents

NEWS

RESEARCH

BIOLOGY

MATH

SPOTLIGHT

ON THE FRONT AND BACK COVERS

Each summer, dozens of student and faculty researchers trek to the University of Notre Dame Environmental Research Center (UNDERC) East property spanning northern Wisconsin and the Upper Pensinsula of Michigan. They are unified in their desire to conduct research on the wide variety of aquatic and terrestrial habitats unique to the area. This year's edition of *Scientia* includes three student research projects conducted at UNDERC. The image featured on this year's front and back covers is taken at Tenderfoot Lake on the UNDERC property.

THE UNIVERSITY OF

NOTRE DAME

The 1,250-acre campus of the University of Notre Dame is located on the north side of South Bend, Indiana, just 90 miles from Chicago. Founded in 1842 by Rev. Edward F. Sorin, C.S.C., a French Holy Cross priest, Notre Dame had very humble beginnings. Now, it is the preeminent Catholic educational institution in the United States, with an annual enrollment of 8,530 undergraduate students, and more than 1,300 professors, who together hold advanced degrees from major universities around the world. Notre Dame's endowment is the 11th largest in the country, and research in science attracts more than $40 million in federal research funds each year.

RESEARCH

Want to get involved in undergraduate research?
For more information about undergraduate research opportunities at the University of Notre Dame, visit science.nd.edu/undergradresearch.
Learn more about the Charles Edison Student Fellowship of *Scientia* on scientia.nd.edu or email scientia@nd.edu for details.

College of Science New Faculty Spotlight

MEGAN HILBERT, EMILY KOZLOWSKI, JULIA ZAPPA

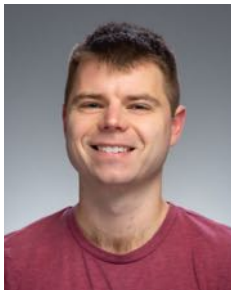
Jenna E. Coalson, Ph.D, Assistant Professor of the Practice for the Department of Biological Sciences at Notre Dame, received her B.A. in Human Biology from Stanford University with a concentration in The Biological and Social Aspects of Infectious Disease. She went on to earn her M.P.H in International Health Epidemiology and her Ph.D in Epidemiologic Sciences from the University of Michigan. Dr. Coalson worked as a postdoctoral researcher at the University of Michigan's School of Public Health before she became a postdoctoral fellow at the University of Arizona's Center for Insect Science, then accepted her current position at the University of Notre Dame. In addition to teaching epidemiology and capstone research seminars for the M.S. in Global Health program, she conducts her own research with a focus on understanding the transmission dynamics of mosquito-borne disease to identify risk patterns and potential intervention strategies. Notable facets of Dr. Coalson's work include studies of long-lasting-insecticide-treated nets in Western Kenya, a project on invasive mosquito survival mechanisms in the American southwest and research on the intransigence of malaria with the International Center of Excellence for Malaria Research in Malawi. In her free time, Dr. Coalson loves cooking, playing tennis, and watching Netflix.

Juan Del Valle, Ph.D, William K. Warren Family Associate Professor, graduated from Carleton College in Minnesota in 1999 with his B.A. in Chemistry. He then went on to earn his Ph.D. in Chemistry from the University of California San Diego in 2004. From 2004-2006 Del Valle worked as a postdoctoral scholar at the University of Montreal. He then taught as an assistant professor at New Mexico State University from 2006-2008 and at the University of South Florida and Moffitt Cancer Center from 2009-2015. In 2015, Del Valle was promoted to associate professor at the University of South Florida and continued to teach in that role until 2019, when he joined the Notre Dame Department of Chemistry and Biochemistry. Research in the Del Valle lab utilizes organic chemistry to address challenges in drug discovery and molecular recognition, and is particularly focused on the structure-based design of biologically active peptidomimetics and small molecules. Del Valle shares that he enjoys research at Notre Dame because of the outstanding colleagues and students as well as the commitment to excellence he has experienced here. In his free time, he likes to play pick-up soccer and try new winter activities with his wife and two sons.

Matthew Champion, Ph.D, Associate Professor in the department of Chemistry and Biochemistry and principal investigator at the Champion Laboratory, earned his B.S. in Microbiology at the University of Iowa and pursued his Ph.D. in Biochemistry at Texas A&M University. Dr. Champion then worked for the Mass-Spectrometry-Proteomics Group at Applied Biosystems (AB Sciex). He remained there for 6 ½ years after graduating, and then accepted a position as a Research Assistant Professor at the University of Notre Dame. He was named a Research Associate Professor and now serves as an Associate Professor at Notre Dame. The Champion Laboratory is in the analytical and biochemistry divisions of the Department of Chemistry and Biochemistry, specializing in Microbial Proteomics and Metagenomics. Their research focuses on the development and exploitation of novel approaches to identify and characterize the components of secreted proteins from virulent microorganisms. Dr. Champion's lab has several ongoing projects in pathogenic mycobacteria, protein translation in E. coli through the PTRN, and quantitative microbial separations measured using capillary electrophoresis.

Susan Del Valle, Ph.D, assistant teaching professor, received her B.A. in Veterinary Pharmacology from the University of Florida in 1996. She then received her M.S. in Chemistry in 2001 and her Ph.D. in Chemistry in 2004 both from the University of California San Diego. Del Valle then worked as a postdoctoral scholar from 2005-2006 at the Universite de Montreal. She worked as an editorial assistant for Organic Letters, published by the American Chemical Society, from 2006-2017. Del Valle then went on to teach as an adjunct professor at the University of Tampa from 2013-2015. She continued to teach at the University of Tampa as an assistant professor of instruction from 2015-2019 before joining the University of Notre Dame Department of Chemistry and Biochemistry in 2019. Del Valle received the American Peptide Society Award for Significant Contributions in 2007 and the 10 Year of Service Recognition Award from the American Chemical Society in 2016. Del Valle's research includes determining how general chemistry course materials and teaching methods can be improved to better foster critical thinking, problem solving, and scientific communication skills. She is also involved in community outreach through her connection with the American Chemical Society.

Guosheng Fu, Ph.D., Robert and Sara Lumpkins Assistant Professor in the Department of Applied and Computational Mathematics and Statistics, earned his B.S. in mathematics from Nankai University in 2011. He then went on to earn both an M.S. in Mathematics and a M.S. in Aerospace Engineering and Mechanics from the University of Minnesota, Twin Cities in 2014, as well as Ph.D. in Mathematics from the University of Minnesota, Twin Cities in 2016, where he also completed a thesis titled, "Devising superconvergent HDG methods by M-decompositions". Fu worked from 2011-2016 as a teaching assistant and research assistant at the University of Minnesota, Twin Cities, and went on to work as Prager Assistant Professor of Applied Mathematics within the Division of Mathematics at Brown University from 2016-2019. Since coming to Notre Dame in July, 2019, Fu has taught both undergraduate and graduate students in Numerical Analysis courses and is currently focusing his research on numerical methods for partial differentials, discontinuous Galerkin methods, high order finite element methods, and computational fluid dynamics and computational mechanics.



Nicholas Edelen, Ph.D., Assistant Professor in the Department of Mathematics, earned his B.Sc. in mathematics from the University of Edinburgh in 2012 and went on to earn his Ph.D. from Stanford University in 2016. Edelen completed his National Science Foundation (NSF) fellowship on "Regularity and free-boundary interaction of mean curvature flow and constant mean curvature surfaces" at MIT where he also worked as a CLE Moore instructor. Here at Notre Dame, Edelen studies geometric analysis and measure theory, where he has a focus on minimal surfaces, especially near their singularities. He also works on the structure of singular sets and mean curvature flow.

Resorbable IVC Filters: Fighting for Better Embolism Protection

NOEL VINCENT

Cardiovascular disease is among the most deadly conditions in the world. Pulmonary embolism (PE) is one type of cardiovascular disease, and arises from blood clots in the lungs. Studies estimate over 1 million people in the United States suffer from PE each year, suggesting that biotechnological innovations within this area have a massive addressable market.

The risk of PE spikes during periods of hypercoagulability, which occurs when the body defensively enhances the blood's ability to clot under certain stressful situations. This means that the individuals with the highest risk profiles include surgical patients, the bed-ridden, and those undergoing chemotherapy. In fact, cancer patients are four times more likely to develop blood clots than the general population.

Traditionally, for patients at risk of PE, physicians deploy a cage-like device in the inferior vena cava (IVC) which would intercept an embolus en route from the deep veins of the lower extremities to the heart and lungs. This IVC filter would keep the emboli away from sensitive organs long enough for the body to naturally dissolve the clot. In recent years, IVC filter use has gained popularity with the advent of retrievable IVC filters — those that can be removed once the period of hypercoagulability has passed. The issue with these retrievable IVC filters is that most patients fail to return for the follow-up extraction procedure. Failure to remove an IVC filter could lead to devastating consequences if not death.

In partnership with the University of Texas MD Anderson Cancer Center, the College of Science at the University of Notre Dame sends a group of students to Houston each summer to take part in research projects carried out at our nation's leading cancer research hospital. In the Department of Interventional Radiology of the University of Texas MD Anderson Cancer Center, the Melancon Lab is developing new resorbable solutions to address PE. "There is a growing need for temporary medical devices. However, most of the ones available in the market are made up of metallic implants that need to be retrieved after the prophylactic period," says Dr. Marites Melancon. "Our goal is to develop devices that could resorb at the time when it is needed and vanish when not needed." These new resorbable IVC filters are made from polydioxanone, the same material that resorbable surgical sutures are made from. Once deployed, these new filters will prevent permeation of emboli during at-risk hypercoagulable period and then dissolve via hydrolysis, thus bypassing the need for a secondary retrieval procedure.

The major difficulty in using these new IVC filters is that, unlike traditional metallic filters that are highly radiopaque, absorbable IVC filters made up of polydioxanone are radiolucent, effectively invisible under radiography. "We also need a way to monitor these implants to determine whether they are in place and working well (or not)," says Melancon. Radiographic confirmation guides the safe deployment and monitoring of IVC filters, and without adequate imaging, physicians could lose track of the filter.

Thus, the development of a resorbable yet high contrast solution to PE became one of the objectives of the Melancon Lab. To induce radiopacity in polydioxanone, the lab infused biocompatible metallic nanoparticles within the polymer. The difference is apparent in the figure below, where a polydioxanone filter infused with bismuth nanoparticles (BiNP-PPDO) is imaged next to a control polydioxanone (PPDO) filter.

By solving the contrast issue, and inducing radiopacity in the filters, this innovative solution allows a resorbable IVC filter to be placed in the body through an image guided procedure. The device then, unlike conventional filters, would dissolve after the high-risk hyper coagulable phase.

Partnership is essential in the development of these devices. Melancon spoke about her collaboration with " biotech companies, such as Adient Medical" which allows them to "easily translate this technology in the clinics." She continued on to say "MD Anderson is also unique because we have multiple core labs where we can do our research." Partnerships like these allow for more efficient innovation, paving the way for a final product that can save lives in the clinic. ■

CXCL5/CDRC2 Signaling Promotes Breast Cancer Metastasis in Bone

MATTHEW GUGGENBILLER

Metastasis describes the spread of cancerous cells from the primary tumor location to distant organs and is the primary cause of morbidity and mortality of cancer, with some estimates suggesting 90% of cancer-related deaths can be attributed to metastasis. Bone is the most common metastatic site for many cancers, since the environment surrounding bone is rich in nutrients and growth factors. While some metastasized cells proliferate quickly, others are able to lie dormant in bone, avoiding typical detection and treatment methods. These dormant cells can be reactivated and subsequently colonize bone upon binding of specific signaling molecules.

Dr. Laurie Littlepage and her team at the Harper Cancer Research Institute focus their research on breast cancer metastasis in bone since bone metastases are correlated with

increased metastasis to other tissues, significantly decreased overall survival, and increased risk for pain associated with tissue destruction. Roughly 73% of women diagnosed with breast cancer display bone metastases at the time of their death, but a thorough understanding of the mechanisms underlying metastasis in bone is lacking. Although general treatment options for metastatic breast cancer have progressed significantly in recent decades, investigation of breast cancer metastasis in bone serves not only to facilitate the development of more efficacious treatment options but also to spur the development of more effective detection and prevention techniques for breast cancer metastasis.

In a paper recently published in *Nature Communications*, Littlepage and her team described the binding of CXCL5, a chemokine, to its receptor, CXCR2, as critical to driving metastasized cells out of dormancy to form tumors in bone. They concluded that cancerous cells secrete more CXCL5 than normal bone cells, which increases proliferative capability of cancerous cells.

Littlepage's work not only highlights potential therapeutic targets in CXCL5/CXCR2 and the signaling they regulate but also describes and highlights a new 3D cell culture system they developed that will be useful in researching the initiation of metastatic colonization of bone tissue.

"Understanding the critical moment — when cancerous cells begin to colonize bone — permits a more thorough understanding of tumor growth and potential ways to prevent or eliminate these tumors," said Littlepage, Campbell Family Assistant Professor of Cancer Research at Notre Dame.

With this model of metastatic proliferation developed, Littlepage and her lab have begun research into potential therapeutic options that may weaken CXCL5/CXCR2 signaling. Subsequent work has demonstrated that inhibitors of CXCR2 decreased the proliferative ability of breast cancer cells in bone. While preventing CXCL5/CXCR2 signaling may be beneficial at stopping bone metastasis, CXCL5/CXCR2 signaling in other unaffected tissues is integral for normal functioning. Hence, as Littlepage describes, "Directing potential therapies only to affected regions may be integral in limiting side effects resulting from aberrant inhibition of CXCL5/CXCR2 interactions." Nonetheless, therapeutic candidates targeting CXCL5/CXCR2 interactions have immense potential in decreasing proliferative ability, especially as "these candidates could work in combination with already utilized therapies."

The Littlepage lab's research into the evaluation of the role of CXCL5/CXCR2 signaling in bone metastases originating from primary sites could provide valuable insight into metastatic proliferation and tumor formation as a whole. Further insight into the mechanism underlying the increased proliferation caused by CXCL5/CXCR2 signaling may provide a solid foundation for research into the proliferative ability in other metastatic locations, especially in identifying key signaling molecules.

This research benefited from the interdisciplinary research environment that is supported by the Harper Cancer Research Institute and the University of Notre Dame. An integral collaborator on this project was Dr. Glen Niebur, professor in the Department of Aerospace and Mechanical Engineering, who also studies bone mechanobiology. Ricardo Romero Moreno, a Notre Dame graduate student in the Littlepage lab, led the completion of the described research with collaboration from other members of the Littlepage and Niebur labs. This research has been supported by generous support from the Kelly Cares Foundation and previously from the Walther Cancer Foundation. ■

Laurie Littlepage, Ph.D. and a student in the Littlepage lab

The First Observation of Fano Interferences Using Electron Microscopy

ALEXANDRA NOBLE

Throughout the long history of scientific breakthroughs and experimentation, a common theme has persisted: The path to scientific discovery is unpredictable. Whether it's decoding complex processes or overcoming a theoretical roadblock, researchers face adversity and hardship on the road to discovery. Researchers from the University of Notre Dame encountered their own scientific struggles as they attempted to observe a specific type of interference, known as a Fano interference. This unique kind of interference is a type of resonant scattering that gives rise to an asymmetric line-shape. For almost a decade, Jon Camden, professor and director of Undergraduate Studies in the Departments of Chemistry and Biochemistry, and his team have been working to observe a Fano interference using electron microscopy, a technique for obtaining high-resolution images of nanoscopic specimens. In general, this project investigated the coupling between plasmonic nanoparticles, which are metallic particles that are highly efficient in absorbing and scattering light.

"This was a long process, longer than most things I've done," said Camden. Since coming up with the idea in

2012, Camden and his team have worked extensively and collaboratively to observe this complicated effect in electron microscopy. Camden credits the success of this work to significant collaboration with a team of researchers across the country. The University of Notre Dame started its work with the Center for Nanophase Materials Science at Oak Ridge National Laboratory (ORNL) in 2009. When working on this particular project, ORNL would conduct the measurements in its ultra-high-resolution scanning transmission electron microscope. From there, the experimentalists collaborated with both David J. Massiello at the University of Washington and Philip D. Rack at the University of Tennessee.

Camden's research team, whose expertise lays in the spectroscopy of plasmonic structures, worked closely with the theoretician David Massiello at the University of Washington. "We came up with some ideas that at first mostly didn't work," said Camden, when reflecting on the group's early meetings. Camden and Massiello would present their ideas to Racks's team of expert material scientists at the University of Tennessee, who would then communicate to the other teams what structures they could and could not fabricate. This allowed the researchers to focus their energy on systems that might work. The constant communication between partnering laboratories and universities capitalized on the expertise of each research group.

During their years of experimenting with different materials to observe the coupling process between the plasmonic particles, the researchers tested this mechanism with different rod lengths, metals, substrates, and combinations. "Rods provide very narrow plasmon resonances — this is necessary to obverse the Fano interference," said Camden. Eventually, they developed what is called the "lollipop structure," which consists of one single rod and a broad disk. An electron probe was located next to the disk and in the opposite direction of the rod. The disk, acting as an antenna, transmitted the energy of the electron probe to the rod. The resulting energy oscillations produced the asymmetric line-shape characteristic of Fano interferences, which is what the research team aimed to observe. Even after the initial discovery of this lollipop structure, it took their group almost a year and a half to refine this nanostructure to most accurately describe the observations of the Fano interferences published in their paper.

When asked what contributed to the successful observation of these interferences, Camden cited the major advancement in the energy resolution of the electron microscope, which improved by about two orders of magnitude during the time period of their research. This advancement allowed researchers to better understand the plasmonic systems that they aimed to observe using electron microscopy.

Reflecting on the trial and error process involved in creating the nanostructure and observing the interference, Agust Olafsson, a graduate student researcher studying analytic and physical chemistry in Camden's research laboratory, notes that the process was more than just trial and error. Olafsson describes their discovery process as one in which "you aren't always sure what you need until you see what you are missing." Fano interferences are an exceptionally complicated process to observe, which contributed to the long duration of this experimental discovery. "It's a delicate effect, and it's complicated, so it's not always the easiest to describe," said Camden.

When asked about his group's work since the publication of the paper in Physical Review Letters, Camden mentioned that his team has "a lot of new ideas that we want to go forward with concerning the capabilities of the new instrument." Specifically, they are very interested in coupling the resonance of these nanoparticles, called plasmons, to the vibrations of molecules. The vibrations of these nanoparticles, which can be light-capturing energy harvesters, allow scientists to selectively drive one reaction over another. In the particular nanostructures used for this research, there were only two particles: a disk and the rod. Now, Camden's team is interested in understanding how energy can flow through a large array of nanoparticles. For these researchers, the first observation of Fano interferences is just the first step to unlocking answers to some of the key questions in this field of study. ■

Malaria DREAM Challenge Revolutionizes Approaches to Scientific Research

EMILY HUNT

Malaria is a neglected infectious disease transmitted through female anopheline mosquitoes. It most commonly affects people in developing countries, predominantly Sub-Saharan Africa, and is a growing global health issue that claimed the lives of nearly half a million people in 2018 according to the World Health Organization. In addition to this high death toll, there is an increasing risk that emerging drug resistance to artemisinin — the main anti-malarial drug — could spread from Southeast Asia to Africa. Geoffrey Siwo is a research Assistant Professor currently specializing in genomic medicine, computational biology and artificial intelligence at the University of Notre Dame. He is the lead organizer for the Malaria DREAM (Dialogue for Reverse Engineering Assessments and Methods) Challenge, a crowdsourcing project uniting scientists, engineers and coders from across the globe to address obstacles in combating malaria.

The goal of the Malaria DREAM Challenge is to develop computational models for predicting and understanding the molecular mechanisms of artemisinin drug resistance. Over the duration of the DREAM Challenge in 2019, 360 teams from over 30 countries answered the call to participate.

The DREAM Challenges approach to solving biomedical problems is not unique to malaria. However, previous DREAM Challenges have not been oriented toward neglected diseases such as malaria. Previous DREAM challenges include understanding single cell signaling in breast cancer, developing risk models for multiple myeloma, and identifying better methods for processing mobile sensor data collected by digital biomarkers for Parkinson's disease.

Siwo is a pioneer in specifically using the platform to address global health issues and neglected diseases in developing countries. While working in research at IBM under Dr. Gustavo Stolovitzky, Siwo was inspired by the "power of the [DREAM Challenge's] wide structure" and utilizing the "wisdom of the crowd [of participants in the Challenge]" to advance research. "If you have a problem, you can basically challenge anyone in the world to solve it," said Siwo. "You can get new ideas from places that you didn't expect, better than what you would've even thought or predicted."

The National Institutes of Health (NIH) supported the grant for the project, which was funded in 2014. With additional support from Sage Bionetworks, biological samples and data from Dr. Michael Ferdig's lab at Notre Dame (Siwo's advisor when he was a Ph.D. student), the Mahidol-Oxford Tropical Medicine Research Unit in Thailand, and the Texas Biomedical Research Institute in San Antonio, Texas, Siwo and his team finally brought the dream for the project to fruition nine years later. The project was also bolstered by a team of young scientists from eight countries in Africa who came together in South Africa for a one-week hackathon financed by the H3Africa Bioinformatics Network and IBM Research Africa to provide a pre-challenge assessment of the datasets finally used in the Malaria DREAM challenge. In March 2019, registration for the challenge officially opened.

DREAM Challenges are especially beneficial for advancing research for diseases in developing countries because of the dearth of computational scientists working on these diseases. According to Siwo, it is difficult to find data scientists with both the necessary computational and biology skills to work on neglected diseases like malaria. "Many people good at [computational] modeling would rather go work on cancer or for companies like Facebook or Google," said Siwo. However, he believes the crowdsourcing method is revolutionizing how researchers are approaching the world's greatest problems in science. "It's straying away from the common 'here's the paper, here's what we did,'" said Siwo. "Now it's 'here's the model, let's show you exactly what we did.'"

Though the Challenge has recruited many teams and received generous funding from well-known donors such as the Bill & Melinda Gates Foundation, there are significantly less resources being allocated towards malaria in comparison to well-known illnesses, such as cancer, that are not concentrated in developing countries. Siwo is hopeful that future projects similar to the Malaria DREAM project will draw more attention to diseases in developing countries. ■

Mimicking Biological Locomotion with Synchronized Coupled Oscillator Dynamics

ANDREW CAMERON

The movements of animals, from running to swimming to flying, have a characteristic rhythm and synchronization dynamics which inspired the development of bio-inspired robots. The secret behind the smooth rhythm of biological movement — including that of humans — lies in specialized neuronal circuits known as central pattern generators (CPGs). In humans, CPGs are located in the lower spinal cord, regulating a host of movements including swallowing, breathing, and walking. A primary function of the CPG is to locally give rise to complex rhythmic patterns for locomotion and seamless gait transition while receiving simple input signals from higher regions in the brain. When running, for example, you don't need to consciously control each step. The brain can deliver modulatory signals to the CPG — to adjust speed or gait, for example — but CPGs are able to synchronize muscles to maintain the rhythm and pattern of running without the central nervous system needing to control every movement.

Most robots currently employ a centralized control system — a single "brain" controlling all components. To develop better bio-inspired models for biological movement of robots, researchers at the University of Notre Dame are using newly-developed hardware to mimic the function of biological CPGs, potentially allowing for a decentralized control system more akin to biological motor systems. In a paper published in Nature Communication in July, entitled "Programmable coupled oscillators for synchronized locomotion," a team of Notre Dame researchers led by Professor Suman Datta, in collaboration with the Georgia Institute of Technology, presented its work on a network of coupled phase-transition nano-oscillators that is able to mimic the functionality of a biological CPG.

Dr. Sourav Dutta, a Notre Dame postdoctoral research associate and primary author of the paper, said that this is not the first time that engineers have tried to replicate biological CPG for robotic control. However, extreme energy-efficiency and compactness in designing the hardware lies at the heart of developing autonomous locomotion for micro-robots that will be capable of real-world tasks such as spatial exploration in energy-constrained environment. Dr. Dutta and his fellow researchers have been trying to acheive this by exploring unconventional methodologies of computing using emerging devices and circuits and using exotic functional materials.

"The entire research comes down to how you can make something in a very small footprint area. The area has to

be very small," he said. "And the energy dissipation has to be very small."

Dutta and his team focused on the construction of oscillators — circuits ubiquitous in clocks and everyday electronic circuits from cell phones to laptops. Dutta said his team has been able to make nano-scale oscillators using only four transistors, where other efforts have required anywhere from 70 to 200.

"There is always a push to go down and down in energy and in size," he said. "So, we looked at this problem and saw that we can actually make this circuit with fewer transistors, which means if you talk about footprint area, the size of your circuit is really small and scalable."

By using novel materials in these circuits, Dutta and his fellow researchers have been able to construct extremely small and energy-efficient oscillator circuits. The oscillators use vanadium dioxide — a unique material that can change from an insulating to a metallic state, and with the right combination of minimum circuitry, they were able to produce self-sustained oscillations. These circuits can be used to maintain or modulate fixed patterns — imitating the function of CPGs in animals. Given their small size and low energy dissipation, Dutta said potential applications could range from wearable exoskeletons for paraplegic patients to microrobots for industrial uses and spatial exploration of unknown terrain.

Dutta also noted that the oscillators he and his colleagues have developed aren't limited to CPG applications. The nano-oscillator circuits may also be used to solve complex optimization problems that current computers struggle with, known as non-deterministic polynomial-time hard problems, or NP-hard problems. An example is the Travelling Salesman problem, where one must determine the shortest route between various cities, visiting each city once before returning to the original point. This is easy to solve for a small number of cities, but as the count increases, the difficulty of the problem also dramatically increases. With large numbers of cities, even powerful computers require long periods of time to produce an optimized solution.

Many approaches to solving NP-hard problems in the past have relied on complicated software algorithms, but Dutta believes that using tailor-made hardware — specifically coupled oscillators-based Ising machine — may provide the key to cracking these problems quickly, drastically reducing the time to produce an optimal solution. ■

Removal of Vegetation Potentially Reduces the Transmission of River Blindness

CAROLINE MYERS

Onchocerciasis, more commonly known as river blindness, is a disease caused by a fly-born worm infection. The disease currently predominates in and around rivers of Sub-Saharan Africa, although it was previously common in regions of Latin America. The disease spreads through bites from black flies, which breed in fast moving rivers and on riverine vegetation. Worm larvae are transmitted through the fly's saliva into the human body where they develop under the skin, causing irritation and infection. These worms can eventually migrate into the human eye and cause blindness. It is estimated that around 25 million people throughout the world are affected by onchocerciasis, with almost one million of those people having some sort of visual impairment.

Onchocerciasis is both treatable and preventable. Although effective organization and development has been shown to effectively combat diseases like river blindness, currently, drugs are being distributed by institutions, such as the World Health Organization, as a mass preventive chemotherapeutic measure to control or interrupt the transmission of onchocerciasis in affected populations. Even with mass treatment, however, not all worms within an infected host are killed and surviving worms can live for up to 10 years. Additionally, although the distribution of drugs is free to the public as of now, it is hard to know if that will continue in the future. Because of this, it is important to consider other sustainable and cost-effective ways to treat and prevent river blindness.

Dr. Edwin Michael, an epidemiologist in Notre Dame's Biological Sciences department, has been researching river blindness and using mathematical models to understand and predict its transmission. Theoretical epidemiologists like Michael and his research group routinely work with control experts in order to evaluate field methods for achieving long-term sustainable disease transmission interruption, and believe that a new riverine vegetation removal technique, called slash-and-burn, along with continuous drug treatment, may show the most promise in terms of effectively reducing transmission of river blindness in Sub-Saharan African communities. The slash-and-burn technique is where trailing riverine vegetation is cut and then thrown on riverbanks to dry, thereby killing any attached and maturing black fly larvae. By removing the vegetation required for larval development, transmission of the parasite would theoretically decrease as "there will be no habitat for the transmitting flies to re-establish so it's a fantastically simple control method in that sense. It's making control very self-sustainable and you're interrupting transmission and preventing inmigration of flies from neighboring sites because there's no more habitat for fly development."

The slash-and-burn method is free and can be organized by community members, making it extremely sustainable, as "anybody in the community can cut that vegetation and there's no cost." The "two-pronged approach" of continued drug treatment with slash-and-burn could "be sustained by the community" and would "empower them" because "they want to get involved." By organizing the communities around the treatment and prevention of the disease, the fight against river blindness becomes much more sustainable and cost-ef-

fective.

When asked whether the slash-and-burn technique would harm the local river ecosystem, Michael replied that not much is known about its true effect on local ecosystems. He stated that "there's always a risk and there's always a consequence. We are moving into natural systems. But so far we have not done that work yet...We haven't looked at the impact on wildlife and other ecosystem effects, but if those effects are there then we need to look at the frequency at which we remove the vegetation." Michael stated that the slash-and-burn technique does not have to be performed every month, rather that his group's models have shown that reduction of transmission of the disease could occur even if they "remove the vegetation just before the transmission season. Following this routine can therefore minimize the risk to the other components of the ecosystem."

Currently, Michael is using mathematical models to predict the effects of the slash-and-burn technique on transmission rates. Because it is difficult to observe the disease progression in humans — especially over the decades that he predicts will be required to see an impact — mathematical models represent a "powerful forecasting tool" to examine the effects of this solution. Michael uses data from field studies examining fly populations in river habitats exposed to the slash-and-burn technique to build his models. Michael believes it will take another "10 to 20 years to get rid of Onchocerciasis in Africa." Although it requires a long term effort and engagement from the community, his models show how the slash-and-burn technique could be extremely effective in preventing river blindness. ■

Understanding the Surplus of Gas in the Milky Way

LAUREL AMMOND

Our understanding of our galaxy has been somewhat limited to stars, interstellar medium, and dust. However in 2009, the Cosmic Origins Spectrograph (COS) was installed onto NASA's Hubble Space Telescope. With this new technology has come entirely new information about galaxies, changing the past understanding of a galaxy's true definition. Dr. Nicolas Lehner, professor of Astrophysics at Notre Dame, has studied gas in and around galaxies throughout his career, which spans over 20 years. Beginning with his doctoral work at The Queen's University of Belfast, Dr. Lehner has taken great interest on how galaxies interact with their gaseous surroundings. He attributes some of his recent understanding of galaxies to the UV light absorption capabilities with the COS instrument on Hubble. Regular imaging techniques are not sensitive enough to pick up much of the gas beyond a galaxy radius. Absorption techniques with UV spectra of stars or quasars are therefore required to pick up signatures from the very diffuse gas around galaxies. Before COS, this was limited to a couple of handfuls of stars and quasars, strongly limiting the results. COS being at least 10 times more sensitive than its predecessors, suddenly astronomers could work with several tens of stars and hundreds of quasars, which have allowed them to better sample the halos of galaxies and our own Milky Way and to produce robust scientific results. These new findings have awakened astronomers to the central role the gas surrounding the galaxies — the circumgalactic medium — plays in the life of a galaxy.

The circumgalactic medium is now understood to be a very diffuse gas surrounding galaxies. This gas is being cycled into and out of the galaxy, aiding in the formation of new stars. As said by Dr. Lehner, "If you take the gas within a galaxy, and there's not some gas coming from anywhere else, after about a billion years, a galaxy like our own Milky Way will have exhausted all its fuel and cannot form stars anymore." However, the presence of young and forming stars tells us that is not the case. The question, then, is where the new gas comes from.

The Hubble Space Telescope has helped answer this question for the Milky Way. First, Dr. Lehner has used the UV light received from nearby stars to determine the presence of nearby circumgalactic gas. The second step was led by Dr. Fox from the Space Telescope Science Institute in collaboration with Dr. Lehner to determine how much of this nearby circumgalactic gas is entering or leaving the Milky Way. Interestingly, and to their surprise, the amount of gas entering the galaxy is not equal to the amount leaving, creating an unexplained surplus of gas within the galaxy. The reasons for this surplus are unknown, but it is likely good news for a growing galaxy. With a gas surplus, the galaxy can continue forming new stars.

Part of Dr. Lehner's future research will involve understanding this surplus. His most recent work provides insight into the gases around Andromeda, the massive galaxy near the Milky Way. Circumgalactic gases have been relatively understudied; however, this has changed and there is still a great deal to be learned: The origin of these gases around the Milky Way is one such mystery. Current work suggests that some gas is "recycled" by the Milky Way, leaving and then reentering the galaxy to form new stars. However, gases with different chemical compositions from those of the Milky Way are also present, a factor that leads Dr. Lehner to believe that some must come from somewhere else in the universe. Whether this is from other galaxies, the intergalactic medium, or someplace entirely different is still up for debate. Other galaxies are also a big question for researchers, and Dr. Lehner is studying them to determine if a similar surplus is present. Considering the recent discovery of the importance of the gases in the life of a galaxy, the future is full of breakthroughs waiting to occur. Dr. Lehner's research is part of understanding the complicated task that is a galaxy. An unexplained surplus of gas is just one more piece of the puzzle of how a galaxy truly works. ■

Single-Cell Gene Expression Profiling Helps Design Novel Combination Therapy for Breast Cancer Patients

MADELEINE ANDREAS

Fundraisers and activists often describe the fight against cancer as a "race for the cure." The unfortunate reality is that there is no magic bullet for cancer. No special pill, single treatment, or vaccine can completely "cure" cancer. Current treatment modalities include surgical resection, chemotherapy, and radiation; all three are typically used to effectively treat cancer. However, many patients will develop resistance to chemotherapy drugs, leading to the progression and eventual spread of the disease to other parts of the body. There is a pressing need for novel therapeutics and treatment regimens that can stop cancer before it spreads to the rest of the body.

One of these novel treatments is called immunotherapy, which uses the body's defense systems to target and kill cancerous cells. These treatment regimens would eliminate the need for devastating chemotherapy and radiation treatments, leading to a better prognosis for cancer patients around the world. The field is still in its infancy, and was recognized in 2018 with pioneering researchers awarded the Nobel Prize in Physiology or Medicine for their seminal contributions to the field. Even with these exciting developments, not every patient is currently eligible for immunotherapy: while immunotherapy works well for tumors with certain characteristics, other cancers do not have the same sensitivity to immunotherapy treatment. This is especially true in the case of breast cancer, for which previous immunotherapy trials have not been successful. Unfortunately, the problems facing breast cancer patients and their oncologists are multifold — not only do they have to endure rigorous therapy regimens that ravage the body, but they also have to deal with tumors that will develop resistance to chemotherapy and result in the eventual spread of the disease. It is difficult to understand the problems within a particular patient's case and develop a treatment in the lab before an adverse outcome occurs; this is a particular problem in clinical trials.

Research in Siyuan Zhang's lab at the University of Notre Dame is uncovering new ways of treating cancer using cutting-edge technology to sensitize breast cancer cells to immunotherapy. The Zhang lab presented their research in a recent 2019 publication in Nature Communications, "Single-cell profiling guided combinatorial immunotherapy for fast-evolving CDK4/6 inhibitor-resistant HER2-positive breast cancer." Within this study they detail the development of their mouse model mirroring the clinical trial in answering their big question: can we sensitize these breast cancer cells to immunotherapy using CDK4/6 inhibitor single-cell profiling?

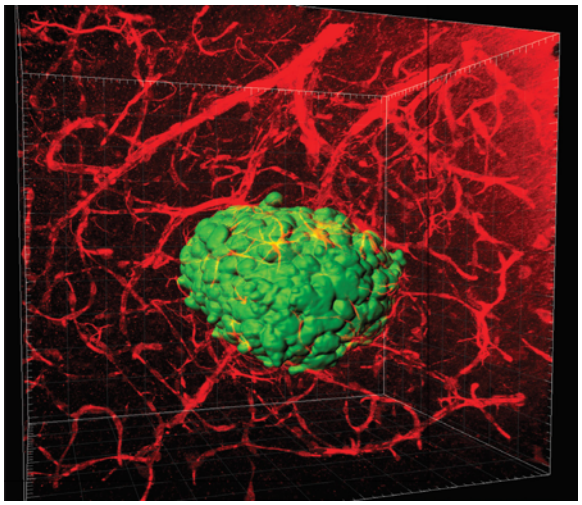
To do so, researchers in the Zhang lab developed a mouse model that closely mimics the conditions of a human clinical trial. Previously, cancer research has been conducted *in vitro* — only using cells in a dish. The Zhang lab's mouse model contains all of the extracellular bits that make a tumor cancerous, including immune cells, cancer-associated fibroblasts, collagen, and other parts of the tumor microenvironment. This *in vivo* model gives the Zhang lab a huge advantage. Zhang describes the model as a "parallel mouse clinical trial with the patient clinical trial," giving the clinical trial team an edge "because with the mouse you can do a lot more molecular level single-cell sequencing, look at the tumor dynamics and you can quickly test combinations...of solutions," instead of waiting for lab biology to catch up with the patient trial. For example, if a patient develops resistance in a clinical trial, researchers mimicking the trial in mice can adjust the therapy regimen in mice first to see how they react. Not only does this new research save valuable time from translating bench research to a clinical trial, but it also helps clinicians effectively treat more patients in real-time.

Another important aspect of the model is that it involves a relatively new understanding of cancer as an ecosystem. No longer do researchers think of cancer as just a block of cells within a tumor. Rather, cancer contains the tumor cells and associated support cells in the tumor microenvironment in a "multi-cell complex ecosystem," as Zhang describes. Cells are subject to evolutionary pressures as well — especially after treatment with chemotherapy. In understanding resistance, the team first "profiled the original naive tumor and looked at their naive cells, tumor cells, how the transcriptome looked like [and how the] genes looked like." After treatment with the CDK4/6 inhibitor, the tumors shrank. But then, these cells developed resistance to the drug and grew again. "Later we looked at the resistance tumors, tumor composition which immune cells are increasing. And if immune cells are increased, what type of tumor has changed in response to treatment," said Zhang. They found that a specific type of immune cell called a myeloid-derived suppressor cell greatly infiltrated the resistant tumors, suppressing the body's immune response. This allowed the tumors to evade detection by the immune system. Zhang said that explains "why those tumors have that response, because they have so many immunosuppressive cells trying to calm things down."

Intrigued by this discovery, they removed the MDSCs and treated the mice with a PDL-1 inhibitor, an FDA-approved immunotherapy currently used in the clinic. This is an exciting finding because breast cancer has historically been difficult to treat with immunotherapy due to a low mutation load in tumors. Breast cancer has been especially difficult because it has a low tumor mutation load — there is not much for the immune system to recognize as different and worthy of attack. Fortunately, this combination of treatments worked. Zhang summarized the final experiments as such: "Interestingly, after you give the first [CDK4/6] treatment, then somehow the immune ecosystem changes. The resistant tumor responds to [immunotherapy] now…We also show this strategy can...treat these resistant tumors for a fairly long time." This has massive clinical applications, as this type of treatment regimen more closely mimics what physicians prescribe to patients in the clinic, as expensive immunotherapy regimens need to be given

out 30 days at a time. The tumor will shrink after treatment, and when it begins to grow again, treatment will begin again. Zhang argues that this regimen is the future of medicine because "this combination is not targeting the tumor cell: It's targeting the remodeled environment, the remodeled ecosystem."

Overall, this work represents a clinically applicable strategy for breast cancer treatment. "It's looking at… [a] patient's tumor [using ecological] and evolutionary paths and...single-cell technology," said Zhang. Clinicians have already begun to reach out to Zhang and his team regarding the implementation of this type of treatment regimen in their clinical trials. Although it is no magic bullet, Zhang's work offers the promise of a brighter future for cancer patients and their families. ■



Modeling of breast cancer metastasis in the brain

Research Lab Uses Nuclear Physics to Detect Toxic PFAS Chemical Class

KARA MIECZNIKOWSKI

One of the main goals of scientific advancement is to improve human quality of life, but sometimes an apparent solution can cause just as many problems as it solves. In the mid-20th century, a group of nearly 5,000 chemicals known collectively as per- and polyfluoroalkyl substances (PFAS) were developed to create waterproof, stain-resistant, and non-stick solutions for everyday items, from clothes to carpet to cookware. These chemicals, which consist of a carbon-fluorine backbone that does not naturally occur in the environment, are incredibly toxic. They have immunosuppressive effects, and exposure is correlated with diseases such as testicular and kidney cancer, hypertension, high cholesterol, preeclampsia, and ulcerative colitis. This toxicity is compounded by their environmental persistence, said Graham Peaslee, concurrent professor of chemistry and biochemistry at the University of Notre Dame, who has been involved with PFAS research for almost seven years. As products with the chemicals are used and thrown away, PFAS bioaccumulate in landfills and enter the landfill leachate system, which introduces them to groundwater used for irrigation and drinking water.

Companies have been using PFAS in their products since the 1940s, but the chemicals' risk to human health did not come to light publicly until the turn of the 21st century. Because of PFAS' widespread use over the past 80 years, a quick and low-cost way of detecting the chemicals is critical to reduce human exposure. Ion Beam Analysis (IBA) is a nuclear physics technique that can do just this. IBA uses an electrostatic accelerator to bombard the surface of a potentially PFAS-contaminated sample with charged particles. This results in the emission of x-rays, gamma-rays or UV-Vis light that can yield important information about the elemental content of the sample. Unlike other techniques, IBA has high sensitivity and — importantly— only requires 180 seconds to analyze a sample.

Peaslee's lab uses an IBA technique called particle-induced gamma ray emission (PIGE) to detect fluorine in samples: the gamma rays given off by this element are unique and allow accurate identification. Peaslee noted that IBA cannot detect PFAS specifically, but if a sample contains fluorine, it can be further investigated for PFAS. "We just measure fluorine, and we can do that quickly...We can save people a lot of time. If there's no fluorine, there's no PFAS. If there is fluorine, we then send that sample out for the complete analysis. And that's where we're trying to make an inroad."

Peaslee emphasized the importance of developing a quick, low-cost way to test for the presence of PFAS. "We're trying to get this process that we've developed commercialized," said Peaslee. Conducting an initial screening for fluorine with an IBA-like test using a medical cyclotron, a type of compact particle accelerator, would allow for nationwide surveying at a fraction of the current cost. "About 80 % of the samples [tested should be fluorine-free]. And instead of paying for a $300, six-week test, we can do it in a day and charge $20," said Peaslee. This technology would ideally be used to address the drinking water market first; it is estimated that somewhere between six and 76 million Americans are drinking water contaminated with PFAS. "Nobody knows exactly how much [is in the water], but we have a measurement technique...that can screen down to all the EPA limits," said Peaslee.

The EPA advisory level is 70 parts per trillion of the combination of PFOA and PFOS, which are just two of the

thousands of PFAS chemicals that exist. PFOA and PFOS are rarely seen alone, and likely comprise only 10% of what is present in the environment, meaning that the remaining 90% are not being taken into account in the EPA's assessment. Peaslee's lab measures every PFAS chemical. "I would say [the current EPA advisory level is] definitely unsafe, but that's the current health advisory limit," said Peaslee. Some states have lowered it, for individual analytes. "If it was 70 part trillion for all PFAS, I'd feel a lot safer. It should be something around the single digits for an individual compound of PFAS. Getting down to that level is going to be really hard to do, because it's so ubiquitous."

Peaslee's lab's research has led to the discovery of PFAS in flame retardants, shoes, fast food wrappers, diapers, and firefighting gear and foam. After the lab's work on PFAS in fast food wrappers was published in 2017, 20 out of 20 companies — including McDonald's and Starbucks — changed their food packaging to non-PFAS wrappers. "To me, that's power through science…[it was] eye opening to me, that a paper can drive policy." Peaslee pointed out that some companies do test their manufactured products for PFAS, but may only check for the long-chain variety of PFAS — not the short-chain, which are just as harmful. "It may be an environmentally conscientious company, perhaps, but they have no idea what's in their supply chain," said Peaslee. "We're working with companies to [help them] make the right choices with their products and supply chain."

One of the lab's current projects includes helping fire departments replace their current uniforms and fire retardant foams, which contain PFAS, with gear that is safer for both firefighters and the local community. "We're talking to firefighters all over the country about how to get rid of the old foam properly," said Peaslee. "[It needs to be] incinerated in a special hazardous waste incinerator. We're working with states and regulators to try and do that." Peaslee continued, "The remarkable thing about this is that it's apolitical. If you drink the water, it doesn't matter whether you're red or blue, it still poisons you. And that has refreshing across the aisle talks going on everywhere."

Alternatives to PFAS include silicones (which are less dangerous but still manmade and environmentally persistent), lanolin (made from sheep wool), and a new technique called microfibering, which creates surface tension that gives a material water resistance. The safety of these alternatives needs to be evaluated, said Peaslee, but they may be good substitutes for PFAS. "We should avoid indiscriminate use of this chemical and just use it where it's needed," said Peaslee. "If you're going up Mount Everest, having the best waterproof jacket probably saves lives. If you're going to the mall, does it have to be the best waterproof jacket? No, probably not." ■

Graham Peaslee, Ph.D.and his research team with one of the lab's particle accelerators

The Role of Synaptic Vesicles in Early Development

JACK HEATHERMAN

Cellular vesicles are essential biological structures involved in various biological processes, including exocytosis, endocytosis, and molecule transport within and between cells. One class of vesicles, synaptic vesicles, contains structures that transport electro-chemical signals within the central nervous system (CNS). Vesicles are so important to our understanding of human biology that the 2013 Nobel Prize in Physiology or Medicine was awarded for the study of these biological materials. While the evolution and development of the synapse itself has been studied for years, few have investigated what roles synaptic vesicles perform before arriving at the synapse. Where do they come from and what function might these vesicles have prior to surfacing at the synapse?

This is the question that undergraduate Evan Nichols sought to answer through his research project at the University of Notre Dame. "The lack of literature describing what the vesicles did before they went to the synapse is what piqued our initial interest," says project advisor and the Elizabeth and Michael Gallagher Assistant Professor of Biological Sciences Cody Smith, Ph.D. Smith explained that previous theories on the vesicle's origin purported that vesicles were either formed at the synapse or shuttled from another part of the cell to the synapse during synaptic development. Since neither of these theories explained what the vesicles' functions were prior to arrival at the synapse, Nichols decided to investigate the process by which axons navigate across the spinal cord during development, in the hope of linking vesicles to the initial breakthrough of axons into the spinal cord.

"We noticed that there were synaptic vesicles sitting right at the region where the axon met the spinal cord, which led us to hypothesize that these vesicles were involved in the initial breach of the axon into the spinal cord," Smith said. Time lapse imaging of the pioneer axons of dorsal root ganglia (DRG), the first axons formed in the DRG, indicated that the the vesicles played a role in guiding the pioneer axon entry into the spinal cord. Nichols then devised an experiment to test the involvement of a class of proteins, called matrix metalloproteinases (MMP), in the axon entry process.

In order to visually capture the spinal cord entry process of pioneer axons, the Smith lab turned to the usage of green and red fluorescent proteins, common proteins used as reporters of gene expression and protein localization. Through the expression of green and red fluorescent proteins in the dorsal root ganglia and axons of zebrafish, Nichols and the Smith lab were able to track the spinal cord entry process of pioneer axons from the peripheral nervous system into the spinal cord, when MMPs were inhibited. "What Evan discovered in the end was that the inhibition of MMPs resulted in multiple errors in axon entry into the spinal cord," stated Smith. "Essentially, segments of the peripheral nervous system of the zebrafish were unable to connect to the central nervous system through the spinal cord, rendering them functionally useless." Nichols' research concluded that synaptic vesicle contents, specifically MMPs, do serve a vital purpose in the unification of the peripheral and central nervous system before they arrive at the synapse.

Following the publishing of his findings in the paper "Synaptic-like Vesicles Facilitate Pioneer Axon Invasion" in early 2019, Nichols further researched the application of his discoveries. Once again, Smith served as his mentor. His fourth paper on the subject, "Functional Regeneration of the Sensory Root Via Axonal Invasion," which was published in January of 2020, focused on how the discovery of these invasion components during early development could lead to the possibility of clinical sensory root regeneration. "During regeneration, usually the invasion components of axons are ineffective in penetrating the spinal cord. Evan's research has shown that by understanding the invasion process during development, we can now utilize them to initiate essential sensory circuit regeneration. With this data, artificial regeneration is a possibility," Smith said.

When asked about Nichols and the project as a whole, Smith was very enthusiastic about his pupil's efforts. "Overall, I am very proud of [Evan's] work. The entire project from start to finish was his idea and his own experiments. He is an excellent example of where curiosity and hard work can take you in the sciences." Smith went on to say that he hopes more students at the University of Notre Dame will be able to tackle projects of similar magnitude during their time as undergraduates. While an undergraduate at Notre Dame, Nichols worked on multiple biological research projects and was the primary author of five papers, all published through the Smith lab. Nichols is a graduate of the class of 2019, was the College of Science Dean's research awardee, and is currently pursuing a Ph.D. degree at Stanford University. ■

Cody Smith, Ph.D. and Evan Nichols in the Smith lab

Spatial Distribution of Opuntia Spp. on Several Galapagos Islands

Rachel Hughes[1]
Advisor: Gary Lamberti[1]
[1]*University of Notre Dame, Department of Biological Sciences*

Abstract

Opuntia spp. is the keystone genus in the arid regions of the Galapagos Islands. As the climate changes, temperatures rise, and weather patterns shift, it is important to understand the factors that limit the growth of Opuntia, as they may provide insight about how to manage Opuntia habitat to protect the cacti and other endemic species. Spatial distribution allows speculation about which factors have the greatest impact on the location of individuals within a species, and can be described as uniform, random, or clumped. This study uses the coefficient of aggregation to predict the spatial distribution of Opuntia spp. on three Galapagos Islands: Santa Fe, Santa Cruz, and South Plaza. When the data were combined, Opuntia displayed a random distribution that may tend towards clumped. Opuntia on Santa Fe and South Plaza displayed a random distribution, but Opuntia on Santa Cruz displayed a clumped distribution. On Santa Cruz, some factor may have made it advantageous for individuals to grow close together, although it is more likely that minimal replication and lack of sample randomization affected results. In addition, a confounding variable, such as cactus age, may have interacted with the spatial distribution of Opuntia.

Introduction

The prickly pear cactus (Opuntia spp.) is a flat-padded cactus that can grow up to 12 m in height, and produces yellow flowers and prickly green fruits. Opuntia dominates the arid regions of the Galapagos Islands (1). Morphologically, the six species and 14 varieties of Opuntia are quite diverse, ranging from tall and tree-like to short and shrubby. Despite this extreme variability in morphology, there is very little genetic variation among Opuntia species (2), and all varieties perform key ecological functions in their ecosystems. Because its spiny pads and prickly fruit are a major food source for Galapagos macrofauna such as tortoises and mockingbirds, Opuntia are the keystone species in the Galapagos' arid regions (3). As anthropogenic climate change becomes a more imminent threat to isolated ecosystems such as the Galapagos, a better understanding of how organisms function (i.e., interact with each other and find nutrients) within these ecosystems will allow a more comprehensive plan for the conservation of these systems in the future. The investigation of Opuntia cacti informs on how arid ecosystems function in the Galapagos.

The spatial distribution of a particular plant species can provide insight into the nature of the species' intraspecific competition (4). Plants exhibit uniform, random, or clumped distributions, each of which has different implications for how individuals of a species interact. A random distribution suggests that there are no competitive factors influencing the location of individual plants. A uniform distribution implies that there is a factor such as need for water driving individuals away from one another. A clumped distribution suggests that a factor such as uneven distribution of soil nutrients draws individuals together. Increasing our understanding of the spatial distribution(s) of Opuntia spp. could supply insight into its intraspecies competition, and the most important factor(s) that drive new plant growth and location. If climate conditions continue to change rapidly, this knowledge could be key to preserving the Opuntia cacti, and managing the arid parts of the Galapagos Islands.

This study examined the spatial distribution of Opuntia spp. on three separate islands to determine the overall distribution of the genus, and investigate whether the distribution varies on different islands. The first hypothesis was that Opuntia spp. has a uniform distribution when calculated from all of the data collected on all three islands. Because Opuntia cacti appear in the driest parts of a very arid area, the prediction was that Opuntia spp. would be uniformly distributed due to competition for water. The second hypothesis was that Opuntia on all islands would have the same coefficient of aggregation, and thus the same distribution. A uniform distribution would suggest that competition for water or nutrients separates the plants, and because they all are found in the same, arid zone on the islands, our prediction was that the coefficient would not change.

Methods and Materials

Location and sites

Opuntia spp. are found in the arid regions of the Galapagos Islands, although they are not found on every island. Over the course of this study, measurements were taken on three of the four islands visited on which Opuntia was found: Santa Fe, Santa Cruz, and South Plaza. The fourth island was North Seymour, but the cacti were far apart and so far off the path that measurements could not be taken given the spatial and temporal limitations of the study.

Data collection and calculations

The method established by Cottam and Curtis (5) was used to quantify the spatial distribution of plants, using a calculation that they called the coefficient of aggregation. This coefficient (A) is based on distance between a plant and a random point (P) and its nearest neighbor (I). The spatial distribution of a plant can be classified as random, clustered, or uniform based on these two measurements and the calculation of the coefficient A, defined as:

$$A = \sum P / \sum I \qquad (1)$$

where $\sum P$ is the sum of all measurements between a random point and the nearest plant, and $\sum I$ is the sum of all measurements between the plant and its nearest neighbor. If coefficient A is about 1, the plants are randomly distributed; a coefficient >1 implies a clumped distribution; and a coefficient <1 implies a uniform distribution.

In order to calculate the coefficient A, two measure-

ments were taken for each cactus: the distance from a random x-coordinate to a cactus, and the distance from that cactus to its nearest neighboring cactus. When possible, a 30-m transect was laid along the footpath and a tape measure used to measure from three random, independent x-coordinates to the nearest cactus. I took measurements from three cacti on each of five transects on Santa Cruz, and one on South Plaza. For the rest of South Plaza and the island of Santa Fe, transects could not be laid, so measurements were taken while hiking. A "random" (i.e. haphazard) point along the path was chosen from which the distance to the nearest cactus was measured. This path-to-cactus distance was recorded as P. Then, the distance from that cactus to its nearest neighbor was measured using a tape measure, and recorded the cactus-to-cactus distance as I. a total of six P/I pairs were measured on Santa Fe, fifteen P/I pairs on Santa Cruz, and six P/I pairs on South Plaza.

These data were entered into Cottam and Curtis's equation (Eq. 1) to calculate A for each island, and an overall A. The coefficient A must be transformed in order to test if it differs significantly from 1 (6). The transformation (Eq. 2) was utilized:

$$x = A / (1+A) \qquad (2)$$

so that A could be compared to the normal distribution. Then the standard deviation was calculated using (Eq. 3):

$$s = 1 / (2*\mathrm{sqrt}(2*n+1)) \qquad (3)$$

where n = the number of distances used to compute x. In a randomly distributed plant population, x will have a mean of 0.5 (6). Using Excel, the probability (p-value) of obtaining the values of x computed from the Opuntia data, given that the null hypothesis (H0: x has a mean of 0.5) was true, was calculated. Because there were two alternative hypotheses (Ha1: Opuntia spp. exhibits a clumped distribution; Ha2: Opuntia spp. exhibits a uniform distribution), the p-value was compared to an alpha of 0.025. If $p < 0.025$, the cacti were distributed in clumps. If $p > 0.975$, the cacti were distributed uniformly. If $0.025 > p > 0.975$, the cacti were randomly distributed.

Results:

The coefficient of aggregation calculated from Opuntia on all three islands was A = 1.3174 (Table 1), suggesting that the cacti may have exhibited a slightly clumped distribution. However, the difference from A = 1 was not significant, so we failed to reject H0 that the cacti were randomly distributed (p = 0.1549, Figure 1). For Opuntia on the island of Santa Fe, a coefficient of A = 1.0783 was calculated, which was not significantly different from A = 1, suggesting that the cacti were randomly distributed (p = 0.4460, Figure 1). The greatest number of P/I pairs were measured on Santa Cruz (n = 15), which had a coefficient of A = 2.1705, (significantly higher than A = 1), suggesting that the cacti on Santa Cruz exhibited a clumped distribution (p = 0.0199, Figure 1, 2). On South Plaza, A = 0.8442, which suggests that the cacti were more uniformly distributed than clumped, but the difference from A = 1 was not significant (p = 0.6197, Figure 1) and we failed to reject H0 of random distribution.

Table 1: *The probability that the transformed coefficient of aggregation (x) differs from a normal distribution with a mean of 0.5 and a standard deviation of s. Using (Eq. 1, 2, and 3), I calculated the probability of obtaining the x values above given the H0 that x was distributed normally with a mean of 0.5 and a standard deviation of s. Only Santa Cruz had a statistically significant p-value (bolded), and because it was lower than p = 0.025, it suggests that the Opuntia on Santa Cruz exhibit a clumped distribution. Santa Fe, South Plaza, and all three islands combined had insignificant p-values (0.025 > p > 0.975), which suggests random distributions of cacti. South Plaza was the only location where A < 1, which suggests a possible uniform distribution, but this was not significant.*

Island	n	A	x	s	p-value	Distribution
Overall	27	1.3174	0.5684	0.0674	0.1539	Random
Santa Fe	6	1.0783	0.5188	0.1387	0.4460	Random
Santa Cruz	15	2.1705	0.6846	0.0199	**0.0199**	Clumped
South Plaza	6	0.8442	0.4578	0.1387	0.6198	Random

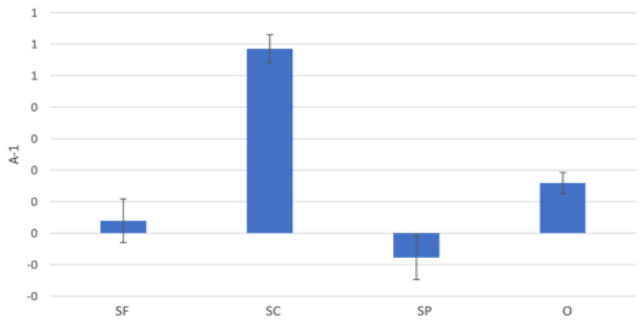


Figure 1: *The coefficient of aggregation (A) – 1 of Opuntia on Santa Fe (SF), Santa Cruz (SC), South Plaza (SP), and overall (O). This graph displays the calculated coefficient of aggregation (A) – 1 ± s (Table 1). Bars that extend up in a positive direction (A > 1) suggest a clumped distribution and bars extending down in the negative direction (A < 1) suggest a uniform distribution. South Plaza tended towards a uniform distribution, but its coefficient did not differ significantly from 1 (Table 1). Santa Cruz was the only island whose coefficient A differed significantly from 1, and it suggested a clumped distribution of Opuntia spp.*

Figure 2: *Observations of cactus size and distribution. On Santa Cruz, I noticed that smaller cacti often grew in closer proximity to each other and between larger cacti. This suggests that age may be a confounding variable in the spatial distribution of Opuntia.*

Discussion

Both of the initial hypotheses were rejected. Opuntia spp. exhibited a random distribution when the data from the three islands sampled were combined. When islands were examined separately, Santa Cruz displayed a different (clumped) distribution than the other two islands. Because the distribution of cacti differed among islands, the conclusion that cacti are randomly distributed overall is not supported. No general conclusion about the spatial distribution of Opuntia spp. on the Galapagos Islands can be made as a result of this study. However, the individual islands may provide insight into patterns about Opuntia's spatial distribution. The distribution on both Santa Fe and South Plaza islands was random, which suggests that there is no pattern in the distribution of individuals. Cacti on Santa Cruz displayed a clumped distribution, suggesting that close proximity between individuals is advantageous to individual survival. More research is needed to establish why this is the case, but the first step would be to repeat this study with more replication and true randomization to determine if these trends remain apparent.

The methods of this study had to be modified in order to accommodate the protection of habitat and other species. The method proposed by Cottam and Curtis (5) requires measurements to be taken from a random x,y-coordinate to the nearest cactus, but transects could only be laid upon predetermined footpaths in order to preserve the integrity of the islands. When time and space permitted, measurements were limited to to a random x-coordinate, while the y-coordinate was set to 0 and resided along the footpath. This limited the sample to cacti growing along the paths, and compromised the integrity of the randomization, but it was more important to not disturb habitat or organisms. If this research project were to be extended, special permission could be secured to stray from the paths, and researchers could learn to identify wildlife signs so that measurements could be taken from true random points without posing a threat to other Galapagos species.

Limited time also affected our ability to collect data, especially on Santa Fe and South Plaza. On each of these two islands, only six data points were collected due to time constraints on each island, and it was often not possible to lay a transect. Therefore, many of the "random" x-coordinates were selected haphazardly as we walked along the islands. The tape measure had a limited range, so the points were usually closer to the nearest cactus than they might have been if they had been truly random. This introduced sampling bias into the study, as the area was more likely to be sampled if there were cacti near enough to the path to be measured. This could have artificially decreased both the P and I measurements for any particular data point.

However, these limitations do not explain why Opuntia spp. exhibited a clumped distribution on Santa Cruz. On this island, the published protocol most closely: five independent transects were laid along the path, and three data points were taken along each transect. The data showed a significant clumped distribution, the opposite result than expected. This result suggests that competition for water is not the primary driver of the location of individual cacti. Age of the cacti may have been a confounding variable. Measurements were taken to the nearest cactus, regardless of the age (approximated by number of pads (7)). Sometimes this meant measuring to a very small cactus, comprised of <5 pads. While taking measurements on Santa Cruz in particular, it was noted that these smaller cacti were normally closer to each other and to the larger cacti than other larger cacti (Figure 2). This may suggest that the younger cacti are the offspring of the older cacti. In addition, there were also no land iguanas on the island of Santa Cruz, which allows the cacti to grow more slowly and/or recruit the population more successfully. On the other hand, South Plaza displayed a distribution that leaned towards uniform, although it did not differ significantly from a random distribution. This island contained numerous bird colonies and land iguanas, both of which may have prevented the successful growth of younger Opuntia through crowding, trampling, or herbivory. This observation suggests that the older Opuntia were more uniformly distributed, but the younger, smaller Opuntia displayed a more random or even clumped distribution where they are allowed to grow more slowly alongside older, potentially parental individuals. Additionally, Mandujano et al. found that 3x more young globose cacti successfully established under a nurse plant than in bare areas due to a decrease in direct sunlight (8). This may indicate that young cacti thrive better under a larger plant, although this may be more related to a commensalism relationship where the young cacti benefit and the older cactus is unaffected, than it is to intraspecific competition for resources. A future study might consider recording the distance between cacti and also the size and/or number of pads for individuals to explore relationships between spacing and age.

The spatial distribution of large plants provides insight into which factors are most important to their growth and reproduction. For example, Béland et al. used spatial distribution to determine that the interaction between birch and pine trees is primarily due to interspecific competition for soil resources (4). Zhang et al. showed that Chinese pine stands are distributed in dense but randomly distributed groups, which makes them a good candidate for thinning to promote greater species diversity (9). Studying the spatial distribution of Opuntia cacti could lead to similar discoveries about the cacti's greatest limiting factors and best management practices. There has been very little research done on the spatial distribution of Opuntia, or other cacti, so further study in this area could fill in a gap in our knowledge of how arid ecosystems function. It also gives us greater predictive power about how global climate change will affect the cacti, and allows us to manage the genus more effectively in the future.

Acknowledgments

Thank you to the University of Notre Dame's Biological Sciences and Civil and Environmental Engineering and Earth Sciences (CEEES) departments for their generous contributions that made it possible to travel to the Galapagos Islands. Special thanks to Gary Lamberti, Ph.D. and Jeremy Fein, Ph.D. for spearheading the trip and their research contributions, to Katherine O'Reilly for her time and attention, and to Luis Rodriguez, our marvelous guide during our time on the islands. Thank you also to Anthony Travel and Rafael Galvao Guerra at Notre Dame International, without whom we never would have made it to Ecuador.

References

1. Hamann, O. 2001. "Demographic studies of three indigenous stand-forming plant taxa (Scalesia, Opuntia, and Bursera) in the Galápagos Islands, Ecuador." Biodiversity and Conservation 10: 223-250.

2. Helson, P., R.A. Browne, D.J. Anderson, P. Verdyck, S. Van Dongen. 2008. "Galapagos' Opuntia (prickly pear) cacti: extensive morphological diversity, low genetic variability." Biological Journal of the Linnean Society 96: 451-461.

3. Hicks DJ, and A. Mauchamp. 1996. "Evolution and conservation biology of the Galapagos Opuntias (Cactaceae)." Haseltonia 4: 89–102.

4. Béland, M., J.M. Lussier, Y. Bergeron, M.H. Longpré, and M. Béland. "Structure, spatial distribution and competition in mixed jack pine (Pinus banksiana) stands on clay soils of eastern Canada." Annals of Forest Science 60.7 (2003): 609-617.

5. Cottam, G. and J.T. Curtis. 1956. "The use of distance measures in phytosociological sampling." Ecological Society of America 37: 451-460.

6. Hopkins, B. 1954. "A new method of determining the type of distribution of plant individuals." Annals of Botany 18: 213-226.

7. Bowers, J.E. "Influence of climatic variability on local population dynamics of a Sonoran Desert platyopuntia." Journal of Arid Environments 61 (2005): 193-210.

8. Mandujano, M.C., A. Flores-Martinez, J. Golubov, E. Ezcurra, 2002. "Spatial distribution of three globose cacti in relation to different nurse-plant canopies and bare areas." The Southwestern Naturalist 47(2): 162-168.

9. Zhang, L., G. Hui, Y. Hu, Z. Zhao. 2018. "Spatial structure characteristics of forests dominated by Pinus tabulaeformus Carr." PLoS ONE 13(4): 1-13.

About the Author

Rachel Hughes is a junior at the University of Notre Dame, majoring in the Program of Liberal Studies and Environmental Science. On campus, she is the social media intern at the Office of Sustainability, and works in Notre Dame's Stream and Wetland Ecology Lab (SWEL). Her work at SWEL centers around investigating the effects of the invasive waterweed Elodea canadensis in south-central Alaskan ecosystems. She spent last summer in Alaska, working for the USDA Forest Service and collecting samples for analysis back on campus. Rachel is also an active member of Lyons Hall, where she has served as outreach executive, academic commissioner, piano accompanist, and lector over the course of her three years. She is currently studying abroad in Angers, France to improve her French language skills. After graduation, Rachel hopes to join the conversation about ecological responsibility and care for our common home, although she is not sure what form that will take yet.

Pycnopsyche (Trichoptera: Limnephilidae) Larvae Influence on Orconectes Propinquus Grazing

Abigail A. White[1]
Advisor: Dr. Todd A. Crowl[2]
[1]*University of Notre Dame, UNDERC, BIOS 35502-01: Practicum in Field Biology*
[2]*Florida International University, Institute for the Environment*

Abstract

Crayfish and caddisflies both play important roles as detritivores in streams. However, crayfish occupy a complex trophic role as omnivores, consuming both plant material and other herbivores. Their relationship with caddisflies exemplifies the crayfish's complex trophic role. Crayfish may either reduce plant material by feeding or increase plant material via top-down control on caddisfly herbivory. It is unknown whether their role as predator or their role as herbivore is more impactful in stream communities. I hypothesized that in the presence of both caddisflies and crayfish, less vegetation would be consumed than in situations where crayfish alone were present as the crayfish would eat caddisflies instead of plant material. Crayfish predation on caddisflies would reduce the herbivory of both the crayfish and the caddisflies. Cages were stocked with plant matter and either no organisms, crayfish only, caddisflies only, or both crayfish and caddisflies. The cages with crayfish had more plant matter consumed than those without, and the presence of caddisflies had no significant impact in any treatment. No caddisflies were consumed by crayfish. Overall, the presence of caddisflies was not shown to reduce crayfish foraging on vegetation. Further study is needed to explore the interaction between crayfish and caddisflies.

Introduction

In streams, many macroinvertebrates keep the water clear and free of rotting organic matter by consuming leaf litter, periphyton, aquatic plants, and various detritus. Northern caddisfly larva (Pycnopsyche spp.) and northern clearwater crayfish (Orconectes propinquus) are two macroinvertebrates common in Midwestern United States streams. Pycnopsyche are important detritivores in cool, woodland streams (Wiggins 1997), consuming allochthonous carbon mostly in the form of fallen leaves (Hutchens et. al 1997). Northern clearwater crayfish inhabit a complex trophic role as omnivores and, in addition to acting as detritivores and herbivores, act as predators on smaller macroinvertebrates. However, it is not well known whether crayfish play a more significant role as herbivores, reducing leaf litter, or as predators, controlling populations of herbivores and detritivores and therefore preserving leaf litter (Lodge et. al 1994).

Caddisfly (Trichoptera) larvae are part of the widely-used EPT (Ephemeroptera Plecoptera Trichoptera) Index used for water quality and their presence generally indicates cleaner water (Masese and Raburu 2017). It is important to understand other impacts on caddisfly presence, such as predation, in order to better understand the extent of their presence as a metric of water quality. For example, if crayfish prey on caddisflies heavily, then a difference in Trichoptera abundance in two streams may be due to the presence of crayfish rather than a disparity in water quality. This study explores the caddisflies' role in the ecosystem, both as consumers and as prey.

Orconectes Propinquus are regarded as ecosystem engineers in forested, headwater streams for their reduction in the abundance of fine particulate matter, which opens habitat for Heptageniid mayfly larvae. They also impact the rate of detrivory in the stream, which influences the benthic habitat by removing decomposing material (Creed 2004). O. propinquus have been found to be important "gardeners" in stream ecosystems, their foraging determining the presence or absence of Cladophora on stream beds (Hart 1992). They scavenge detritus and a variety of other macroinvertebrates including other crayfish (Capelli 1980). Omnivorous crayfish have been found to be key in breaking down leaf litter in their streams (Zhang et. al 2004), an important part of incorporating allochthonous carbon into a stream ecosystem. It is becoming more important to study the role of O. propinquus as the invasive rusty crayfish (Orconectes rusticus) spreads throughout Michigan and Wisconsin and outcompetes O. propinquus (Capelli and Munjal 1982). This study seeks to investigate whether the availability of caddisflies as prey for crayfish impacts their role as grazers in Tenderfoot Creek, a stream in Michigan inhabited by both organisms. This information would shed light on the mechanisms by which crayfish engineer their ecosystems.

I predicted that the presence of caddisflies would decrease the amount of leaf material that crayfish eat because they would prey on caddisflies, satiating the crayfish in addition to controlling the amount the caddisflies eat. The statistical null hypothesis is that groups of crayfish alone, caddisflies alone, crayfish and caddisflies together, and a control group with neither crayfish nor caddisflies will all see the same amount of leaf material consumed over a six-day period. The biological null hypothesis is that the group with crayfish and caddisflies will consume the same amount of leaf material as the sum of the crayfish only group and the caddisfly only group.

Methods and Material

Study site

The portion of Tenderfoot Creek used is located on the University of Notre Dame's Environmental Research Center, a facility in Michigan's upper peninsula that is largely reserved for research purposes and is free from significant human development. Tenderfoot Creek is a first order stream flowing out of Tenderfoot Lake and a tributary of the Ontonagon River Watershed. This study took place in early July 2019.

Study organisms

We collected all study organisms in the Ontonagon River Watershed. We collected the caddisfly specimens from the stream by hand under rocks, on sticks, and on the skeleton of a white-tailed deer, which suggests that they feed on a variety of detritus along with their main diet of leaf litter. We also found and collected crayfish by hand in rocky areas of shallow streambed.

Experimental apparatus

We placed 20 15 cm by 15 cm unglazed clay tiles in fine wire mesh cages that excluded all but the smallest macroinvertebrates in a semi-shaded riffle in Tenderfoot Creek. We placed one leaf of romaine lettuce cut to the same length as the tile in each cage and weighed them down with a small, dry, clean rock from the shoreline. We chose lettuce for its ease of acquisition and because of its large leaves, which were easy to measure and to prevent from floating in the cages. We weighed each lettuce piece before placing it in its cage.

Estimating initial dry weight of experimental lettuce

We weighed another, similar group of lettuce pieces and dried them at 80˚C until they reached a constant weight after one day, then weighed them again. We used a linear regression between these initial weights and dry weights to estimate what the initial dry weight of the experimental lettuce used in the cages would have been had it been dried (Figure 1).

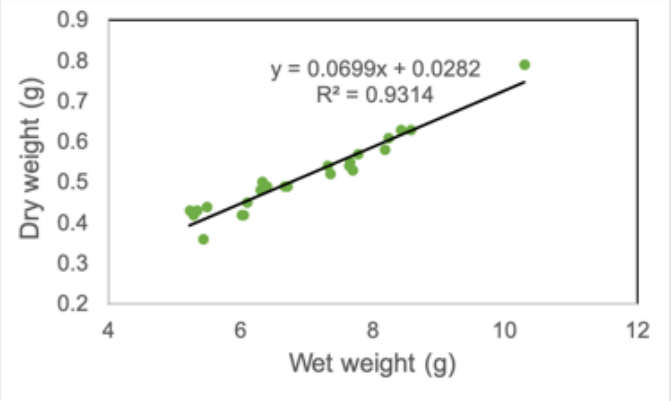


Figure 1. *Linear regression shows the relationship between the initial wet weight of a group of romaine lettuce leaves and their dry weight after being dried at 80˚C until reaching a constant weight after one day. This expression (Dry weight = 0.0699 * Wet weight + 0.0282) was used to estimate the initial dry weight of the lettuce used in the experiment.*

We used this estimation to avoid drying the lettuce before putting it in the stream, where it would likely have disintegrated and would not have imitated the fresh vegetation that foragers generally find in streams.

Experimental design and procedure

We randomly assigned each experimental cage one of four treatments: control, caddisflies only, crayfish only, or both caddisflies and crayfish. The control group had only the lettuce, the tile, and the rock in the cages with no other organisms added. The caddisflies only group had two caddisflies placed inside the cage in addition to the control setup. The crayfish only group had one crayfish added in addition to the control setup, and the caddisflies and crayfish group had two caddisflies and one crayfish added in addition to the control setup. The crayfish used had an average carapace length of 18.06 ± 1.36 mm.

Over the course of the experiment, the water level dropped in the stream due to the summer heat. We checked the cages every two days and moved them closer to the center of the creek in order to keep the bottoms of the cages in approximately 5 cm of water. One crayfish died in the course of the experiment and was replaced. Two dead caddisflies in the caddisfly-only experimental group were replaced in a similar manner. If a caddisfly had died in the cages with both crayfish and caddisflies, we would not have replaced them as they would have been assumed to have been predated by the crayfish. The cages remained in the stream for six days, when the lettuce that had been eaten the most was close to disappearing. After retrieval from the stream, we lightly rinsed the lettuce to remove silt while maintaining structural integrity, dried it at 80˚C until it reached a constant weight after one day, and weighed it.

The difference between initial estimated dry weight of the lettuce and the final dry weight of the lettuce from each cage was considered the mass of lettuce consumed. We conducted a one-way ANOVA to determine if there was a significant difference between the mass of lettuce consumed between the treatments using RStudio (3.5.2, RStudio, Boston, Massachusetts).

Results

After confirming the normality of the mass of lettuce consumed data for all 20 cages with a Shapiro-Wilk normality test (p=0.6625), a one-way ANOVA showed that there was a significant difference between the mass of lettuce consumed in different experimental groups (mean mass of lettuce consumed ± standard deviation; control = 0.19 ± 0.08 g; crayfish only = 0.40 ± .08 g; caddisflies only = 0.20 ± 0.10 g; both crayfish and caddisflies = 0.37 ± 0.17 g; $F_{3,16}$=4.866, p=0.0157, Figure 2).

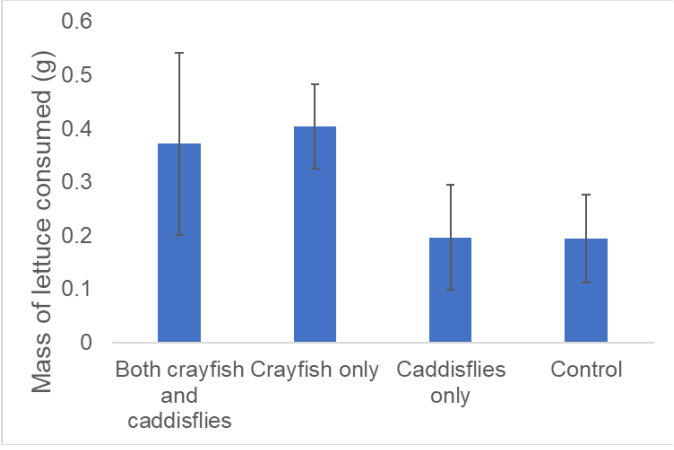


Figure 2. *Bar graph of mass of lettuce consumed (g) for each experimental group. The error bars indicate standard deviation. The two groups with crayfish consumed significantly more lettuce than those without crayfish.*

A Tukey's Honestly Significant Difference Test showed that the crayfish-only group and the group with both caddisflies and crayfish did not differ significantly (p=0.9753). The control group did not differ significantly from the group with only caddisflies (p=1.0000). The crayfish only group significantly differed from the caddisfly only group (p=0.0520), as well as from the control group (p=0.0520). The group with both caddisflies and crayfish differed from the caddisfly only group (p=0.1118) and from the control group (p=0.1118), although only marginally significantly with alpha set to 0.1.

On average, the cages that contained a crayfish had 0.39 ± 0.13 g of lettuce consumed, while the cages that did not contain crayfish had 0.19 ± 0.09 g of lettuce consumed.

Discussion

This study sought to investigate the impact of the presence of caddisflies on the trophic role of crayfish. The results of this study indicate minimal interaction between crayfish and caddisflies, at least as regards predation. Also, the caddisflies ate a negligible amount of vegetation compared to the crayfish. As for the EPT index, it appears as though predation by O. propinquus does not have an impact on the presence of caddisflies in the genus Pycnopsyche. These caddisflies do not appear to be a component, much less an important one, of the crayfish diet. Because no crayfish ate caddisflies, it is impossible to tell whether giving the crayfish prey to feed on would decrease the amount of vegetation eaten in the system. This limits the scope of this study to the impact of caddisfly larvae presence on crayfish herbivory, which appears to be none. Our statistical null hypothesis has been rejected, but the biological null hypothesis has not. While there may be secondary interactions between the two species as crayfish engineer their environments, this study shows no direct interaction.

Unsurprisingly, this study saw that cages with crayfish differed from those without. However, the caddisfly group did not differ from the control. Caddisflies did not significantly reduce lettuce mass through feeding. In the field, I observed small holes in the lettuce of the cages with only caddisflies, which I believe to be evidence of herbivory. However, it is possible these holes were the result of microbial decomposition. I recommend further studies with greater numbers of caddisflies to investigate whether they consume a significant amount of leaf material. It could also be the case that caddisflies do not eat lettuce specifically but would eat another source of leaf material. Although the caddisflies did not significantly diminish the leaf material provided for them, they do play a role as consumers of leaf litter in their ecosystems (Tornwall and Creed 2016), and I expect I would have seen evidence of this had the experiment been run for longer or with more caddisflies.

An unexpected result was that there was no predation of caddisflies by crayfish throughout the duration of the study. Given that crayfish thrive on a diet with both macroinvertebrates and vegetation or macroinvertebrates only over a vegetation only diet, I expected that crayfish would prey on the available macroinvertebrates (Hills et. al 1993). There are several possible explanations for this lack of predation. While the crayfish are opportunistic scavengers that feed on aquatic insects, I could not find record of them specifically feeding on Trichoptera larvae (Capelli 1980). Perhaps caddisflies are too well defended by their cases for the small crayfish to extract them. It is also possible that the crayfish were satiated by the lettuce, which was easy to access, and did not feel pressure to exert themselves by hunting the caddisflies. It is possible that if the crayfish had run out of lettuce before the end of the experiment, they would have preyed on the caddisflies. A future study could place crayfish and caddisflies together with no alternate food source for the crayfish to see if crayfish prey upon caddisflies when they are their only option for food.

Additionally, the caddisflies sometimes wedged themselves between the tile and the cage wall, which could be an effective hiding technique. However, crayfish would also wedge themselves in this area of the cage, so I find it unlikely that the caddisflies would have been able to evade the crayfish had they decided to attack except by receding into their cases. The cases of the caddisflies not only provide a physical shield from attack, but probably also allow the caddisfly to elude the attention of crayfish due to their nondescript appearance (Duffield et. al 1977). The caddisflies' defenses appear to have served them well as they evaded predation in a small enclosure with a crayfish for six days. Another study could present crayfish with caddisflies with and without their cases to test the efficacy of the case in deterring crayfish predation.

Another possible explanation is that the crayfish worried more about being eaten themselves than about eating other macroinvertebrates. This study used small crayfish, which are predated by native fish (Didonato and Lodge 1993). Schools of small fish did occasionally swim in the vicinity of the experimental cages during this study. Perhaps crayfish were more cautious and hid under the lettuce to avoid fish instead of actively hunting. Another study should use different sizes of crayfish to see if there is a relationship between size and predation on caddisflies.

This experiment focused solely on the interaction of crayfish and caddisflies on the grazing potential of both species. However, this is a limited view of the whole stream ecosystem. Further observational studies of areas with and without crayfish and caddisflies are needed to fully understand how this relationship functions in the context of the stream. Other avenues of potential future research include offering the crayfish different prey to get a clearer picture of how hunting impacts crayfish herbivory and comparing the behavior of different species of crayfish. Specifically, I recommend mayfly larvae as the offered prey because they have been found in the stomachs of O. propinquus (Capelli 1980).

Acknowledgments

Thank you to Todd Crowl, Ph.D. for invaluable advice and help with experimental design, field work, and lab work. I am grateful to all UNDERC 2019 staff and students for support this summer, and especially for crucial help in crayfish collection provided by the UNDERC Class of 2019. Special thanks to Matthew Donahue who assisted in many field expeditions and cage constructions. Thank you to Harrison Wojtas and Carolina Quiles-Bengochea for keeping an eye on the crayfish for me. A warm thanks also to Shannon Jones for the prompt procurement

of the proper project materials. Thank you to Dr. Michael Cramer and Dr. Gary Belovsky, who direct the program that made this project possible. This project was funded by the generous support of the Bernard J. Hank Family Endowment.

References

Capelli, Gregory M. 1980. Seasonal variation in the food habits of the crayfish Orconectes propinquus (Girard) in Trout Lake, Vilas County, Wisconsin, U.S.A. (Decapoda, Astacidea, Cambaridae). Crustaceana 38:82-86.

Capelli, Gregory M and BL Munjal. 1982. Aggressive interactions and resource competition in relation to species displacement among crayfish of the genus Orconectes. Journal of
Crustacean Biology 2:486-492.

Creed, RP and JM Reed. 2004. Ecosystem engineering by crayfish in a headwater stream community. Journal of the North American Benthological Society 23(2): 224-236.

Didonato, GT and DM Lodge. 1993. Species replacements among Orconectes crayfishes in Wisconsin lakes- the role of predation by fish. Canadian Journal of Fisheries and Aquatic
Sciences 50:1484-1488.

Duffield, RM, MS Blum, JB Wallace, HA Lloyd, and FE Regnier. 1977. Chemistry of the defensive secretion of the caddisfly Pyncnopsyche scabripennis (Trichoptera: Limnephilidae). Journal of Chemical Ecology 3(6): 649-656.

Hart, David. 1992. Community organization in streams: the importance of species interactions, physical factors, and chance. Oecologia 91:220-228.

Hills, AM, DM Sinars, and DM Lodge. 1993. Invasion of an occupied niche by the crayfish Orconectes rusticus- potential importance of growth and mortality. Oecologia 94(3): 303-
306.

Hutchens, John J, EF Benfield, and JR Webster. 1997. Diet and growth of a leaf-shreddingcaddisfly in southern Appalachian streams of contrasting disturbance history. Hydrobiologia 346:193-201.

Lodge, DM, MW Kershner, JE Aloi, and AP Covich. 1994. Effects of an omnivorous crayfish (Orconectes rusticus) on a fresh-water littoral food-web. Ecology 75:1265-1281.

Masese, Frank Onderi and RO Raburu. 2017. Improving the performance of the EPT Index to accommodate multiple stressors in Afrotropical streams. African Journal of Aquatic
Science 42(3):219-233.

Tornwall, Brett M, and Creed, RP. 2016. Shifts in shredder communities and leaf breakdown along a disrupted stream continuum. Freshwater Science 35(4): 1312-1320.

Wiggins, Glenn B., 1977, Larvae of the North American Caddisfly Genera (Trihoptera), University of Toronto Press, Toronto and Buffalo, Canada, 284 p.

Zhang, Yixin, JS Richardson, and JN Negishi. 2004. Detritus processing, ecosystem engineeringand benthic diversity: a test of predator-omnivore interference. Journal of Animal
Ecology 73(4): 756-766.

About the Author

Abigail White is a junior at Notre Dame. She is majoring in environmental sciences with a minor in theology, and is a member of the Glynn Family Honors Program. Her research was conducted at the University of Notre Dame Environmental Research Center in the upper peninsula of Michigan as part of a summer program. She has also done research in the Tank Lab on stream macroinvertebrates, and will undertake a senior thesis on the same topic. After graduation, she hopes to attend graduate school to continue in her passions of ecology and museum studies.

Carbon Cycling in UNDERC Lentic Ecosystems: Understanding Wetland and Lake Dissolved Organic Carbon (DOC) Uptake

Mariana Silva[1]
Advisor: Ceara Talbot[1]
[1]*University of Notre Dame, Department of Biological Sciences*

Abstract

Lakes and wetlands have different mechanisms for processing carbon, which is important for understanding the world's capacity to store carbon as a part of the global carbon cycle. This research explores mineralization rates in one lake and one wetland on property at the University of Notre Dame Environmental Research Center (UNDERC). The first hypothesis was that wetland and lake ecosystems would demonstrate distinct mineralization rates. Second, it was hypothesized that carbon mineralization in water sample vials would not be affected if these vials were placed in opposite ecosystem locations from their respective origins (which primarily isolates temperature and light as key factors). A "home-versus-away" experimental design was implemented in Morris Lake and a neighboring wetland. After collecting absorbance measurements from each of the samples, exponential decay models were created and their k-constants were compared. Paired t-tests (parametric) and paired Mann-Whitney tests (non-parametric) were performed to compare mineralization rates from the four possible scenarios combining two water sample types with two environmental settings. The sampling time frame was unfortunately too long, and DOC mineralization was overtaken eventually by heterotroph mortality and breakdown. While results were not significant, regression was found to be more reliable in lake water absorbance measurements while wetland water did not demonstrate easily predictable carbon uptake curves. This is especially true due to a lag in uptake noticed in lab-incubated wetland water. A difference was found between wetland and lake water absorbance values, which did not change when incubated in opposite sites. This study has implications for the future of bettering lakes and wetlands to potentially sequester more carbon and would ideally warrant future exploration that includes sampling more lakes and wetlands across multiple times in the summer.

Introduction

Scientific understanding has vastly improved regarding the processes driving chemical and biological constituent quantities in lakes, wetlands, and other inland waters (Travnik et al. 2018). Carbon is an important focus for study, since it is at the forefront of climate research. The current understanding of carbon cycling is that inland waters receive, process (Jansson et al. 2000), emit (Butman and Raymond 2011), and store carbon on a global scale (Einsele et al. 2001); this has evolved from the belief that lakes are completely isolated systems (Forbes 1887). An exchange exists between upstream waters, downstream lentic (still-water) bodies such as lakes or wetlands, the atmosphere, and the sediment sink (Kling et al. 1991). In the context of global warming and climate change, at the most basic level it is important for scientists and community members alike to know where carbon goes and in what forms. This way, we can hope to more efficiently mitigate carbon's effect on a diverse, global environment subject to increased anthropogenic pressure.

Dissolved Organic Carbon (DOC) is the most common carbon form in most lakes; it is available as a resource for heterotrophs that, unable to photosynthesize, alternatively build up the carbon as biomass and excrete it as dissolved carbon dioxide (Pace et al. 2004). Running waters such as rivers and streams carry degradable organic material, are able to export CO2 to the atmosphere, contribute to outflux from downstream transport, and participate in sediment burial of carbon and other nutrients (Butman and Raymond 2011). Additionally, some interaction may exist along interfaces between wetlands, lakes, and other nearby habitats. This could affect the chemical characteristics of these areas and thus the rates of DOC uptake (or mineralization for the purposes of this study), depending on the pathways with which carbon enters the body of water (Pace et al. 2004).

Specifically, the question that this study seeks to investigate is: How does the rate of carbon mineralization differ across lentic aquatic ecosystems?

Determining the rates of carbon processing in lentic ecosystems is a good way to understand carbon cycling and how these different areas might "respond" to an influx in atmospheric carbon, or carbon in other forms. Other research may benefit from this exploration, especially considering the creation of more optimally engineered carbon sequestration systems in natural environments. Further investigation in this area can also contribute to the development of strategies for ecosystem conservation and adaptation to changing climates in wetlands, lakes, streams, or other sites. At UNDERC specifically, large-scale investigations occur regarding lake ecology and the influence of increased DOC levels, dubbed "lake browning" (Jones and Lennon, 2015). The insights from these experiments would benefit from having more knowledge about the carbon-uptake mechanisms of specific lakes, and how mineralization may change in the future.

There are two hypotheses being tested. First, that wetland and lake ecosystems will demonstrate distinct mineralization rates; second, that carbon mineralization in water sample vials would not be affected if these vials were placed in opposite ecosystem locations from their respective origins (which primarily isolates temperature and light as key factors). More specifically, these hypotheses lead to the following predictions: first, that the rate of carbon mineralization will be higher in wetland ecosystems than in lake ecosystems; second, after

following a "home-versus-away" experimental design, the rate for wetland DOC mineralization will remain unaffected when placed in lake ecosystem conditions, and likewise for lake DOC mineralization in wetland conditions. With regard to the second prediction, seeing no change is ideal in order to demonstrate that the chemical and biological factors present in each site are in fact driving carbon uptake mechanisms, rather than more general and variable qualities of aquatic habitats like temperature or sunlight.

Methods and Materials

Study site

Operating within the constraints for this program's 10-week mixed class-and-research time frame, only one site from each ecosystem (lake and wetland) was selected for experimentation. I selected Morris Lake, a relatively small research lake on the northern end of property, which neighbors an accessible wetland to its east that does not receive regular attention and is undisturbed by road traffic (Figure 1).

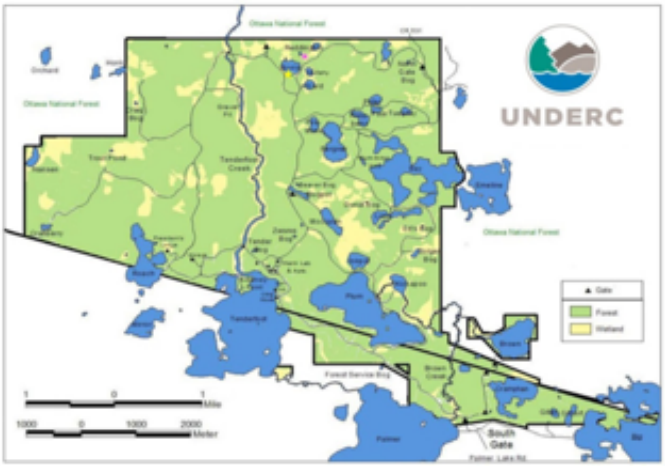


Figure 1. *A map of UNDERC property; in the north of the property, the yellow star represents the Morris Lake study site and the pink star represents the nearby wetland site.*

Morris Lake is designated for research, and is safe from potential disturbance from recreational activities. The experimental site was located close enough to the shore to reach easily with waders, but still less at risk to be disturbed in more open water. The lake itself is eutrophic, with "extensive littoral vegetation in the form of emergent grasses, submerged macrophytes (especially Elodea) growing from the lake's muddy bottom, and floating patches of lily pads extending outward from the shore" (UNDERC, n.d.).

The wetland adjacent to Morris Lake can be characterized as a freshwater marsh, as it contains emergent soft-stemmed aquatic plants, a shallow-water regime, and shallow to nonexistent peat deposits (Mitsch and Gosselink, 2015). Notable flora include wild calla (Calla palustris), tall grasses, mosses, bedstraw (Galium), and sensitive fern (Onoclea sensibilis). The site is a five- to ten-minute walk in waders through wooded territory, giving way to muddy areas as trees decrease in abundance, which finally clears out entirely where only grasses and patches of standing water are prevalent.

Phase 1: Experimental design

Seventy eight plastic, 40mm vials were filled in the field with water; half of the vials were filled at Morris Lake, and the other half were filled at the neighboring wetland site. The vials were separated and left in the field in four "home-versus-away" groups: 1) lake water in a lake setting, 2) lake water in a wetland setting, 3) wetland water in a wetland setting, and 4) wetland water in a lake setting (Figure 2).

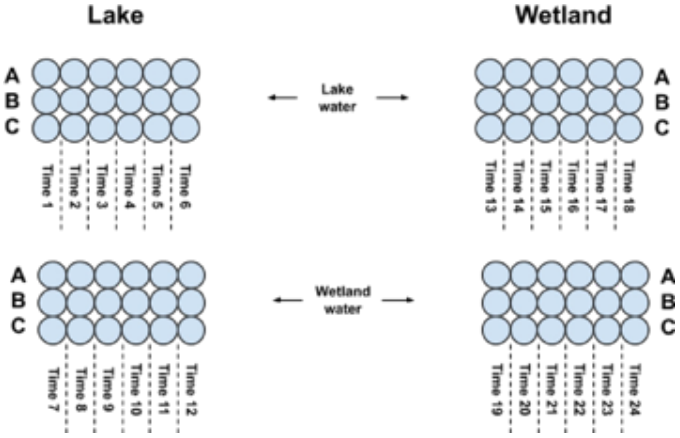


Figure 2. *A schematic describing the experiment design. Blue circles represent individual vials and are split into groups of three (A, B, and C) to be tested over three-day time intervals.*

The vials were held secure in a plastic cage apparatus to allow for light to pass through and for water to circulate freely around the vials (Figure 3).

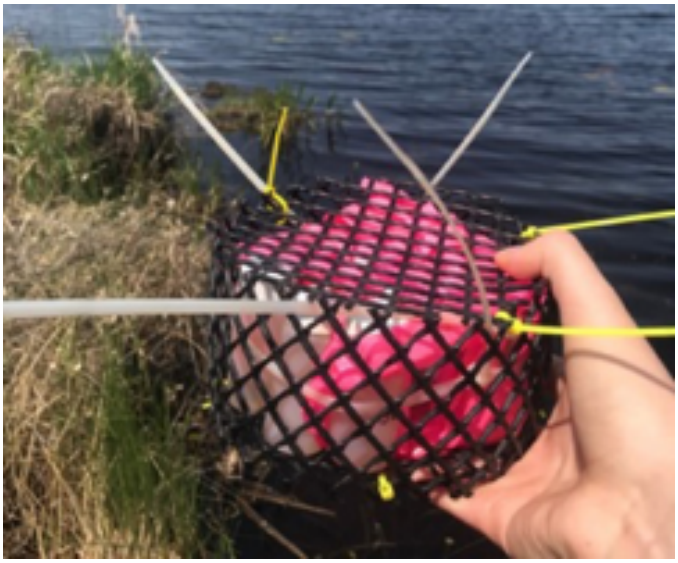


Figure 3. *Experimental apparatus, which was affixed to a wooden stake and left for incubation. Morris Lake is pictured in the background. Note: the pink flagging tape was removed after this photo was taken.*

Vials were collected from each apparatus at regular intervals and brought back to the lab for analysis. Vials were refrigerated if they could not be analyzed immediately. Taking three vials from the sites at a time provides field replication, resulting in 26 groups with which to determine average measurements. Additionally, every time the vials were taken from

the field, a probe was used to measure water temperature in Celsius and Dissolved Oxygen content (DO) in mg/L, using a combined temperature and DO meter. These readings provided additional information for reference when data was being synthesized. Then, in the laboratory, the color in the water samples was analyzed by measuring absorbance on a UV-Vis spectrophotometer at 440 nanometers. This is widely used as a proxy for DOC concentration (Findlay, 2006). First, the water was filtered through a glass-fiber filter with a pore size of 0.5µm. Then, the absorbance for each sample was measured and recorded. This process was repeated for all of the 78 vials. Finally, R was used to compare changes in absorbance/DOC concentration at each time point to determine rates of DOC mineralization between the ecosystem locations.

Phase 2: Lab-incubation verification

After synthesizing some of the data and creating preliminary plots from Phase 1 an ad hoc "lab-incubation" experiment was performed in hopes of providing a more standard regression curve (Figure 4). This was done by shortening the sampling interval to 12 hours in a controlled setting. This second phase was not implemented outside because of meteorological differences (e.g. ambient temperatures, precipitation, etc.) between mid-June and mid-July sampling time frames; more importantly, it was not feasible to return to the field every 12 hours without proper preparation, given the demands of the UNDERC program.

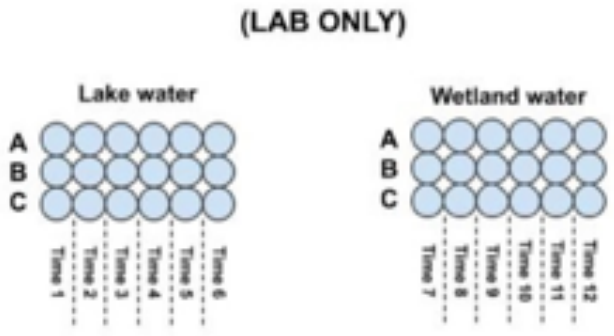


***Figure 4**. A schematic describing the ad hoc "lab-incubation" design. Blue circles represent individual vials and are split into groups of] three (A, B, and C) to be tested over 12-hour time intervals.*

Methods from Phase 1 were repeated, except the lab setting did not involve a home-versus-away component. Instead, half of 42 vials were filled with lake water, and the other half with wetland water, sampled from the same locations as in Phase 1. The vials were kept collectively in a tray of water indoors, submerged with room for water flow, at approximate room temperature - though it was a bit cooler, which helped mirror the water temperatures measured earlier in the summer (Tables 1 and 2). After an initial set of samples were run through the spectrometer, six sampling iterations occurred over three days at 8:15 A.M. and 8:15 P.M.

Statistics

To test the first hypothesis, four exponential decay curves were fitted to each of the home-versus-away scenarios, and two additional curves were fitted to each of the lab-incubation scenarios. The rate of carbon mineralization is calculated by finding the k-constant of the exponential model of the form:

$$y=a\cdot e^{(-k\cdot t)} \qquad [1]$$

Where a is a constant and t represents time. The R2 value for each regression was recorded to verify the assumption that DOC is mineralized approximately exponentially, which has been shown through much previous literature. Then, the k-constants were compared without statistics to provide a baseline understanding of how the rates may differ. The lab-incubation curves were intended to be utilized as references for all home-versus-away scenarios, and to add additional verification if the k-constant is potentially unchanged by external conditions.

To test the second hypothesis, paired t-tests (parametric) and paired Mann-Whitney tests (non-parametric) were performed to compare: (1) lake "home" versus lake "away;" (2) wetland "home" versus wetland "away;" (3) lake "home" versus wetland "home;" and (4) lake "away versus wetland "away." Scenarios 1 and 2 compare site location while controlling for water type, which scenarios 3 and 4 compare water type while controlling for site location. All statistics and figures were performed and built in RStudio.

Results

Part 1: Regression

After creating preliminary plots of all data points, it was observed that the absorbance values for all scenarios begin to trend upwards toward the end of the "decay" period; this is most notable for the last two time periods (Figure 5).

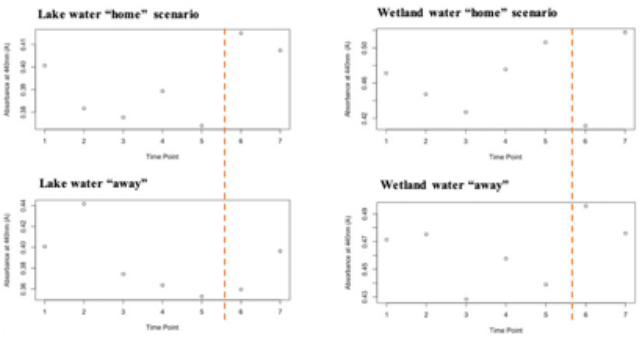


***Figure 5.** A summary of "home-versus-away" plotted as averages for each time point. The red dashed line denotes where a decreasing trend stopped for all scenarios except Wetland "home," which experienced a shift as early as Time Point 4.*

Bacteria and other heterotrophs in the confined vials of water very likely died and after a period of DOC consumption began to break down, contributing to a rise in absorbance levels. This was a clear indication that the time frame for Phase 1 of the experiment had been too long. Because of this, the last two points were dropped from analysis after recording initial observations from plots of the full dataset.

After being split into the four home-versus-away scenario groups, exponential models were fitted. For all regres-

sion models, no significant p-values were found. For the lake "home" scenario, k = -.03262 with an R2 of .5741 (Figure 6, top); for the lake "away" scenario, k = -.09871 with an R2 of .4918 (Figure 6, bottom).

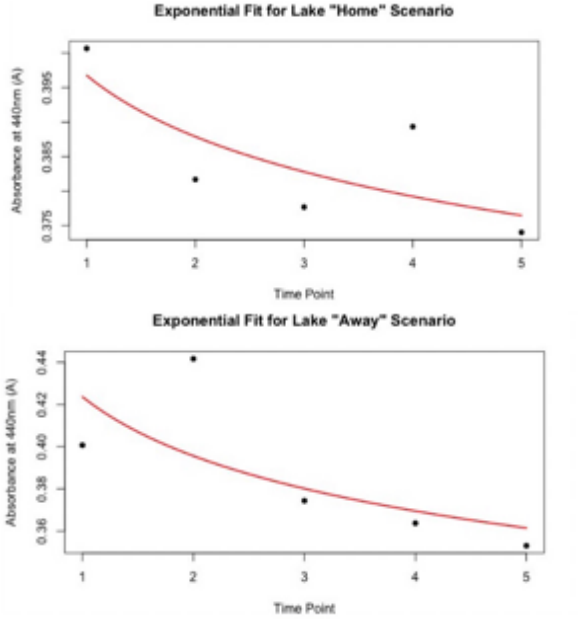


Figure 6. *"Home" and "away" scenario averages for lake water, including exponential regression curves. For the lake "home" (top), k = -.03262, R2 = .5741 (F-statistic: 4.044 on 1 and 3 DF, p-value: 0.1379). For lake "away" (bottom), k= -.09871, R2=.4918 (F-statistic: 2.903 on 1 and 3 DF, p-value: 0.1870).*

Wetland rates from observation of the scatter plot still did not seem to follow any reliable decay curve. For the wetland "home" scenario, k = positive 0.03126 with an R2 of 0.09443 (Figure 7, top); for the wetland "away" scenario, k = -0.01079 with an R2 of 0.02323 (Figure 7, bottom).

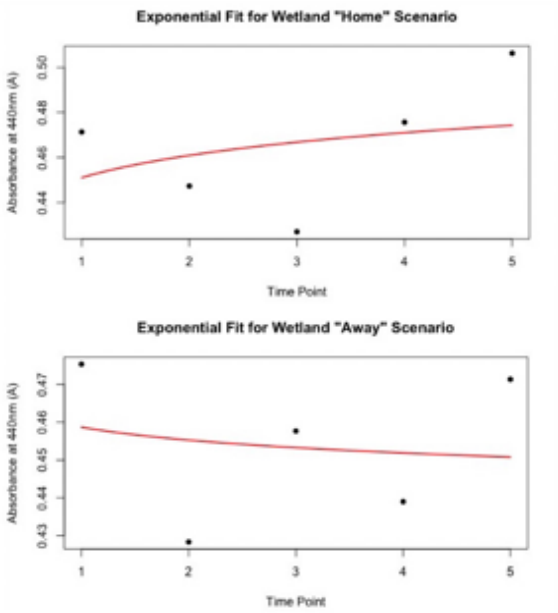


Figure 7. *"Home" and "away" scenario averages for wetland water, including exponential regression curves. For the wetland "home," k = 0.03126, R2 = 0.09443 (F-statistic: 0.3128 on 1 and 3 DF, p-value: 0.615). For wetland "away," k = -0.01079, R2 = 0.02323 (F-statistic: 0.07135 on 1 and 3 DF, p-value: 0.8067).*

It is thus more difficult to compare k-constants between the different home-versus-away scenarios beyond simple observations. With this is mind, it is worth noting that lake water k-constants were observed to be more negative than wetland water k-constants, though the data across time points for wetland water was much less consistent at showing any trend compared to lake water data.

Then, regression analysis was performed on the wetland water and lake water lab incubation rates to provide a more reliable snapshot of decay with shorter time intervals. Though still not significant, the p-values for the lab-incubation are notably lower. For lake water, k = .06971 with an R2 of 0.4461 (Figure 8, top). For wetland water, k = -.05983 with an R2 of 0.5069; importantly, the regression curve for the wetland water did not follow what appeared to be a lag in the decrease in absorbance (Figure 8, bottom).

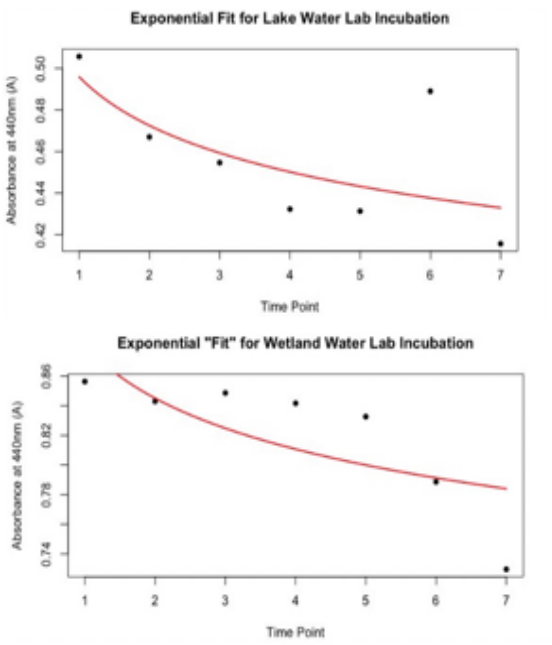


Figure 8. *Lab-incubation averages for both lake and wetland water types, including exponential regression curves. For the lake (top), k = -.09871, R2 = .4918 (F-statistic: 2.903 on 1 and 3 DF, p-value: 0.187). For the wetland (bottom), k = -0.01079, R2 = 0.02323 (F-statistic: 0.07135 on 1 and 3 DF, p-value: 0.8067).*

Part 2: Paired t-tests

A Shapiro-Wilk test was performed on the dataset as a whole, and received a significant result, meaning data was not normal (W = 0.89626, p-value = 1.071e-05). However, once data were subset into groups according to both site and water type, the lake "home," lake "away," wetland "home," and wetland "away" scenarios separately were all normal (in that order: W = 0.93843, p-value = 0.6548; W = 0.91454, p-value = 0.4953; W = 0.96998, p-value = 0.8751; W = 0.96517, p-value = 0.8434). Thus, four paired t-tests were performed to compare each of the subsets.When comparing both "home" scenarios for each water type, a significant difference exists between absorbance values across all paired time points (t = -4.4072, df = 6, p-value = 0.004533). This is also true for comparing both "away" scenarios where water type differs (t = 6.476, df = 6, p-value = 0.0006438). Next, when comparing actual home-versus-away scenarios for the lake water samples, no significant difference was found (t = 0.60464, df = 6, p-value = 0.5676); the same was found for the wetland water samples (t = -0.10541, df = 6, p-value = 0.9195).

Discussion

All points for discussion regarding the findings of this experiment are based off of nonsignificant differences; however, there is still much to learn from the general trends and the overall process of the sampling/analysis. For the first hypothesis, I expected that the k-constant for lake water DOC decay would be smaller in magnitude than the k-constant for wetland water DOC decay. There is a generally lower DOC concentration in lakes than in wetlands, and this should result in a lower rate for lake DOC decay because fewer organisms are available to mineralize carbon quickly (Dodds 2002). This would have indicated a faster carbon uptake process in wetlands. However, because the wetland scenarios' absorbance averages were so erratic with time, "decay" was almost indistinguishable. Thus, when comparing the k-constants it was actually the lake scenarios that appeared to be decaying more quickly. It would be extremely difficult to definitively make this conclusion without much more thorough experimentation and many more significant results, since it would contradict a host of prior literature. We can most likely attribute this observation to other variables that could not have been accounted for while the vials incubated in the field. For example, the wetland water where the vials were submerged could potentially have been shallow enough that a change in water level may have left the vials not fully submerged for some period of time between the three-day intervals. Also, assuming that carbon mineralization does occur faster in wetlands, it would make sense then that the wetland scenarios would become less reliable more quickly — this is most evident in the wetland "home" scenario (Figure 6, bottom), where only the first three time points display a steady decreasing curve before absorbance begins to increase again.

Also, samples from time point seven were kept in the fridge for a longer period of time during class modules and analyzed later. The vials were very small; thus, it is likely that the carbon was taken up very quickly. Along with the time spent in the cold may have allowed for the uptick in absorbance values towards the end, likely because heterotrophs or bacteria confined to the vials would eventually die and decompose, contributing to additional DOC levels.

Instead of being able to reliably compare mineralization rates, investigation shifted more to look at why lake water decayed more predictably than wetland water. This is why the lab incubation, though it was not brought about a priori, became such an integral part of making this study a more holistic perspective. Interestingly, the k-constant for lake water decay in the lab incubation exponential model was still of a greater magnitude than the k-constant for wetland water decay. However, the wetland water curve again does not demonstrate the expected exponential decrease, and instead appears to lag for the first few time points of the decay process. Once the absorbance values truly begin to decrease, they display a more dramatic decay than that of the lake water (Figure 8). It would not be advisable to only test the last few points for a more desirable decay curve, because we are still unsure why the lag is occurring and cannot justify excluding these data. There are some differences with the lab-incubation sampling that may account for this observation.

Note that the water for Phase 2 was taken out of the lake/wetland much later than Phase 1
(mid-July compared to late May into June), which has the potential to change DOC concentrations in the water, and possibly mineralization rates. Also, the wetland as a whole became much less wet when I returned to obtain water for Phase 2. Reeds, cattails, and grasses had grown significantly higher, and I was forced to walk farther than where I had initially set up Phase 1 field incubation because no standing water remained at that location. This gives rise to speculation on how wetland areas might "respond" to increases in summer temperatures due to

climate change, if the observed lag could be replicated and studied further. Also based off of this observation, it would be interesting to see if shorter-term decay rates would change temporally over the summer months.

Tracking carbon cycling clearly does not take into account the nitrogen and phosphorus content of lakes or wetlands. These constituents greatly impact the potential for organisms to thrive in the area, and would perhaps play an indirect role in mineralization as well. In future research at UNDERC, if a more extensive experimentation is possible, it would be useful to measure DOC, total nitrogen, and total phosphorus all at the same time to investigate any interaction that may exist.

This study has implications for the future of bettering lakes and wetlands to potentially sequester more carbon. However, the smaller the scale of carbon uptake, the more difficult it becomes to observe a generalizable impact, because the constituents of individual lake and wetland ecosystems can vary drastically even in close proximity. A key limitation of this study is surely the lack of multiple lake sites and wetland sites. Ideally, future work can be done on Morris Lake again that would include other research lake sites to understand UNDERC property more fully. Researching sequestration potential in dystrophic bogs on property would be an additional wetland area of interest, along with finding other freshwater marshes.

Acknowledgments

A huge thank-you to Ceara Talbot, my mentor, for allowing me to explore biogeochemistry under her wing, and Joey Jaros, my fellow mentee under Ceara, for spending time with me during the many quiet hours spent with the spectrometer. I'm appreciative of all of the support and companionship from the entire UNDERC class, as well as from the class TAs, [Tall] Matt Gregory and Jasper Leavitt. Shannon Jones, the UNDERC lab technician, played an integral role in keeping my life organized. Thank you also to Michael Cramer, and Gary Belovsky for selecting me to have the amazing research experience that I did this summer, and to the Hank Family who make the whole program happen every year.

References

Butman, D., and P. A. Raymond. 2011. Significant efflux of carbon dioxide from streams and rivers in the United States. Nat. Geosci. 4:839–842.

Dodds, W. K. 2002. Freshwater ecosystems. In Freshwater Ecology: concepts and environmental applications. Eds. W. R. Heyer, M. A. Donnely, R. W. McDiarmid, L. C. Hayek, and M. S.
Foster. Smithsonian Institution Press, Washington, D. C.

Einsele, G., J. P. Yan, and M. Hinderer. 2001. Atmospheric carbon burial in modern lake basins and its significance for the global carbon budget. Glob. Planet. Change 30: 167–195.

Findlay, S. 2006. Dissolved Organic Matter. In Methods in Stream Ecology: second edition. Eds. Hauer, F.R.; Lamberti, G., Elsevier: Oxford, pp. 239-248.

Forbes, S. A. 1887. The lake as a microcosm. Bulletin of Peoria Scientific Association, pp. 77–87.
Reprinted in Bulletin of the Illinois State Natural History Survey 15 (1925): 537–550.

Jansson, M., A.-K. Bergström, P. Blomqvist, and S. Drakare. 2000. Allochthonous organic carbon and phytoplankton/ bacterioplankton production relationship in lakes. Ecology 81: 3250–3255.

Jones, S. E. and J. T Lennon. 2015. A test of the subsidy-stability hypothesis: the effects of terrestrial carbon in aquatic ecosystems. Ecology 96: 1550-1560.

Kling, G. W., G. W. Kipphut, and M. C. Miller. 1991. Arctic lakes and streams as gas conduits to the atmosphere: Implications for tundra carbon budgets. Science 251: 298–301.

Mitcsh, W. J. and J. G. Gosselink.

Pace, M. L., J.J. Cole , S.R. Carpenter, J.F. Kitchell, J.R. Hodgson, Van de Bogert, M.C., Bade, D.L., Kritzberg, E.S., Bastviken, D. 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. Nature 427:6971.

Travnik, L. J.; Cole, J. J.; Prairie, Y. T. The study of carbon in inland waters—from isolated ecosystems to players in the global carbon cycle. Limnol. Oceanog. Letters, 3:41-48.

UNDERC. "Morris Lake." Unpublished. Obtained via https://underc.nd.edu/assets/203884/chemmaps.pdf.Accessed 7/18/2019.

About the Author

Mariana Silva is a junior at the University of Notre Dame. She is a Brennan Family Scholar, majoring in environmental engineering, with a minor in theology. last summer, she participated in the University of Notre Dame Environmental Research Center (UNDERC) summer research program, where her interest in wetland ecology and ecological engineering found its roots. After graduation, she seeks to earn a master's degree and hopefully a Ph.D. within the areas of constructed wetlands, wetland restoration, and carbon sequestration.

Exploring the Impact of Dissolved Organic Carbon on Bluegill (Lepomis macrochirus) Behavior: Why Live Subjects are Important for Scientific Study

Matthew A. Aubourg[1]
Advisor: Amaryllis Adey[1]
[1] *University of Notre Dame, Department of Environmental Sciences*

Abstract

Global browning in aquatic systems is a phenomenon, augmented by anthropogenic climate change, that is caused by increasing concentrations of dissolved carbon, making bodies of water brown, decreasing visibility. Higher levels of dissolved organic carbon (DOC) alter the absorbance of solar radiation throughout a water column and organisms living in the water column, especially visual predators, may be impacted, exhibiting altered behaviors to adapt to variation in visibility. For example, greater aggression or exploration may improve the ability to capture prey when visibility is hindered. The objective of this study was to explore the effects of varying concentrations of DOC upon the aggressive and explorative behaviors of bluegill (Lepomis macrochirus) fish. Bluegill were kept in nine mesocosms containing three different concentrations of DOC. After an acclimation period of five weeks, two behavioral assays focusing on aggression and boldness/exploration were conducted upon each fish. We found that aggression and boldness were not significantly related to DOC concentration ($p=0.4658$ and $p=0.2294$, respectively). These findings highlight a need for further studies to determine the impacts of increasing DOC concentrations on fish behavior.

Introduction

The ecological impact of anthropogenic activity has focused mainly on the increases in atmospheric carbon concentrations from fossil fuel emissions. As a result, terrestrial carbon uptake has also been found to increase, cycling excess carbon out of the atmosphere and into other planetary cycles (1). In both marine and freshwater systems, phytoplankton play a significant role in CO2 uptake through photosynthesis in what is often called the biological pump (2, 3). Phytoplankton convert the inorganic, atmospheric carbon into organic carbon, through photosynthesis, and their subsequent decomposition increases levels of dissolved organic carbon (DOC) (4). Heightened levels of atmospheric carbon dioxide also prompt greater uptake by terrestrial plants. Terrestrial systems have been historically known as major carbon sinks, and this trend extends to today with the augmentation of climate change (5, 6). Therefore, increased carbon concentrations in the decomposed matter have led to greater levels of DOC in lakes and other bodies of water via runoff (7). Although DOC occurs naturally, these increased concentrations due to global warming can alter the structure of lake ecosystems (8).

DOC absorbs varying wavelengths of incoming solar radiation (9). Therefore, higher levels limit light penetration at greater depths (10, 11). Primary producers within these systems then receive altered levels of light for their energy production (12). Consumers' predator-prey interactions might also be affected by faster light extinction due to reliance on visual stimuli (13). Previous studies have found fish to be more aggressive and territorial as well as less tolerant of other individuals near them with higher visibility (14). Visibility, especially in captivity, has been found to affect the stress responses of fish; lower visibility leads to lower activity levels, higher metabolic rates, and altered swimming performance (15, 16, 17). Bluegill (Lepomis macrochirus) are common secondary predators that can be found across the globe and are visual predators, likely affected by the global browning phenomenon. Higher DOC may impact bluegill through changing phytoplankton behavior or a direct behavioral response to the lesser visibility. In daily competition for food and space, they have been observed to exhibit aggression towards other organisms (18). The interaction between predators, prey, and other consumers is the foundation for food web dynamics. Understanding how these dynamics fluctuate with climate change is necessary for effective decision making with fishery management and conservation.

In this study, we analyzed the relationship between concentrations of DOC and the behavioral response of bluegill fish, focusing on aggression and boldness, often referred to as exploration. We hypothesized that bluegill exposed to lower levels of DOC would be more aggressive and more explorative due to their higher visibility in comparison to the bluegill accustomed to greater concentrations of organic carbon.

Methods and Materials

Study Site

This experiment was conducted at the University of Notre Dame Environmental Science Center (UNDERC), which is a private research facility covering around 3035 hectares overlapping the state lines of Wisconsin (Vilas County) and the Upper Peninsula of Michigan (Gogebic County:46° 13' N, 89° 32' W). Bluegill collection occurred at Bay Lake, a 67.3-hectare lake with a maximum depth of approximately 40 meters that is primarily oligotrophic and low DOC (absorbance of 0.162 at 440nm) (Figure 1). Bay Lake is mainly surrounded by the research facility and one other private property; it is fished regularly with low fishing pressure.

Figure 1. *Map of Bay Lake, where all bluegill collections occurred. At each of the red circles, Fyke nets set there caught fish that were utilized in this study.*

Bluegill Collection

Bluegill were collected using Fyke nets set near shore at seven locations over 18 days from June 2, 2019 to June 19, 2019. Due to difficulty capturing the number of fish needed for the study, sites to place the nets were chosen based off of known areas of high bluegill abundance (Figure 1). Individuals less than 16 centimeters long were released to ensure further development of young bluegill in their natural environment.

Experimental Design

After collection in the field, the fish were maintained and studied at the UNDERC Aquatic Lab for three weeks. At the lab, fish were processed by caudal fin clippings, nose to tail length measurements, and sex determination for later identification. They were then randomly sorted into nine mesocosms (3 fish/mesocosm), which were prepared during the week before bluegill collection. Each of the nine mesocosms (Pipe Top Round Tanks, Freeland Industries Inc., Portage, WI) was filled with water from Tenderfoot Lake, another lake on the UNDERC property. There were three levels of DOC controlled by three varied amounts of "Super Hume," a humic acid solution commonly used in DOC research (19). The lowest DOC level contained water from Tenderfoot Lake without any of the Super Hume (absorbance of 0.154 at 440 nm). The moderate DOC level had an average absorbance of 0.7073 at 440 nm and, the highest DOC level had an average absorbance of 1.338 at 440 nm (spectrophotometer, Spectronic GENESYS 2, Thermo Electron Co., Madison, WI). Twice per week, this water was changed, the mesocosms were scrubbed clean, and three cups of salt were added after each water change. Fish within each mesocosm were uniquely marked for subsequent identification via unique caudal fin clips within each mesocosm. The bluegill were left to acclimate to their respective DOC level for three weeks while being fed freeze-dried bloodworms (approximately 0.3191g, Tetra GmbH, Melle, Germany). During this period, there were a series of bluegill fatalities due to the stress of being placed in the mesocosms. As a result, we added new fish to the mesocosms to take their place. These fish were each kept in tanks with equivalent DOC concentrations before being added to the experimental mesocosms.

Behavioral Analysis

To determine whether DOC impacted the behavioral response of these bluegill, we conducted aggression and boldness/exploration assays. We used a mirror image assay, which tests individuals for aggressive behavior (18). We determined that a fish had noticed its reflection when they turned their eyes toward the mirror and then backed away from or approached the mirror. A mirror was placed at one side of the container and the fish was added to the other. Fish acclimated in the study container for two minutes with the mirror concealed. Thereafter, we observed the fish for ten minutes, recording the latency time to recognize itself in the mirror and exhibit any aggressive behavior with a maximum time of 10 minutes. Additionally, we recorded whether this display was overt or restrained (19) (Table 1, Figure 2). Overt aggression was displayed through ramming or biting the reflection in the mirror. Restrained behaviors still were displays of aggression but did not involve an attempt to cause harm to the reflection. Some examples of restrained aggression included swimming back and forth along the mirror or displaying the lateral view. In total, nine behaviors were looked for with each fish.

Table 1. *Table of the behaviors focused on in the mirror assay of aggression. Biting and ramming are the two behaviors considered to be overtly aggressive, meaning to do some sort of harm to an opponent.*

1	Biting attempt, touching the mirror	Overt
2	Fast approach with physical contact to mirror, mouth closed	
3	Swimming at high speed towards reflection, opercula are spread	Restrained
4	All fins are maximally spread; fish is close to reflection	
5	Body held stiffly in a bent position along the longitudinal axis	
6	Body inclined downwards. Unpaired and pelvic fins are spread.	
7	Swimming repeatedly back and forth along the mirror, maintaining contact with snout to the mirror	
8	Swimming repeatedly backand forth along the mirror, body parallel to mirror at close distance.	
9	Fish still for a while, showing lateral view	

To test boldness/exploration, fish were placed into a container with an opaque divider in the middle, obscuring their view of the other side of the container (20). On the side opposite the fish, we placed a novel object, a Lego car attached to a rock. Each fish was allowed a two-minute acclimation period. Then, we removed the divider and recorded the latency time for the fish to enter the novel environment as well as whether it interacted with the novel object (Figure 2), with a maximum observation time of ten minutes. Assays were conducted in low DOC water because I was unable to see the bluegill at any higher DOC level.

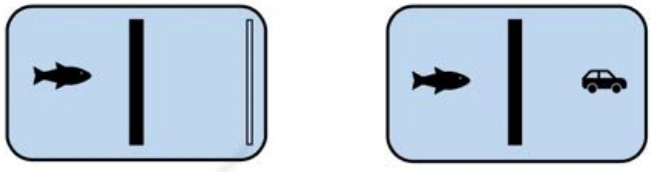


Figure 2. *Diagram of the set up for the mirror assay of aggression (left) and the novel object assay of boldness/exploration (right). For the mirror test, the fish was initially placed in the container in the left side of the partition and the mirror was on*

the opposite side. Similarly, in the novel object test, the fish was initially placed on the left side of the partition and the novel object (Lego car) was placed on the opposite side.

Handling Bacterial Fin Rot in Assays and Mesocosms

Halfway through the acclimation period, every tank was cleaned with bleach due to the observation of bacterial fin rot in several of the tanks. In behavioral assays, individuals that were caught with or developed bacterial fin rot were excluded from the experiment. Disease inherently hinders behavior, which serves as a critical source of error for this experiment.

To combat the continued spread of fin rot, we changed the water in every mesocosm twice per week and added three cups of salt to each mesocosm after the water change. Although bluegill are freshwater fish, adding salt to the mesocosms provides the fish with further electrolytes to better handle the stressful environment and aid in the elimination of bacteria or fungus (21, 22). During the second week of acclimation, we bleached and scrubbed every mesocosm to eliminate as much bacterial or fungal buildup as possible. Fish were kept in tanks of their respective DOC levels during the cleaning process.

Statistical Analyses

To analyze the results of the aggression assay, a Kruskal-Wallis one-way ANOVA was used to determine the relationship between time taken to exhibit aggression and DOC level, sex, and expression of overt or restrained aggression.

Similarly, the boldness/exploration assay was analyzed using a one-way ANOVA to determine the relationship between time taken to explore the novel object and DOC level, sex, and whether contact was made with the Lego car. All analyses were conducted in R Programming version 1.2.1335.

Results

Mirror Test of Aggression

There was no significant difference in latency time between DOC levels (mean ± SE; low DOC, 133 ± 78.7; medium DOC, 364 ± 112; high DOC, 214 ± 100; H=1.5278; p=0.4658; Figure 3).

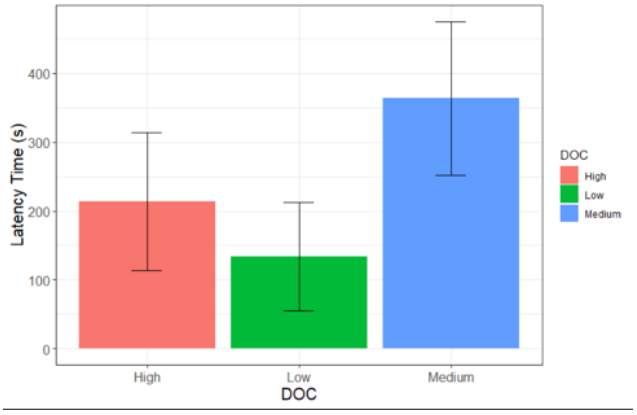


Figure 3. *Bar plot of latency times in the mirror assay of aggression by DOC level. Results of a Kruskal-Wallis test revealed no significant difference in latency times between the DOC levels (H=1.5278, p=0.4658).*

No significant difference was found in latency time between sexes (mean ± SE; male, 370 ± 68.4; female, 355 ± 95.5; H=1.7646; p=0.1841; Figure 4).

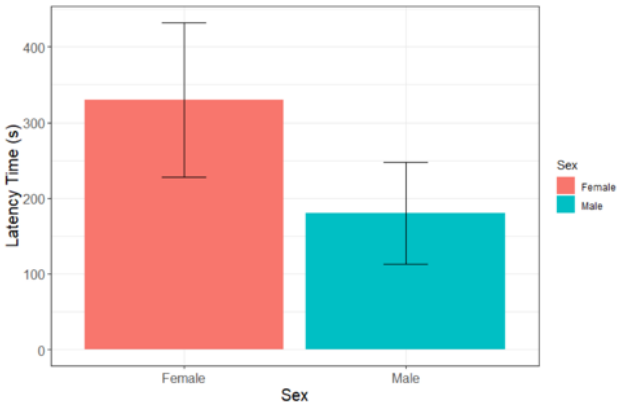


Figure 4. *Bar plot of latency times in the mirror assay of aggression between sexes. Results of a Kruskal-Wallis test revealed no significant difference in latency times between male and female individuals (H=1.7646, p=0.1841).*

There was a significant difference found in latency time between individuals that expressed none, overt, or restrained aggression (mean ± SE; none, 600 ± 0; restrained, 57.6 ± 12.5; overt, 53.4 ± 11.7; H=13.885; p<0.001; Figure 5). A post-hoc Dunn's Test showed that latency time of individuals expressing no aggressive behavior differed significantly from the overt and restrained aggression individuals (Z=3.182; p=0.00146 and Z=3.270; p=0.00108, respectively). However, latency times of overt and restrained aggression did not differ significantly from each other (Z=0.0878; p=0.930).

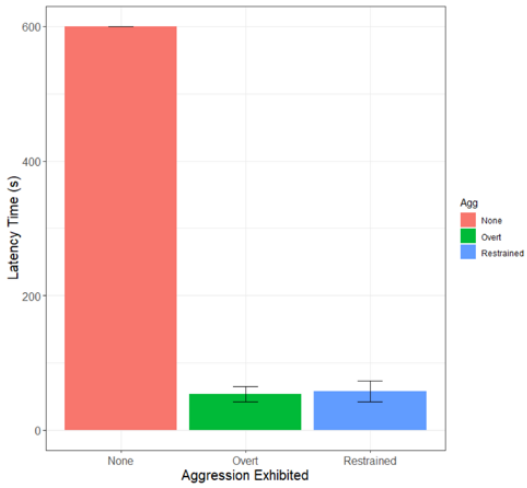


Figure 5. *Bar plot of latency times in the mirror assay of aggression between individuals that exhibited no, overt, or restrained aggression. Results of a Kruskal-Wallis test did reveal a significant difference in latency times between no aggression and both the overt and restrained aggression (H=1.5278, p=0.4658; Dunn's test, Z=3.182; p=0.00146 and Z=3.270; p=0.00108, respectively). No significant difference was found between overt and restrained aggression (Z=0.0878, p=0.930).*

Novel Object Test of Boldness/Exploration

There was no significant difference found in latency time between DOC concentrations (mean ± SE; low DOC, 234 ± 79.1; medium DOC, 465 ± 90.0; high DOC, 395 ± 103; H=2.9444; p=0.2294, Figure 6).

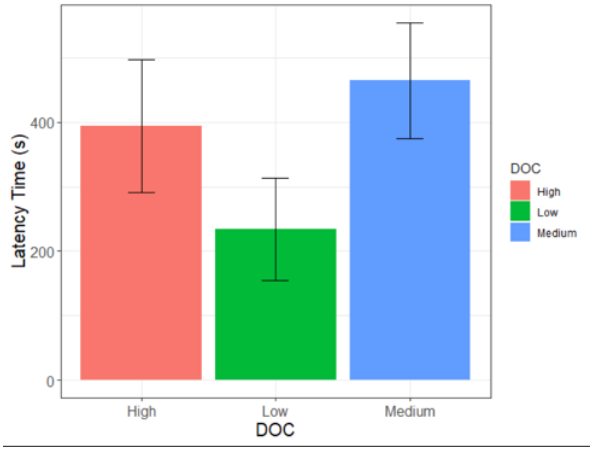


Figure 6. *Bar plot of latency times in the novel object assay of boldness/exploration between DOC levels. Results of a Kruskal-Wallis test revealed no significant difference in latency times between low, medium, and high DOC levels (H=2.9444, p=0.2294).*

There was no significant difference found in latency time between sexes (mean ± SE; male, 370 ± 68.4; female, 355 ± 95.5; H=2.7153; p=0.09939; Figure 7).

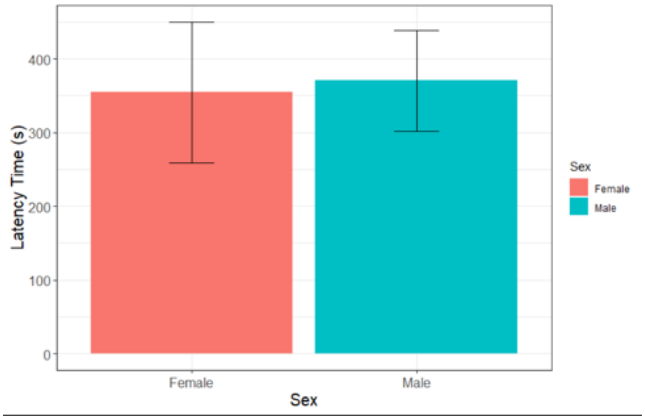


Figure 7. *Bar plot of latency times in the novel object assay of boldness/exploration between sexes. Results of a Kruskal-Wallis test revealed no significant difference in latency times between male and female individuals (H=2.7153, p=0.09939).*

There was no significant difference found in latency time between individuals that did and did not make physical contact with the novel object (mean ± SE; contact, 389 ± 57.3; no contact, 131 ± 12.5; H=1.3022; p=0.2538; Figure 8).

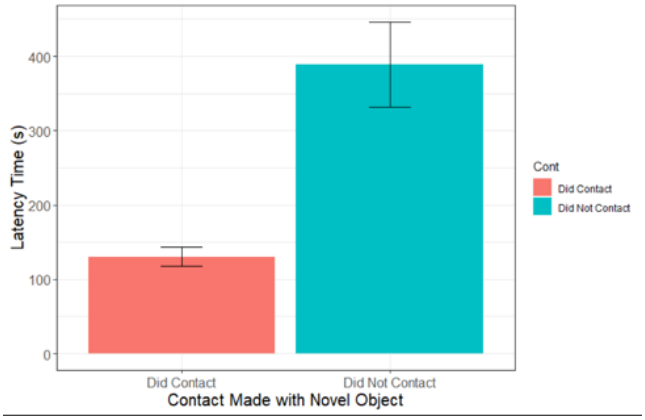


Figure 8. *Bar plot of latency times in the novel object assay of boldness/exploration between individuals that did or did not make physical contact with the novel object. Results of a Kruskal-Wallis test revealed no significant difference in latency times (H=1.3022, p=0.2538).*

Discussion

In this study, we aimed to determine if variation in concentration of DOC altered aggression and boldness/exploration in bluegill. With the global browning phenomena being perpetuated by climate change, it is crucial to understand how aquatic organisms' behaviors may change and influence food web dynamics. The results obtained from this study did not support our hypotheses; there does not appear to be an effect of DOC level upon bluegill aggression and boldness/exploration (p=0.4658, p=0.2294, respectively). However, with greater replication and minimization of stress from the mesocosms in future studies, this hypothesis may gain more support.

We expected to find that bluegill exposed to low DOC with high visibility would have lower latency times and more overt aggression, shown in the results of the mirror test. However, we found no significant relationships in this study that contributed to answering this question. Although significance was found in latency time between aggression exhibited (none, overt, or restrained), this is because the individuals that exhibited no remarkable behavior had a set latency time of 600 seconds with no error or deviation. Therefore, no aggression exhibited was significantly different, but overt and restrained were not significantly different from each other, which is the more important result for this study. Fish in the medium DOC concentration exhibited the highest latency times, which was in direct contrast to my expected results.

In the novel object test of boldness/exploration, we expected to find lower latency time and more contact with the novel object from bluegill in the low DOC level. Similarly, to the aggression assay, there were no significant results found. This is likely due to the same concerns and stresses that were previously mentioned that affected the aggression assay results. Although latency times were lower in both tests with low DOC fish, no significance was able to be determined.

When removing an organism from its natural habitat and placing it into a new environment that may not meet all its needs, it is natural for the individual to be put under stress,

especially with younger organisms (23). Fish, especially, are known for their inability to tolerate high levels of stress when placed into mesocosms, such as what was used in this study (24). Stressed fish have altered metabolic rates and are less likely to be confrontational to conserve energy for flight (25, 18). Being in stressful conditions also suppresses fishes' immune systems, leaving them more susceptible to bacterial and fungal infections (26, 27, 28). These issues related to stress induction were evident in this study with several study organisms dying from extensive "fin rot." Fin rot may occur from several sources, such as physical abrasion from contact with rough surfaces, aggression from other fish, nutritional deficiencies, or bacterial and fungal infection (29, 30). This is a potential explanation for unexpectedly high latency times in the medium DOC level and lack of aggressive or explorative responses observed in assays. Numerous individuals across all DOC concentrations were exposed to stress when they were transported to the laboratory, resulting in high bluegill mortality due to bacterial infection and fin erosion. Although individuals with fin rot were not used in this study, individuals not visibly impacted by fin rot themselves may, however, have been affected by the proximity of deteriorating bluegill in the same mesocosms, although not much research has been done to support this.

After cleansing the mesocosms, there was a decrease in mortality of the fish in every DOC level, and some fish even showed signs of recovery over time. I observed that the bluegill in the medium DOC level were the most affected by the fin erosion and the most fatalities were from the three mesocosms in this level. The reasoning for this is unclear; however, this is likely the reason that their latency times were unexpectedly high in comparison to the extreme DOC levels. Uncertain but natural variability such as aforementioned can be mitigated by greater replication, which should be implemented in future studies.

In addition to the use of more replicates, future studies should attempt to minimize the stress of individuals in mesocosms using larger containers and including elements of the bluegills' natural habitats in them such as rocks and vegetation. As opposed to mesocosms, future studies could sample bluegill from bodies of water with a variety of DOC levels and test their aggression, boldness, and exploration, observing specific behaviors and latency time. That way, fish are likely under the lowest amount of stress because they are being tested right from their natural environment. Greater replication in future studies also may aid in coming to significant conclusions and determine if DOC truly influences fish behavior.

Despite the obstacles of this study, further studies that analyze how aquatic organisms are impacted by increasing DOC is of great importance, especially as global browning is expected to continue to increase with climate change. With atmospheric CO2 being a primary anthropogenic climate change driver, levels of dissolved carbon in both marine and freshwater systems are also increasing (31, 32, 33). Greater dissolved carbon causes further global browning, and this is suspected of having effects on the behaviors of both producers and consumers (34). Ecosystem-wide behavioral fluctuation can lead to changes in food web dynamics and biodiversity in freshwater systems globally (35). Understanding these impacts is essential for aquatic management in response to climate change, and they also may affect freshwater aquaculture, directly impacting human activity. In the realm of how DOC affects secondary and tertiary consumers, there has not been extensive study. However, as time progresses, we must consider these impacts more closely to maintain the beauty and biodiversity of our natural, freshwater ecosystems for future generations to study and appreciate.

Acknowledgments

I want to truly thank Amaryllis Adey and Joe Nowak for all their help and support through all the fish collection and maintenance, writing of this paper, and for helping me to maintain sanity and patience when this experiment was not going as expected. I also want to thank the UNDERC staff and students for their teaching, friendship, and help with this study. And lastly, a huge thank you to the Bernard J. Hank Family Endowment for their generosity in funding my research and providing access to and housing at this beautiful property.

References

1. Joos, F., I.C. Prentice, S. Sitch, R. Meyer, G. Hooss, G.K. Plattner, S. Gerber, and K. Hasselmann. 2001. Global warming feedbacks on terrestrial carbon uptake under the Intergovernmental Panel on Climate Change (IPCC) emission scenarios. Global Biogeochemical Cycles 15(4):891-907.

2. Engel, A. (2002). Direct relationship between CO2 uptake and transparent exopolymer particles production in natural phytoplankton. Journal of Plankton Research 24(1):49-53.

3. Low-Decarie, E., Fussmann, G. F., & Bell, G. (2014). Aquatic primary production in a high-CO2 world. Trends in Ecology & Evolution 29(4):223-232.

4. Basu, S., & Mackey, K. (2018). Phytoplankton as Key Mediators of the Biological Carbon Pump: Their Responses to a Changing Climate. Sustainability 10(3).

5. Ciais, P., Tans, P. P., Trolier, M., White, J. W. C., & Francey, R. J. (1995). A large northern hemisphere terrestrial CO2 sink indicated by the 13C/12C ratio of atmospheric CO2. Science 269(5227):1098-1102.

6. Ciais, P., Tan, J., Wang, X., Roedenbeck, C., Chevallier, F., Piao, S. L., ... & Peng, S. (2019). Five decades of northern land carbon uptake revealed by the interhemispheric CO 2 gradient. Nature 568(7751):221.

7. Monteith, D.T., J.L. Stoddard, C.D. Evans, H.A. De Wit, M. Forsius, T. Høgåsen, A. Wilander, B.L. Skjelkvåle, D.S. Jeffries, J. Vuorenmaa, and B. Keller. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. Nature 450(7169):537.

8. Solomon, C.T., S.E. Jones, B.C. Weidel, I. Buffam, M.L. Fork, J. Karlsson, S. Larsen, J.T. Lennon, J.S. Read, S. Sadro, and J.E. Saros. 2015. Ecosystem consequences of changing inputs of terrestrial dissolved organic matter to lakes: current knowledge and future challenges. Ecosystems 18(3):376-389.

9. Kirk, J.T., 1994. Light and photosynthesis in aquatic ecosystems. Cambridge university press.

10. Pérez-Fuentetaja, A., P.J. Dillon, N.D. Yan, and D.J. McQueen. 1999. Significance of dissolved organic carbon in the prediction of

thermocline depth in small Canadian Shield lakes. Aquatic Ecology 33(2):127-133.

11. Houser, J.N. 2006. Water color affects the stratification, surface temperature, heat content, and mean epilimnetic irradiance of small lakes. Canadian Journal of Fisheries and Aquatic Sciences 63(11):2447-2455.

12. Thrane, J.E., D.O. Hessen, and T. Andersen. 2014. The absorption of light in lakes: negative impact of dissolved organic carbon on primary productivity. Ecosystems 17(6):1040-1052.

13. Horppila, J., A. Liljendahl-Nurminen, and Malinen, T. 2004. Effects of clay turbidity and light on the predator prey interaction between smelts and chaoborids. Canadian Journal of Fisheries and Aquatic Sciences 61(10):1862-1870.

14. Valdimarsson, S.K. and N.B. Metcalfe. 2001. Is the level of aggression and dispersion in territorial fish dependent on light intensity?. Animal Behaviour 61(6):1143-1149.

15. Strand, Å., Alanärä, A., Staffan, F., & Magnhagen, C. (2007). Effects of tank colour and light intensity on feed intake, growth rate and energy expenditure of juvenile Eurasian perch, Perca fluviatilis L. Aquaculture 272(1-4):312-318.

16. Barton, B. A., & Schreck, C. B. (1987). Metabolic cost of acute physical stress in juvenile steelhead. Transactions of the American Fisheries Society 116(2): 257-263.

17. Bonga, W. se, 1997. The Stress Response in Fish. Physiol. Rev 77.

18. Balzarini, V., M. Taborsky, S. Wanner, F. Koch, and J.G. Frommen. 2014. Mirror, mirror on the wall: the predictive value of mirror tests for measuring aggression in fish. Behavioral ecology and sociobiology 68(5):871-878.

19. Lennon, J.T., S.K. Hamilton, M.E. Muscarella, A.S. Grandy, K. Wickings, and S.E. Jones. 2013. A source of terrestrial organic carbon to investigate the browning of aquatic ecosystems. PLoS One 8(10):e75771.

20. Wilson, A.D. and J.G.J. Godin. 2009. Boldness and behavioral syndromes in the bluegill sunfish, Lepomis macrochirus. Behavioral Ecology 20(2):231-237.

21. Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... & Knowlton, N. (2007). Coral reefs under rapid climate change and ocean acidification. science 318(5857):1737-1742.

22. Caldeira, K., & Wickett, M. E. (2003). Oceanography: anthropogenic carbon and ocean pH. Nature 425(6956):365.

23. Koakoski, G., Oliveira, T. A., da Rosa, J. G. S., Fagundes, M., Kreutz, L. C., & Barcellos, L. J. G. (2012). Divergent time course of cortisol response to stress in fish of different ages. Physiology & behavior 106(2):129-132.

24. Marçalo, A., Pousão, Ferreira, P., Mateus, L., Duarte Correia, J. H., & Stratoudakis, Y. (2008). Sardine early survival, physical condition and stress after introduction to captivity. Journal of Fish Biology 72(1):103-120.

25. Sloman, K. A., Motherwell, G., O'connor, K., & Taylor, A. C. (2000). The effect of social stress on the standard metabolic rate (SMR) of brown trout, Salmo trutta. Fish Physiology and Biochemistry 23(1):49-53.

26. Maule, A. G., Tripp, R. A., Kaattari, S. L., & Schreck, C. B. (1989). Stress alters immune function and disease resistance in chinook salmon (Oncorhynchus tshawytscha). Journal of Endocrinology 120(1):135-142.

27. Snieszko, S. F. (1974). The effects of environmental stress on outbreaks of infectious diseases of fishes. Journal of Fish Biology 6(2):197-208.

28. Walters, G. R., & Plumb, J. A. (1980). Environmental stress and bacterial infection in channel catfish, Ictalurus punctatus Rafinesque. Journal of Fish Biology 17(2):177-185.

29. Latremouille, D. N. (2003). Fin erosion in aquaculture and natural environments. Reviews in Fisheries Science 11(4):315-335.

30. Schneider, R., & Nicholson, B. L. (1980). Bacteria associated with fin rot disease in hatchery-reared Atlantic salmon (Salmo salar). Canadian Journal of Fisheries and Aquatic Sciences 37(10):1505-1513.

31. Carneiro, P. C. F., & Urbinati, E. C. (2001). Salt as a stress response mitigator of matrinxã, Brycon cephalus (Günther), during transport. Aquaculture Research 32(4):297-304.

32. Tomasso, J. R., Davis, K. B., & Parker, N. C. (1980, March). Plasma corticosteroid and electrolyte dynamics of hybrid striped bass (white bass x striped bass) during netting and hauling. In Proceedings of the World Mariculture Society 11(1:4):303-310.

33. Mitsch, W. J., Bernal, B., Nahlik, A. M., Mander, Ü., Zhang, L., Anderson, C. J., ... & Brix, H. (2013). Wetlands, carbon, and climate change. Landscape Ecology 28(4):583-597.

34. Creed, I. F., Bergström, A. K., Trick, C. G., Grimm, N. B., Hessen, D. O., Karlsson, J., ... & Senar, O. E. (2018). Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. Global change biology 24(8):3692-3714.

35. Modenutti, B., Albariño, R., Bastidas Navarro, M., Díaz Villanueva, V., Souza, M. S., Trochine, C., ... & Balseiro, E. S. T. E. B. A. N. (2010). Structure and dynamic of food webs in Andean North Patagonian freshwater systems: organic matter, light and nutrient relationships. Ecología Austral 20(2):95-114.

About the Author

Matthew Aubourg is a junior at Notre Dame majoring in environmental sciences with a minor in sustainability. He is a member of the Balfour-Hesburgh Scholars Program and the Department of Biological Sciences Honors Program. His research was conducted at the University of Notre Dame Environmental Research Center during a summer research internship. However, during the semester, he works with Neil Lobo, Ph.D. and his research group studying malaria vector bionomics and interventions. After graduating from Notre Dame, Matthew is hoping to attend graduate school for global health and pursue a career in combating food insecurity and pursuing environmental justice.

An Elementary Proof of the Prime Number Theorem

Andrew Burke
Advisor: Richard Birkett, Nicholas Lohr
University of Notre Dame, Department of Mathematics

Abstract *In number theory, the prime number theorem describes the asymptotic distribution of the prime numbers. It states that $\pi(x) \sim x/\log x$. The prime number theorem was first proved in 1896 using the tools of complex analysis. This paper, assuming no background in number theory from the reader, presents an elementary proof of the theorem.*

Introduction

The sequence of prime numbers

$$2,3,5,7,11,13,17,\ldots$$

has long been a source of great mystery for mathematicians. Prime numbers are fundamental in the sense that every number can be decomposed as a product of its prime factors. They are the building blocks out of which all numbers are built. However, it has proved to be exceedingly difficult to find patterns within the primes.

It is natural to consider the number of primes less than or equal to a given number x. We will denote the quantity by $\pi(x)$; then

$$\pi(x) = \sum_{p \leq x} 1$$

where the index p runs over all primes less than or equal to x. Around the year 1800, Gauss was among the first to suggest $x/\log x$ as an approximation for $\pi(x)$ where the logarithm is base e. The idea that e is the base of the logarithm is quite remarkable, seeing as the primes have no obvious connection with e, or, indeed, with any of calculus. This approximation implies that an arbitrary number x has about a $1/\log x$ chance of being prime. In more precise language, the claim that

$$\lim_{x\to\infty} \frac{\pi(x)}{x/\log x} = 1$$

is what we today call the prime number theorem. Little progress was made on the conjective until the middle of the 19th century when Chebyshev provided an important bound on the size of $\pi(x)$. Finally, nearly 100 years after Gauss, the theorem was proved independently by Jacques Hadamard and Charles-Jean de la Vallée Poisson in 1896. Both proofs involved complex numbers, which also have no obvious connection with the primes.

Some mathematicians still wanted an elementary proof, one that avoided complex numbers, in the hope that it would provide a clearer explanation for why the theorem holds. This proof was given by Atle Selberg [1] and Paul Erdös in 1949. It succeeded in avoiding complex analysis, but it is still far from easy. This paper will present an elementary proof of the prime number theorem, assuming no background in number theory from the reader. Some background in calculus and proof techniques will be useful.

The remainder of this section will focus on elementary properties of the primes, as well as give a heuristic argument for the prime number theorem [6]. Section 2 will present a theorem due to Chebyshev [4], [2]. Sections 3, 4, and 5 mirror [5], a more modern presentation of Selberg's argument. Section 3 introduces Möbius inversion, section 4 proves lemmas for later use, and section 5 deduces Selberg's inequality, a result used in all known elementary proofs of the prime number theorem. Finally, sections 5 and 6 prove the prime number theorem [3].

Throughout this paper, x, y refer to real numbers, n, m, j, k to natural numbers and $p, q, r, \ldots$ to primes unless otherwise specified. The symbol $\lfloor x \rfloor$ refers to the greatest integer less than or equal to x. The notation $f(x) = O(g(x))$ means that there exists a constant C such that $|f(x)| \leq Cg(x)$ for all sufficiently large x and the notation $f(x) = o(g(x))$ means that $\lim_{x\to\infty} f(x)/g(x) = 0$.

In school, one learns that every number is representable as a product of primes. The elementary proof is omitted and will be used as a fact.

Theorem 1. *Any number $n \geq 2$ is expressible as the product of primes.*

This shows that every number can be written as the product of primes in at least one way. Could there be multiple ways? The answer is yes, and since the proof is simple, we treat it as a fact.

Theorem 2. *Any number can be written as the product of primes in exactly one way*

This result is called the fundamental theorem of arithmetic. Now that we know that every number is built out of primes, how many building blocks are there? In chemistry, for example, there are only finitely many elements on the periodic table. The building blocks of the numbers, however, are not so simple (This proof is done by Euclid and is simple. One can find it on their own):

Theorem 3. *There are infinitely many primes*

You probably already had an intuitive feeling for the above theorems before seeing the proof. However, the statement of the prime number theorem is far from intuitive. It was originally conjectured based off of numerical evidence alone. I hope here to provide a theoretical reason for why one might expect such a result to hold.

You will have hopefully learned in calculus that the harmonic series $\sum_{n=1}^{\infty} 1/n$ diverges. However, for $s > 1$, the sum $\sum_{n=1}^{\infty} 1/n^s$ converges. The function $\zeta(s) = \sum_{n=1}^{\infty} 1/n^s$ is called the Reimann zeta function.

Now consider, for $s > 1$, the product extending over all primes

$$\begin{aligned}\prod_p \frac{1}{1-\frac{1}{p^s}} &= \prod_p (1 + \frac{1}{p^s} + \frac{1}{p^{2s}} + \ldots) \\ &= (1 + \frac{1}{2^s} + \frac{1}{2^{2s}} + \ldots)\cdots(1 + \frac{1}{p^s} + \frac{1}{p^{2s}} + \ldots)\cdots\end{aligned}$$

where the simplification follows from the formula for an infinite geometric series and the properties of exponents. Multiplying

the product out, we see that the sum is over terms of the form $1/(p_1^{a_1}p_2^{a_2}\cdots)^s$. Each term of $\sum_{n=1}^{\infty} 1/n^s$ is of the form $1/n^s$. In light of the fundamental theorem of arithmetic, we conclude that $\zeta(s) = \prod_p \frac{1}{1-1/p^s}$. For a rigorous argument, see page 183 of Stein and Shakarchi's "Complex Analysis".

Now we begin a simple but imprecise argument for the prime number theorem. One can determine the number of primes less than or equal to x as follows: take a list of the first x natural numbers and cross out all multiples of 2 greater than 2, then all remaining multiples of 3 greater than 3, of 5, and so on for all primes less than or equal to x. The remaining numbers are all prime. The first step removes about $1/2$ of the numbers, the second $1/3$ and so on. We would expect to have approximately

$$x\prod_{p\le x}\left(1-\frac{1}{p}\right)$$

remaining primes. By the discussion above, it is hopefully believable that the reciprocal of the product

$$\prod_{p\le x}\frac{1}{1-\frac{1}{p}} \approx \sum_{n\le x} 1/n \approx \log x$$

by basic calculus and so

$$x\prod_{p\le x}\left(1-\frac{1}{p}\right) \approx \frac{x}{\log x}$$

This suggests that $\pi(x) \approx \frac{x}{\log x}$. The remainder of this paper is dedicated to making this result precise.

Chebyshev's Theorem

We can define two new prime-counting functions as

$$\vartheta(x) = \sum_{p\le x} \log p$$

$$\psi(x) = \sum_{p^k\le x} \log p$$

where the second sum is extended over all powers of primes that are less than or equal to x.

In addition, for later, use, define the Von Mangoldt function, Λ, by

$$\Lambda(x) = \begin{cases} \log p & \text{if } x = p^k \text{ for some prime } p \text{ and } k \ge 1 \\ 0 & \text{otherwise} \end{cases}$$

Lemma 1.

$$\psi(x) = \sum_{n\le x} \Lambda(n)$$

The advantage of $\vartheta(x)$ and $\psi(x)$ is that, as we will see, they have similar magnitude to $\pi(x)\log x$ while being easier to work with due to the algebraic properties of the logarithm. In this section we aim to prove a theorem of Chebyshev, which, when published in 1850, was the strongest evidence in favor of the prime number theorem.

We use the prime factorization of certain binomial coefficients to bound the prime number counting functions and the following lemma can be proved by induction. We omit the proof since this is an elementary fact.

Lemma 2. *For all $x \ge 2$, we have $\vartheta(x) \le x\log 4 < 3x/2$*

Lemma 3.

$$\liminf_{x\to\infty} \frac{\pi(x)\log x}{x} \ge \log 2 > \frac{1}{2}$$

We now put everything together, as well as show that $\pi(x)\log x$, $\vartheta(x)$, and $\psi(x)$ have the same limiting behavior.

Theorem 4 (Chebyshev's Theorem)**.** *The following equations hold:*

$$\liminf_{x\to\infty} \frac{\vartheta(x)}{x} = \liminf_{x\to\infty} \frac{\psi(x)}{x} = \liminf_{x\to\infty} \frac{\pi(x)\log x}{x} \ge \log 2 > \frac{1}{2}$$

and

$$\limsup_{x\to\infty} \frac{\vartheta(x)}{x} = \limsup_{x\to\infty} \frac{\psi(x)}{x} = \limsup_{x\to\infty} \frac{\pi(x)\log x}{x} \le \log 4 < \frac{3}{2}$$

Proof. Let $x \ge 2$. Note that $p^k \le x$ if and only if $k \le \log_p x = \log x/\log p$. Therefore,

$$\begin{aligned}\vartheta(x) = \sum_{p\le x} \log p \le \psi(x) &= \sum_{p^k\le x} \log p \\ &= \sum_{p\le x} \left\lfloor \frac{\log x}{\log p} \right\rfloor \log p \le \log x \sum_{p\le x} 1 = \pi(x)\log x\end{aligned}$$

Consequently,

$$\liminf_{x\to\infty} \frac{\vartheta(x)}{x} \le \liminf_{x\to\infty} \frac{\psi(x)}{x} \le \liminf_{x\to\infty} \frac{\pi(x)\log x}{x}$$

$$\limsup_{x\to\infty} \frac{\vartheta(x)}{x} \le \limsup_{x\to\infty} \frac{\psi(x)}{x} \le \limsup_{x\to\infty} \frac{\pi(x)\log x}{x}$$

We now prove the other side of the inequalities. Let $0 < \delta < 1$. Then

$$\begin{aligned}\vartheta(x) &\ge \sum_{x^{1-\delta}<p\le x} \log p \ge \sum_{x^{1-\delta}<p\le x} (1-\delta)\log x \\ &= (1-\delta)(\pi(x) - \pi(x^{1-\delta}))\log x \\ &\ge (1-\delta)\pi(x)\log x - x^{1-\delta}\log x\end{aligned}$$

This inequality can now be used to show that $\vartheta(x)$ has the same limiting behavior has $\pi(x)\log x$. We have that

$$\frac{\vartheta(x)}{x} \ge \frac{(1-\delta)\pi(x)\log x}{x} - \frac{\log x}{x^\delta}$$

Since $\lim_{x\to\infty}(\log x/x^\delta) = 0$,

$$\liminf_{x\to\infty} \frac{\vartheta(x)}{x} \ge (1-\delta)\liminf_{x\to\infty} \frac{\pi(x)\log x}{x}$$

$$\liminf_{x\to\infty} \frac{\vartheta(x)}{x} \ge \liminf_{x\to\infty} \frac{\pi(x)\log x}{x}$$

By the same reasoning,

$$\limsup_{x\to\infty} \frac{\vartheta(x)}{x} \ge \limsup_{x\to\infty} \frac{\pi(x)\log x}{x}$$

Consequently,

$$\liminf_{x\to\infty} \frac{\vartheta(x)}{x} = \liminf_{x\to\infty} \frac{\psi(x)}{x} = \liminf_{x\to\infty} \frac{\pi(x)\log x}{x}$$

$$\limsup_{x\to\infty} \frac{\vartheta(x)}{x} = \limsup_{x\to\infty} \frac{\psi(x)}{x} = \limsup_{x\to\infty} \frac{\pi(x)\log x}{x}$$

By lemmas 2 and 3, this concludes the proof. □

Chebyshev's theorem shows that, if $\lim_{x\to\infty} \frac{\pi(x)\log x}{x}$ exists, then it is between $\log 2$ and $\log 4$. In fact, Chebyshev also showed that if the limit exists, it must necessarily be equal to one. We will not prove this claim, though it is worthwhile to note that the hardest part of the prime number theorem is showing that the limit converges to some value, not in determining what it converges to. In addition, we have learned that the prime number theorem is equivalent to the assertion that

$$\lim_{x\to\infty} \frac{\psi(x)}{x} = 1$$

This is the form we strive to prove.

Möbius Inversion Here, we introduce an important concept called Möbius inversion. Suppose G is a function that can be expressed in the form

$$G(x) = F(x) + F(x/2) + F(x/3) + \ldots + F(x/\lfloor x \rfloor) = \sum_{n\le x} F(x/n)$$

for some function F. Is it possible to invert this relation, namely to express the function F in terms of G? The way to do so is suggested by the following:

$$\begin{aligned} G(x) &= F(x) + F(x/2) + F(x/3) + F(x/4) + F(x/5) + \ldots \\ G(x/2) &= \quad F(x/2) \quad + F(x/4) \quad + F(x/6) + \ldots \\ G(x/3) &= \quad\quad F(x/3) \quad\quad + F(x/6) + \ldots \end{aligned}$$

To solve for F, one might take $G(x)$, subtract $G(x/2)$ to clear out the $F(x/2)$ term and then subtract $G(x/3)$ to clear out the $F(x/3)$ term. At this point, the $F(x/4)$ term is already gone, so subtracting $G(x/4)$ is unnecessary. Then one would subtract $G(x/4)$, add $G(x/6)$, and so on, in general clearing out the $F(x/n)$ by use of the equation for $G(x/n)$. When finished the equation for F will be of the form

$$F(x) = \sum_{n\le x} \mu(n) G(x/n)$$

where μ is some uniquely defined function on the natural numbers. We call μ the Mobius function. We now determine the value of $\mu(n)$ for arbitrary n. Substituting the definition of G into the preceeding equation,

$$F(x) = \sum_{j\le x} \mu(j) \sum_{k\le x/j} F(\frac{x}{jk}) = \sum_{jk\le x} \mu(j) F(\frac{x}{jk}) = \sum_{n\le x} F(\frac{x}{n}) \sum_{j|n} \mu(j)$$

where the the sum after the second equality is over all ordered pairs (j,k) with $jk \le x$. From the array, we note that $\mu(1) = 1$. Moreover, the above becomes an identity if

$$\sum_{j|n} \mu(j) = 0$$

for $n > 1$. We note that the preceding equation uniquely determines the function μ.

Lemma 4. *Let f be a function that satisfies $f(1) = 1$ and, when $n = p_1^{a_1} \ldots p_m^{a_m}$ is the prime factorization for $n > 1$, it follows that*

$$f(n) = \begin{cases} 1 & \text{if } a_1 = \ldots = a_m = 1 \text{ and } m \text{ is even} \\ -1 & \text{if } a_1 = \ldots = a_m = 1 \text{ and } m \text{ is odd} \\ 0 & \text{otherwise} \end{cases}$$

Then $f = \mu$.

Proof. By (7), it suffices to show, for $n > 1$, that $f(n)$ satisfies $\sum_{j|n} f(j) = 0$. By the definition of f,

$$\sum_{j|n} f(j) = \sum_{j|p_1\cdots p_m} f(j)$$

If $m = 1$, the identity holds since $f(1) + f(p_1) = 1 - 1 = 0$. If $m > 1$, note that

$$\sum_{j|p_1\cdots p_m} f(j) = \sum_{j|p_1\cdots p_{(m-1)}} (f(j) + f(jp_m))$$

Each term in the preceding sum is zero, and so the claim follows. □

Summarizing, we have

Theorem 5. *If $G(x) = \sum_{n\le x} F(x/n)$, then $F(x) = \sum_{n\le x} \mu(n) G(x/n)$ where μ is given by $\mu(1) = 1$ and if $n = p_1^{a_1} \ldots p_m^{a_m}$ is the prime factorization for $n > 1$, then*

$$\mu(n) = \begin{cases} 1 & \text{if } a_1 = \ldots = a_m = 1 \text{ and } m \text{ is even} \\ -1 & \text{if } a_1 = \ldots = a_m = 1 \text{ and } m \text{ is odd} \\ 0 & \text{otherwise} \end{cases}$$

Proof. □

We now develop an example of a Möbius inversion.

Lemma 5.

$$\Lambda(x) = \sum_{n|x} \mu(n) \log\left(\frac{x}{n}\right)$$

Proof. Let x be factored as $x = p_1^{a_1} \cdots p_m^{a_m}$. Then

$$\log x = a_1 \log p_1 + \ldots + a_m \log p_m = \sum_{j|x} \Lambda(j) = \sum_{n\le x} \Lambda(x/n) \quad (1)$$

since $\Lambda(x/n)$ can be nonzero only when x/n is an integer. However, this equation is true only when x is an integer; otherwise, the right side is zero. To make the equation true for all x, we temporarily redefine $\log x = 0$ for nonintegral x. Then, by Möbius inversion,

$$\Lambda(x) = \sum_{n\le x} \mu(n) \log(x/n) = \sum_{n|x} \mu(n) \log(x/n)$$

The right side of the equation only takes on integral values for log and so this coincides with its standard definition, which we return to for the rest of the paper.

□

Next, we prove an important result for later.

Theorem 6 (Tatuzawa-Iseki Identity)**.** *Let F, G be given as in theorem 5. Then*

$$F(x)\log x + \sum_{n\le x} F(x/n)\Lambda(n) = \sum_{n\le x} \mu(n) \log(x/n) G(x/n)$$

Proof. Define an auxiliary function

$$\begin{aligned} H(x) &= \sum_{j\le x} \mu(j)\log(x/j)G(x/j) \\ &= \sum_{j\le x} \mu(j)\log(x/j) \sum_{k\le x/j} F(\frac{x}{jk}) \\ &= \sum_{jk\le x} \mu(j)\log(x/j)F(\frac{x}{jk}) \\ &= \sum_{n\le x} F(x/n)\sum_{j|n}\mu(j)\log(x/j) \end{aligned}$$

We would now like to apply lemma 5. Before doing so, we make use of the identity $\log(x/j) = \log(x/n) - \log(n/j)$. We have

$$\begin{aligned} H(x) &= \sum_{n\le x} F(x/n)\log(x/n)\sum_{j|n}\mu(j) - \sum_{n\le x} F(x/n)\sum_{j|n}\mu(j)\log(n/j \\ &= F(x)\log x + \sum_{n\le x} F(x/n)\Lambda(n) \end{aligned}$$

where the last step uses (7). Comparing this result with the original definition of H concludes the proof. □

We present one last example of Möbius inversion for later use.

Lemma 6. *Set $T(x) = \sum_{n\le x}\log n$. Then*

$$\psi(x) = \sum_{n\le x}\mu(n)T(x/n)$$

A Few Lemmas

This section proves a few lemmas that will be used later on. Here we consider a discrete analogue of integration by parts, as well as a way of converting sums into integrals. The following lemma follows from computation. We use it as a fact.

Lemma 7. *Let $f(t)$ have a continuous derivative, $f'(t)$, for $t \ge 1$. Let (a_n) be a sequence and let $A(t)$ be its partial sum: $A(t) = \sum_{n\le t} a_n$. Then*

$$\sum_{n\le x} a_n f(n) = f(x)A(x) - \int_1^x f'(t)A(t)dt$$

and

$$\sum_{n\le x} f(n) = \int_1^x f(t)dt + \int_1^x (t-\lfloor t\rfloor)f'(t)dt + f(1) - (x-\lfloor x\rfloor)f(x)$$

We can now apply the preceding lemma to prove some number theoretic results. The following lemma comes directly from applying (1) to $f(t) = \frac{1}{t}$, and we use it as a fact.

Lemma 8.

$$\sum_{n\le x}\frac{1}{n} = \log x + \gamma + O(1/x)$$

where γ is a constant (and, in fact, is Euler's constant).

The lemma follows from applying (1) to $\log(t)$.

Lemma 9.

$$T(x) = \sum_{n\le x}\log j = x\log x - x + O(\log x)$$

Finally, we combine a few previous results to get the following:

Lemma 10.

$$\sum_{n\le x}\frac{\Lambda(n)}{n} = \log x + O(1)$$

Proof. From the proof of lemma 6, we have $T(x) = \sum_{n\le x}\log n = \sum_{jk\le x}\Lambda(j)$. If we sum this expression in the opposite way than we did before, we get

$$\begin{aligned} T(x) &= \sum_{j\le x}\Lambda(j)\sum_{k\le x/j} 1 = \sum_{j\le x}\Lambda(j)\left\lfloor\frac{x}{j}\right\rfloor \\ &= x\sum_{j\le x}\frac{\Lambda(j)}{j} - \sum_{j\le x}\Lambda(j)\left(\frac{x}{j} - \left\lfloor\frac{x}{j}\right\rfloor\right) \end{aligned}$$

We note that

$$0 \le \sum_{j\le x}\Lambda(j)\left(\frac{x}{j} - \left\lfloor\frac{x}{j}\right\rfloor\right) \le \sum_{j\le x}\Lambda(j) = \psi(x) = O(x)$$

by lemma 1 and Chebyshev's theorem. Using lemma 9, it follows that $x\sum_{j\le x}\Lambda(j)/j = x\log x - x + O(x)$ and so $\sum_{j\le x}\Lambda(j)/j = \log x + O(1)$.

□

Selberg's Inequality

We now prove the key results in Selberg and Erdös' proof of the prime number theorem. It is hard to directly find asymptotic relations for the prime number functions. However, there is a roundabout way of doing so. From lemma 6, we have a formula for ψ in terms of the function T. We wish to estimate the value of ψ. Therefore, it may be helpful to find another Mobius transform

$$F_1(x) = \sum_{n\le x}\mu(n)G_1(x/n)$$

such that $G_1(x)$ is close to $T(x)$. Then we would be able to plug $F(x) = \psi(x) - F_1(x)$ and $G(x) = T(x) - G_1(x)$ into the Tatuzawa-Iseki identity. Ideally, using the closeness of G_1 to T, we would be able to estimate ψ. The only remaining question is what to set equal to F_1. Since we are trying to show that $\psi(x) \sim x$, it is natural to want to set $F_1(x) = x$. However, this is not quite enough.

We set $F_1(x) = x - C$ for some, as of yet, undetermined constant C. Then, borrowing notation from section 3,

$$\begin{aligned} G_1(x) &= \sum_{n\le x} F_1(x/n) \\ &= x\sum_{n\le x}\frac{1}{n} - C\sum_{j\le x} 1 \\ &= x\log x + \gamma x - Cx + O(1) \\ &= x\log x - (C-\gamma)x + O(1) \end{aligned}$$

by lemma 8. Then, motivated by the similarities of the above equation to lemma 9, we set $C = \gamma + 1$. Now, following our plan above by making use of the Tatuzawa-Iseki identity, we get

$$(\psi(x)-x+C)\log x+\sum_{n\le x}(\psi(x/n)-(x/n)+C)\Lambda(x/n)=$$
$$\sum_{n\le x}\mu(n)\log(x/n)(T(x/n)-G_1(x/n))$$

We now estimate the right side. By lemma 9,
$T(x)-G_1(x)=O(\log x)$ and so
$(\log x)(T(x)-G_1(x))=O(\log^2 x)=O(\sqrt{x})$.
Therefore, using the fact from elementary calculus that
$\sum_{n\le x}1/\sqrt{n}=O(\sqrt{x})$ as well as $\mu(x)=O(1)$, it follows that

$$\sum_{n\le x}\mu(n)\log(x/n)(T(x/n)-G_1(x/n))=O(x)$$

.
Thus we can simplify to get

$$(\psi(x)\log x-x\log x)+\sum_{n\le x}(\psi(x/n)-(x/n))\Lambda(n)=O(x)$$

where we use the fact that $\sum_{n\le x}\Lambda(n)=\psi(x)=O(x)$ by Chebyshev's theorem. By lemma 10, this implies that

$$\psi(x)\log x+\sum_{n\le x}\Lambda(n)\psi(x/n)=2x\log x+O(x)$$

Using lemma 7 with $a_n=\Lambda(n)$ and $f(t)=\log t$, we get that

$$\sum_{n\le x}\Lambda(n)\log n=\psi(x)\log x-\int_1^x\frac{\psi(t)}{t}dt=\psi(x)\log x+O(x)$$

where we again use the result that $\psi(x)=O(x)$ from Chebyshev's theorem.
Finally,

$$\sum_{jk\le x}\Lambda(j)\Lambda(k)=\sum_{j\le x}\Lambda(j)\sum_{k\le x/j}\Lambda(k)=\sum_{j\le x}\Lambda(j)\psi(x/j)$$

so if we define a new function Λ_2 by

$$\Lambda_2(x)=\Lambda(x)\log x+\sum_{jk=x}\Lambda(j)\Lambda(k)$$

it follows that

$$\begin{aligned}\sum_{n\le x}\Lambda_2(n)&=\sum_{n\le x}\Lambda(n)\log n+\sum_{jk\le x}\Lambda(j)\Lambda(k)\\&=\sum_{n\le x}\Lambda(n)\log n+\sum_{j\le x}\Lambda(j)\psi(x/j)\\&=\psi(x)\log x-\psi(x)\log x+2x\log x+O(x)\\&=2x\log x+O(x).\end{aligned}$$

This is the final form of Selberg's inequality. Even though there is an equal sign, it is an inequality because the above really says that, for some C,x_0, we have $\sum_{n\le x}\Lambda_2(n)-2x\log x\le Cx$ so long as $x\ge x_0$.

We summarize this section's results below.

Theorem 7 (Selberg's Inequality)**.** *The following hold:*

$$\psi(x)\log x+\sum_{n\le x}\Lambda(n)\psi(x/n)=2x\log x+O(x)$$

$$\sum_{n\le x}\Lambda(n)\log n=\psi(x)\log x+O(x)$$

$$\sum_{n\le x}\Lambda_2(n)=2x\log x+O(x)$$

Big Lemma
Set $a_n=\Lambda(n)\log n$, $b_n=\sum_{jk=n}\Lambda(j)\Lambda(k)$, and $r(x)=\psi(x)-x$. Calling on the results from the previous section and Chebyshev's theorem,

$$\frac{1}{2}x\log x\le\sum_{n\le x}a_n=\sum_{n\le x}\Lambda(n)\log n\le\frac{3}{2}x\log x$$

for sufficiently large x.
Further, we see that,

$$\begin{aligned}\sum_{n\le x}(a_n+b_n)&=\sum_{n\le x}\Lambda(n)\log n+\sum_{jk=n}\Lambda(j)\Lambda(k)\\&=\sum_{n\le x}\Lambda_2(n)\sim 2x\log x\end{aligned}$$

and it follows that

$$\frac{1}{2}x\log x\le\sum_{n\le x}b_n\le\frac{3}{2}x\log x$$

for sufficiently large x. Finally, if $x\ge y$, the relation

$$r(x)-r(y)=(\psi(x)-x)-(\psi(y)-y)\ge-(x-y)$$

holds because ψ is an increasing function.

What does this give us? The following theorem is long so we have devoted an entire section to it. The reader may, at this point, prefer to skip to the next section to see how it is used to reach a proof of the prime number theorem.

Lemma 11. *Suppose $(a_n)\ge 0$ and $(b_n)\ge 0$ are sequences that satisfy*

$$\frac{1}{2}x\log x\le\sum_{n\le x}a_n\le\frac{3}{2}x\log x \tag{2}$$

and

$$\frac{1}{2}x\log x\le\sum_{n\le x}b_n\le\frac{3}{2}x\log x$$

for all large x and

$$\sum_{n\le x}(a_n+b_n)\sim 2x\log x$$

as $x\to\infty$. Also, suppose that $r(x)$ is a function such, given $0<\beta\le 1$,

$$|r(x)|\le\beta x \tag{3}$$

for all sufficiently large x and

$$r(x)-r(y)\ge-(x-y)$$

whenever $x\ge y$. Then

$$|\sum_{n\le x}(a_n-b_n)r(x/n)|\le(\beta-\frac{\beta^2}{100}+o(1))x\log^2 x \tag{4}$$

Proof. Without loss of generality, we may assume that the hypotheses hold for all $x \geq 1$. This is because we may, if necessary, adjust the definitions of a_n, b_n, and r for x smaller than some fixed number so that they meet the hypotheses for all $x \geq 1$. By (3) and lemma 8, the value of the left side of (4) will only change by $O(x \log x)$. Also, it suffices to show that

$$\sum_{n \leq x} (a_n - b_n) r(x/n) \leq (\beta - \frac{\beta^2}{100} + o(1)) x \log^2 x$$

since the full inequality can then be derived by changing the roles of a_n and b_n. Upon expansion, the left side of the preceding equation takes the form

$$\beta x \sum_{n \leq x} \frac{a_n + b_n}{n} - \sum_{n \leq x} a_n \left(\frac{\beta x}{n} - r(x/n) \right) - \sum_{n \leq x} b_n \left(\frac{\beta x}{n} - r(x/n) \right)$$

We can estimate the first term of this expansion. Using lemma 7, set $f(t) = 1/t$. Then

$$\begin{aligned} \sum_{n \leq x} \frac{a_n + b_n}{n} &= \frac{1}{x} \sum_{n \leq x} (a_n + b_n) + \int_1^x \frac{1}{t^2} \sum_{n \leq t} (a_n + b_n) dt \\ &\sim 2 \log x + \int_1^x \frac{2 \log t}{t} dt \sim 2 \log x + \log^2 x \sim \log^2 x \end{aligned}$$

so we get the final estimation

$$\sim \beta x \log^2 x - S_A - S_B$$

where S_A and S_B represent the asymptotic behavior of the second and third terms of the above equation, respectively. Note, that $S_A, S_B \geq 0$. We need to show that

$$S_A + S_B \geq \left(\frac{\beta^2}{100} + o(1) \right) x \log^2 x$$

As we will show, it suffices to prove that

$$\begin{aligned} \sum_{y < n \leq 16y} a_n \left(\frac{\beta x}{n} - r(x/n) \right) + b_n \left(\frac{\beta x}{n} + r(x/n) \right) \\ \geq \frac{1}{16} \beta^2 x \log y \end{aligned} \tag{5}$$

for y bigger than some fixed constant. For if we sum (5) over $y = x16^{-k}$ as k ranges over $1 \leq k \leq \lfloor \log_{16} x \rfloor$, noting that $\log_{16} x - 1 < \lfloor \log_{16} x \rfloor \leq \log_{16} x$, we get

$$\begin{aligned} S_A + S_B &= \sum_{n \leq x} a_n (\frac{\beta x}{n} - r(x/n)) + \sum_{n \leq x} b_n (\frac{\beta x}{n} - r(x/n)) \\ &= \sum_{\substack{y = x16^{-k} \\ 1 \leq k \leq \lfloor \log_{16} x \rfloor}} (\sum_{y < n \leq 16y} a_n (\frac{\beta x}{n} - r(x/n)) + \sum_{n \leq x} b_n (\frac{\beta x}{n} - r(x/n))) \\ &\geq \sum_{\substack{y = x16^{-k} \\ 1 \leq k \leq \lfloor \log_{16} x \rfloor}} \frac{1}{16} \beta^2 x \log y \\ &= \sum_{1 \leq k \leq \lfloor \log_{16} x \rfloor} \frac{1}{16} \beta^2 x (\log x - k \log y) \\ &\geq \frac{1}{16} \beta^2 x \log x \log_{16} x - \frac{1}{16} \beta^2 x \log 16 \frac{\lfloor \log_{16} x \rfloor (\lfloor \log_{16} x \rfloor + 1)}{2} \\ &\geq \frac{1}{16} \beta^2 x \log x (\log x / \log 16) \\ &\quad - \frac{1}{16} \beta^2 x \log 16 \frac{\log x / \log 16 (\log x / \log 16 + 1)}{2} \\ &\geq (\frac{\beta^2}{100} + o(1)) x \log^2 x \end{aligned}$$

because $1/(16 \log 16) - 1/(16 \log^2 16) \geq 1/100$. We will prove (5) by cases.

Case 1: Suppose that $|r(x)| \leq (1/2)\beta x$ for all $x \in [x/(16y), x/(4y)]$. Then $r(x/n) \leq (1/2)\beta x/n$ for all $n \in [4y, 16y]$, and so

$$\begin{aligned} \sum_{y < n \leq 16y} a_n \left(\frac{\beta x}{n} - r(x/n) \right) &\geq \sum_{4y < n \leq 16y} a_n \left(\frac{\beta x}{n} - r(x/n) \right) \\ &\geq \frac{1}{2} \beta x \sum_{4y < n \leq 16y} \frac{a_n}{n} \geq \frac{\beta x}{32} \sum_{4y < n \leq 16y} a_n \end{aligned}$$

The sum on the right side can be written $\sum_{n \leq 16y} a_n - \sum_{n \leq 4y} a_n \leq 8y \log 16y - 6y \log y > 2y \log y$ by (2). Therefore,

$$\sum_{y < n \leq 16y} a_n \left(\frac{\beta x}{n} - r(x/n) \right) \geq \frac{\beta x \log y}{16}$$

Since $\beta \leq 1$ and $S_B \geq 0$, this proves (5).

Case 2: Similarly, we now suppose that $|r(x)| \geq (-1/2)\beta x$ for all $x \in [x/(4y), x/y]$. Then $r(x/n) \geq (-1/2)\beta x/n$ for $n \in [y, 4y]$. Using the same argument as before, we find that

$$\sum_{y < n \leq 4y} b_n \left(\frac{\beta x}{n} - r(x/n) \right) \geq \frac{1}{2} \beta x \sum_{y < n \leq 4y} \frac{b_n}{n} \geq \frac{\beta x}{8y} \sum_{y < n \leq 4y} b_n \geq \frac{\beta x \log y}{16}$$

Since $\beta \leq 1$ and $S_A \geq 0$, (5) follows.

Case 3: Finally, suppose that there exists $x_1 \in [x/(16y), x/(4y)]$ with $r(x_1) > (1/2)\beta x_1$ and $x_2 \in [x/(4y), x/y]$ with $r(x_2) < (-1/2)\beta x_2$.
Set $A = \{x \geq x_1 | r(x) \leq -1/2 \beta x\}$ and

$$x_3 = \inf A = \inf \{x \geq x_1 | r(x) \leq -1/2 \beta x\}$$

We show that $r(x_3) = -(1/2)\beta x_3$. Of course this would be easy if r were continuous, but r need not be, and, indeed, is not in the

case in which we apply this lemma. Suppose that $r(x_3) > -(1/2)\beta x_3$, say $r(x_3) + (1/2)\beta x_3 = \delta > 0$. Let y be any number with

$$y \in [x_3, x_3 + \frac{\delta}{1-(1/2)\beta}]$$

Then $y - (1/2)\beta y < x_3 - (1/2)\beta x_3 + \delta$ and so $\delta - (1/2)\beta x_3 - y + x_3 > -(1/2)\beta y$.
Then, by (3),

$$r(y) \geq r(x_3) - (y - x_3) = +\delta - \frac{1}{2}\beta x_3 - (y - x_3) > -\frac{1}{2}\beta y$$

In particular, for all y in the above interval, $y \notin A$, which contradicts the definition of x_3 as $x_3 = \inf A$.

Next, suppose $r(x_3) < -(1/2)\beta x_3$; we will similarly derive a contradiction. Say $-r(x_3) - (1/2)\beta x_3 = \delta > 0$. Let

$$y \in [x_3 - \frac{\delta}{1-(1/2)\beta}, x_3]$$

Then $-\delta - (1/2)\beta x_3 + x_3 - y < -(1/2)\beta y$
and so

$$r(y) \leq r(x_3) + (x_3 - y) = -\delta - (1/2)\beta x_3 + x_3 - y < -(1/2)\beta y$$

which again contradicts $x_3 = \inf A$. Hence it follows that $r(x_3) = -(1/2)x_3$.
Now set

$$x_4 = \frac{1-(1/2)\beta}{1+(1/2)\beta} x_3$$

Note that $x_4 < x_3$ and $-(1/2)\beta x_3 + x_3 - x_4 = (1/2)\beta x_4$.
Let $y \in (x_4, x_3]$. Then

$$\begin{aligned} r(y) \leq & r(x_3) + (x_3 - y) = -(1/2)\beta x_3 + x_3 - y \\ & < -(1/2)\beta x_3 + x_3 - x_4 = (1/2)\beta x_4 < (1/2)\beta y \end{aligned} \quad (6)$$

In particular, $x_1 \notin (x_4, x_3]$ and so $x_1 \leq x_4$.
In summary, we have

$$\frac{x}{16y} \leq x_1 \leq x_4 \leq x_3 \leq x_2 \leq \frac{x}{y}$$

and, for $y \in (x_4, x_3]$,

$$|r(y)| \leq \frac{1}{2}\beta y$$

by $x_3 = \inf A$ as well as (6).
Hence

$$\begin{aligned} & \sum_{y < n \leq 16y} a_n(\frac{\beta x}{n} - r(x/n)) + b_n(\frac{\beta x}{n} + r(x/n)) \\ & \geq \sum_{(x/x_3) < n \leq (x/x_4)} a_n(\frac{\beta x}{n} - r(x/n)) + b_n(\frac{\beta x}{n} + r(x/n)) \\ & \geq \frac{1}{2}\beta x \sum_{(x/x_3) < n \leq (x/x_4)} \frac{a_n + b_n}{n} \\ & = \left(\frac{1}{2}\beta + o(1)\right) x(\log^2(\frac{x}{x_4}) - \log^2(\frac{x}{x_3})\Big). \end{aligned} \quad (7)$$

In order to estimate the final expression, note that

$$\log\left(\frac{x}{x_4}\right) - \log\left(\frac{x}{x_3}\right) = \log\left(\frac{1+(1/2)\beta}{1-(1/2)\beta}\right)$$

by the definition of x_4. We can use a general Taylor series to estimate the expression

$$\begin{aligned} \log\left(\frac{1+x}{1-x}\right) &= \log(1+x) - \log(1-x) \\ &= \sum_{n=1}^{\infty} (-1)^{n+1}\frac{x^n}{n} + \sum_{n=1}^{\infty} (-1)^{n+1}\frac{x^n}{n} \\ &= \sum_{n=1}^{\infty} \frac{x^n}{n}(1 + (-1)^{n+1}) = \sum_{n=1}^{\infty} \frac{2x^{2n-1}}{2n-1} \end{aligned}$$

and so

$$\log\left(\frac{x}{x_4}\right) - \log\left(\frac{x}{x_3}\right) = \sum_{n=1}^{\infty} \frac{\beta^{2n-1}}{(2n-1)2^{2n-2}} > \beta$$

Also, since $x_3, x_4 \leq x/y$, it follows that $\log(x/x_4) + \log(x/x_3) \geq 2\log y$. Hence (7) is

$$\geq (\frac{1}{2}\beta + o(1))x(2\beta \log y) = (\beta^2 + o(1))x\log y$$

This expression satisfies (5) for big y and so we are done. □

The Proof of the Prime Number Theorem

We now have the necessary tools for proving the prime number theorem. What remains is to check that $\psi(x)$ is close to x for large x, which is what we are trying to prove. This suggests that, if we try a proof by contradiction, we may be able to satisfy the final condition and apply the lemma. To do so, we consider

$$\begin{aligned} \sum_{n \leq x} (a_n - b_n) r(x/n) &= \sum_{n \leq x} \big(\Lambda(n)\log n \\ &\quad - \sum_{jk=n} \Lambda(j)\Lambda(k)\big)\big(\psi(x/n) - (x/n)\big) \\ &= \sum_{n \leq x} \Lambda_2(n)(\psi(x/n) - (x/n)) \end{aligned}$$

Simplifying this expression, we get

$$(\psi(x) - x)\log x + \sum_{n \leq x} (\psi(x/n) - (x/n))\Lambda(n)) = O(x) \quad (8)$$

Replacing x with x/m for arbitrary m, it follows that

$$(\psi(x/m) - (x/m))\log(x/m) + \sum_{n \leq x/m} (\psi(\frac{x}{nm}) - (\frac{x}{nm}))\Lambda(n) = O(x)$$

If we multiply the preceding equation by $\Lambda(m)$ and sum over $1 \leq m \leq x$, we get

$$\begin{aligned} & \sum_{m \leq x} (\psi(x/m) - (x/m))\Lambda(m)\log(x/m) \\ & + \sum_{mn \leq x} (\psi(\frac{x}{nm}) - (\frac{x}{nm}))\Lambda(m)\Lambda(n) = O(x\log x) \end{aligned} \quad (9)$$

Now multiply (8) by $\log x$ and subtract (9) to get

$$(\psi(x)-x)\log^2 x+\sum_{m\le x}(\psi(x/m)-(x/m))\Lambda(m)\log m$$
$$-\sum_{mn\le x}(\psi(\frac{x}{nm})-(\frac{x}{nm}))\Lambda(m)\Lambda(n)=O(x\log x)$$

We can now simplify part of the left side:

$$\sum_{m\le x}(\psi(x/m)-(x/m))\Lambda(m)\log m$$
$$-\sum_{mn\le x}(\psi(x/nm)-(x/nm))\Lambda(m)\Lambda(n)$$
$$=\sum_{m\le x}(\Lambda(m)\log m-\sum_{jk=m}\Lambda(j)\Lambda(k))(\psi(\frac{x}{nm})-(\frac{x}{nm}))$$
$$=\sum_{n\le x}\Lambda_2(n)(\psi(x/n)-(x/n))$$

which is what we wanted. It follows that

$$|\sum_{n\le x}(a_n-b_n)r(x/n)|=|r(x)\log^2 x|+O(x\log x)\qquad(10)$$

We now suppose that the prime number theorem is false: let $\alpha=\limsup_{x\to\infty}|r(x)|/x$. Then $\alpha>0$. We also know, by Chebyshev's theorem, that $\alpha\le 1/2$. Thus, it is possible to choose $0<\beta\le 1$ with the property that $\beta-\beta^2/100<\alpha<\beta$. Then, by assumption, $|r(x)|\le\beta x$ for sufficiently large x. Lemma 11 applies, so we get

$$|\sum_{n\le x}(a_n-b_n)r(x/n)|\le(\beta-\frac{\beta^2}{100}+o(1))x\log^2 x$$

In conjunction with (10), this yields

$$|r(x)|\log^2 x\le(\beta-\beta^2/100)x\log^2 x$$

for sufficiently large x. But then $\alpha=\limsup_{x\to\infty}|r(x)|/x\le(\beta-\beta^2/100)$, a contradiction. Hence, our assumption was wrong. The prime number theorem is, therefore, true:

$$\lim_{x\to\infty}\frac{\pi(x)}{x/\log x}=1$$

Acknowledgements

I would like to thank the Notre Dame College of Science for supporting my research this summer as well as Nicholas Lohr and Richard Birkett for teaching me and helping me with this paper.

References

[1] Atle Selberg *An Elementary Proof of the Prime-Number Theorem* Annals of Mathematics, Second Series, Vol. 50, No. 2 (Apr., 1949), pp. 305-313

[2] Ciarán O'Rourke. *The Prime Number Theorem: Analytic and Elementary Proofs.* 2013.

[3] Hugh Montgomery and Robert C. Vaughan *Multiplicative Number Theory I. Classical Theory.* Cambridge University Press. 2005.

[4] Melvyn M. Nathanson *Additive Number Theory The Classical Bases* Springer. 1996.

[5] Norman Levinson. *A Motivated Account of an Elementary Proof of the Prime Number Theorem.* The American Mathematical Monthly, Vol. 76, No. 3 (Mar., 1969), pp. 225-245

[6] Paul Sinclair. Mathematics Stack Exchange. https://math.stackexchange.com/questions/2144940/intuition-for-the-prime-number-theorem.

About the Author

Andrew Burke is a sophomore from Phillipsburg, New Jersey living in Keenan Hall. He is a math major with honors and computing concentrations and is a member of the Glynn Family Honors Program. Andrew is on the club water ski team and does data analytics for the football team. His research was conducted at the University of Notre Dame last summer as a part of the College of Science's SURF program. After graduating from Notre Dame, Andrew intends to go to graduate school and to study algebra or number theory.

Student Spotlight

Several Notre Dame students publish their research in renowned peer-reviewed journals every year. The Student Spotlight section is one of the newest additions to the journal to celebrate and learn from some of these students.

We interviewed junior neuroscience and behavior and economics major Jake Berg, sophomore physics major with astrophysics and applied physics concentrations Andrew Langford, and senior science preprofessional and science, technology, and values major Alex Richard for insight into their research and publication experiences.

How did you get involved in research?

Jake: My journey into scientific research began during my first semester on campus. I wasn't entirely sure what research entailed but I did know that universities liked to place heavy emphasis on it. Then, sometime around October, the College of Science sponsored the Fall Undergraduate Research Fair for upperclassmen to showcase the work they have done. I decided I would stop by and briefly see what it was about. I was excited by the students I spoke with; the research being conducted felt purposeful and each student seemed highly passionate about their projects. I was inspired by what I saw and began reaching out to professors around campus, inquiring about possible research positions in topics I was interested in. I have been involved in research every single semester and I plan to bring my research background into my post-graduation plans.

Andrew: One aspect of Notre Dame which stood out to me while applying was the opportunities for undergraduate research. During my first semester, I reached out to my physics professor about getting involved with the observatory on campus. By the early second semester, I was training on the .8-meter Sarah L. Krizmanich telescope on the Jordan Hall of Science roof while learning about the type of stars I observed, cataclysmic variable stars. My interest in research greatly increased while continuing my research projects over the summer of 2019.

Alex: I have been volunteering in the emergency department at Rhode Island Hospital since high school, and became interested in clinical research because of my experiences there. I reached out to a trauma surgeon at the hospital with some project ideas, and she agreed to work with me.

What kind of research do you do? In what lab?

Jake: I applied to the Mayo Clinic Summer Undergraduate Research Fellowship this past fall and was selected to join the neuroinformatics lab of Dr. Mohamad Bydon. Our lab primarily studies the impacts of surgical intervention and the treatment of injury or diseases of the spine. Building off of this, I used my interest in mental health disorders to design my own research project. Over the course of 10 weeks, I learned how to amass relevant medical data from national databanks to study various outcomes in mental health patients following common spinal procedures. Using this data, I was then able to generate a patient cohort and use STATA software to run statistical tests on thousands of entries to determine significant relationships between variables of interest. At the end of the summer, I had the opportunity to present my research as a poster which was then later featured at the 9th Annual Mayo Clinic Neurosurgery Research Forum. Looking back, it was an incredible experience that taught me so much about working in biomedical research. Back on campus, I am also engaged in cognitive neuroscience research with Dr. Joshua Koen. Our lab focuses on using behavioral and imaging techniques to study critical cognitive processes such as aging and memory. Both experiences have given me valuable training in reading, presenting, and working with scientific data and research methods.

Andrew: My observational astronomy research is focused on cataclysmic variable stars. This type of star system is formed when two stars orbit each other so closely that the strong gravitational pull of one star rips off the atmosphere of the other. In particular, I research magnetized cases of the mass-transfer process between the two stars. My current research project utilizes data from NASA's Kepler Space Telescope while I continue to observe other transient systems with Notre Dame's Sarah L. Krizmanich Telescope. These works are advised by Dr. Peter Garnavich and Colin Littlefield.

Alex: I designed a chart review clinical research project to examine incidence and trends of rhabdomyolysis and renal failure secondary to illicit drug use/overdose. As part of the study, I reviewed the records of 1,200 patients diagnosed with rhabdomyolysis to determine if drug use contributed to this diagnosis (by screening urine toxicology tests, reading history of present illness/discharge information.) I also extracted creatinine laboratory values from this cohort and analyzed differences in lab values between patients who developed rhabdo secondary to drug use and patients who developed rhabdo because of other non-drug related reasons. I also compared trends in initiation of hemodialysis as a measure of acute failure between these two groups. I also do bench research on campus working in the Lieberman lab. I work on the Illicit Drug Paper Analytical Device (idPAD,) although the clinical research was what I presented at FURF.

To what journal have you submitted/do you plan to submit your work to?

Jake: My recently submitted paper, Mental Illnesses Among Patients Undergoing Elective Anterior Cervical Diseconomy and Fusion: Analysis from the National Readmissions Database, was accepted for publication in Clinical Neurology and Neurosurgery. I currently have a second paper that is in its final stages of preparation for submission.

Andrew: The Astronomical Journal.

Alex: To be determined.

What advice would you give to other ND students looking to get involved in research and also publish some day?

Jake: Developing both a sense of flexibility and resilience were two things that definitely helped me get to where I am now. When conducting research, not only is it important to cultivate a passion, but it is equally important to remain open to advice and learn from your network of peers and mentors. Others may have valuable perspectives or suggestions that can build upon your ideas and strengthen your results. At the same time, it is important to use rejections and defeat as a learning point for future improvement. If something doesn't work the first time, that's okay! Significant data won't just manifest on your first time running an experiment and publications don't suddenly appear overnight. Just keep working toward your goals and everything will eventually work out.

Andrew: I would encourage ND students to find a lab in which the day to day work is enjoyable for them. This will increase the chance of a student sticking with the research long term and accumulating findings to publish.

Alex: If you're interested in research, don't be afraid to reach out to professors or doctors to get involved. Being involved with research has been one of the most rewarding parts of my undergrad experience, and something that I plan to continue to engage with next year in medical school and eventually as a physician. I've been incredibly grateful to have supportive and passionate mentors (both Dr. Lieberman here at Notre Dame and Dr. Lueckel at Rhode Island Hospital) that have really helped me grow as a researcher.

Jake Berg, '21

Andrew Langford, '22

Alex Richard, '20

Alumni Spotlight

MEGAN MCCABE

Dr. Elizabeth Berry-Kravis graduated from Notre Dame in 1979, majored in chemistry with biochemistry concentration, and lived in Farley Hall. Her interest in science and specifically in neuroscience began at a young age when she was fascinated by the mechanisms of the human brain. She has strong ties to Notre Dame as she grew up in South Bend. Her father and grandfather were both professors at Notre Dame, and her mother was very involved in the development of Notre Dame's Robinson Community Learning Center. After Notre Dame became coed in 1972, she was among some of the first women to attend Notre Dame, let alone study in a STEM field. After graduating from Notre Dame, she attended the University of Chicago for an M.D./Ph.D. program, and completed a residency in pediatrics and a fellowship in pediatric neurology. Dr. Berry-Kravis created a Fragile X Clinic and Research Program at the University of Chicago, and then she moved to Rush University Medical Center in Chicago in 1992. Since then, she has not only done groundbreaking research with Fragile X Syndrome, but also has expanded her work to cover many other disorders such as Niemann-Pick Type C, Angelman syndrome, and other autism spectrum disorders. At Rush, she works in the department of Pediatric Neurology where she not only treats patients on a regular basis, but also continues to research drug efficacy and genetic mechanisms behind various rare, genetic, and neurological disorders. Dr. Berry-Kravis presents at numerous conferences each year, and her list of publications is extensive. She has won various awards and honors for her clinical and research work. She is the epitome of a compassionate, persistent, and brilliant clinician, and has countless strong relationships with patients and their families. Dr. Berry-Kravis has continued to tie her academic roots back to Notre Dame with her work in parallel with Notre Dame's Ara Parseghian Medical Research Foundation through research in a clinical trial for a drug treating Niemann-Pick Type C disease. She continues to visit Notre Dame multiple times a year for Rare Disease Day in the spring, and for football games in the fall.

At Notre Dame, what helped you form your career goals? Were you involved in research?

I went to Notre Dame knowing I wanted to go to medical school and work on the brain (neurologist or neurosurgeon). I did research at Notre Dame that helped solidify my decision that I wanted to do research as well as clinical medicine. I worked in the lab of Dr. Michael Gould (not currently at Notre Dame). I studied the effects of various food additives on the uptake of neurotransmitters (dopamine and norepinephrine) into synaptic vesicles to determine if a mechanism existed based on impaired uptake through which food additives might promote ADHD symptoms. These experiments did not show a clear effect, but the idea of doing research that might inform a clinical problem and allow decisions about treatment for brain disorders really stuck with me. I decided I did want a career that combined research and clinical medicine which led to me apply to a medical scientist training program.

What was your motivation for receiving both an M.D. and a Ph.D.?

You can do research with an M.D. and some fellowship training but to really get good training in how to think about a research problem, whether clinical or basic research, it really helps to do the Ph.D. upfront. Since I was sure I wanted a career in academic medicine and research, it made sense and was most efficient to do the combined program.

What inspired you to go into pediatric neurology specifically studying and treating rare, genetic, and neurological disorders?

In medical school I did rotations in neurosurgery, neurology and pediatric neurology in my 3rd/early 4th year. I did not like the drawn-out nature of surgery training and did not feel like the surgery schedule was as conducive to research development as neurology. Rotating on pediatric neurology and adult neurology back to back made it very clear to me that I like working with kids best. I thought that new research that might change the course of pediatric brain disease could have a bigger effect as the kids were at the beginning of life and were likely to have more plasticity and potential for improvement. I started out doing work in Battens disease and Fragile X syndrome, with Fragile X as my main focus. After building a program in Fragile X, it was clear there are so many overlaps between these different conditions in terms of challenges of developing new treatments, so I branched out into multiple rare disease areas. I really enjoy working with these populations because the families are so involved and well-informed and it's really a team approach and a partnership with the families to try to beat the disease threatening and impairing their children. Also with rare diseases we are usually dealing with a single gene and very specific genetic mechanisms of disease, which offer the chance to target a specific mechanism to try to treat the disease. More general pediatric neurology diseases like seizures, ADHD and autism have such complex genetics that it's much harder to conceive of mechanism-directed treatment and one has to rely on more general types of new treatments which may not be as effective.

Elizabeth Berry-Kravis, M.D., Ph.D.,'79

Can you describe a bit about your connection with Notre Dame's Parseghian Foundation in relation to your current research with Niemann-Pick Type C Disease (NPC)?

The Parseghian Foundation has really laid the basis for all current research in NPC. They funded the early work that helped understand what causes the disorder and led to the identification of the gene. When I would read papers on NPC it seemed that almost all of them would list the Parseghian Foundation as a funder. I could see their huge impact. Later when I started treating NPC patients, I could see how important the Parseghian Foundation was in terms of bringing researchers together to prompt collaborations, and funding seed projects. I lived in Farley with Cindy Parseghian when I was at ND. We played on the same interhall basketball team. We went our separate ways after ND and only re-connected when I started treating patients and came to the conference well into my career. She is an ongoing inspiration in the same way all the families I treat are to me.

What advice do you have for undergraduates who want to be involved with scientific research?

Do research in a lab or in several labs, and explore what you are interested in so you can decide early in medical school if you want to be in a research track.

Talk Science

September 19, 2019

Kevin Vaughan, Ph.D.
Department of Biological Sciences

Challenges in Rare Disease Research

Helen Streff
Biological Sciences, Bioengineering, STV ’20

Using the Common Approach of CRISPR to Study Lysosomal Rare Diseases

October 10, 2019

Olaf Wiest, Ph.D.
Department of Chemistry

Organic Synthesis in the Age of Artificial Intelligence

Aviva Lund
Biological Sciences ’23

The Effect of PAM-3 on Trophoblast Cells and Human Endometrial Stromal Cells

November 21, 2019

Kevin Lannon, Ph.D.
Department of Physics

Can Artificial Intelligence Help Us Discover New Particles?

Veronica Kalwajtys
Biochemistry and Liturgical Music Ministry ’20

Antimicrobial Peptides as Alternative Antibiotics

January 30, 2020

Jessica Brown, Ph.D.
Department of Chemistry

Using MALAT1 to Crack Open Triple-Stranded RNA Biology

Michael Sokolowski
Biological Sciences ’20

The Role of SGK-1 Mediated ATP Generation in Cellular Survival of ECM Detachment

February 20, 2020

Annette Pilkington, Ph.D.
Department of Mathematics

Wrestling with Rings and Rankings

Andrea Lebron
Biological Sciences ’21

Characterizing natural products active against antibiotic-resistant bacteria of clinical significance

JOIN US

Interested in publishing your research in the next issue of *Scientia* or presenting at the next Talk Science seminar?

Want to help publish the next issue of *Scientia*? We are currently seeking reviewers, editors, news columnists, and graphic designers for the upcoming semester.

Email scientia@nd.edu for details.


UNIVERSITY OF
NOTRE DAME
SCIENCE