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Journal of Undergraduate Research



Fall 2022, Volume Twenty-one Issue One



The Journal of Undergraduate Research (JUR) is dedicated to providing the student body with intellectual perspectives from various academic disciplines. JUR serves as a forum for the presentation of original research, thereby encouraging the pursuit of significant scholarly endeavors.

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Letter from the Editors

The Journal of Undergraduate Research was founded in the fall of 2002 with the mission of serving as a forum for original student research. Between those pages have been every possible academic discipline our university has to offer, yet reflecting on how the face of JUR has changed over the past 20 years, one feature has remained consistent: the quality and depth of the works published.

Fundamentally, conducting research requires embarking on a quest for answers. How can we improve existing computer software systems? What happens when different languages first come in contact with each other? What are the most effective methods to assess student burnout? Armed with this knowledge, we can contribute to the betterment of humanity. But it becomes challenging to see the big picture when met with protocols to troubleshoot, funding to obtain, and exams to take. It would be natural for any student to ask, 'is this pursuit worthwhile?'. Becoming an adept researcher, able to think critically and persist through these challenges, to refine one's pursuit, and importantly, to ask for help when needed, requires time and years of practice. That is why we are continuously inspired by the tenacity and grit of our student researchers to not only develop these skills but to reaffirm, year after year, their worth — to contribute to our collective body of knowledge.

In this issue, we highlight nine articles that represent the wide breadth and diversity of research being conducted at the University. Evan Bushinsky conducts a compelling analysis of carcinogens in Monroe County's drinking water supply (p. 22). Chesna Apere uncovers the role of TRMT1L in tRNA modification (p. 58), and Sasha Murray translates her findings on Zora Neale Hurston's impact on dance to the stage (p. 13). We also feature an interview with Dr. Anne Meyer and feature the 2021 iGEM team's innovative development of a device to detect sepsis (p. 9).

We would be remiss if we did not express our gratitude to our outstanding editorial board, including our managing editors and content editors for their hard work ensuring our articles were of the highest quality, as well as our layout chair, Catherine Lan, and layout editors for assembling the journal design. Every single contribution is prized and appreciated. Finally, we are ever grateful for the support of the Office of Undergraduate Research, Dr. Sina Ghaemmaghami and Ms. Ann Robinson, without which this publication would not be possible.

Research as a student endeavor is a difficult but rewarding undertaking, and we are proud to continue to be the platform that showcases the intellectual capacity of our student body — as we have for the last 20 years, and as we plan to for many more. To our readers, we hope you enjoy the end result of their hard work.

Sincerely,

Jocelyn Mathew & Ryan Hecker

Editors-in-Chief

Journal of Undergraduate Research

Journal of Undergraduate Research

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This issue of the Journal of Undergraduate Research was assembled on macOS Big Sur using Affinity Publisher. Microsoft Word and Google Docs were used for text editing and review. Fonts used include Minion Pro, the main font for body text, and Myriad Pro, the main font for headings and decorative text. This physical version of this journal was bound by Emerald Print Management of Rochester, NY.

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Professor Interview

Anne S. Meyer, PhD

Associate Professor Department of Biology University of Rochester



Dr. Anne S. Meyer is an Associate Professor of Biology at the University of Rochester, USA. Dr. Meyer received her Ph.D. In Biological Sciences at Stanford University (USA) in 2005. She was a post-doctoral fellow in the Department of Biology at the Massachusetts Institute of Technology (USA). Dr. Meyer served as an Assistant Professor in the Department of Bionanoscience at TU Delft in The Netherlands, prior to moving her research group to the University of Rochester in September, 2018. She has served as the lead advisor for eight iGEM (International Genetically Engineered Organisms) teams, which have won numerous awards including the 2015 Grand Prize. Her research focuses on using quantitative techniques in the fields of biochemistry, microbiology, and biophysics to study structural dynamics, macromolecular interactions, and physiological responses of organisms to environmental stressors. She also uses tools of synthetic biology to engineer novel functions into microorganisms, with a particular focus on the production of improved, tunable biomaterials and the development of new tools for 3D patterning of bacteria.

JUR: How did you first get involved with iGEM?

Dr. Meyer: When I first became a professor, it was at a different university in the Netherlands, it had an iGEM team, and they were looking to add extra advisors to help give advice on the wet lab work. The advisor of that team asked me if I would join as a secondary advisor. I first went to the Jamboree, which is what they call the final competition to present their projects. I was blown away by the creative, differing types of projects. How ambitious the ideas were — I really got the iGEM bug, and it made me want to keep going.

JUR: How many years were you involved with iGEM before moving here?

Dr. Meyer: I was head advisor of five teams, and then there was this extra year I was the secondary advisor there. Here, I run three teams, and we're starting a fourth one next year.

JUR: In terms of the functioning of the teams, was there a big difference in your role coming here?

Dr. Meyer: The thing I'd say is different, just kind of based on the nature of the academic programs, is that the iGEM students in the Netherlands, and a lot of the European countries are like this, mostly were first-year Masters students, which means they had already done a full, required three months or longer research project to get their Bachelor's degree. Since everyone on the team had done an extensive research project, they had a lot of experience with wet lab techniques, and a lot of experience reading literature developing it.

Whereas here, some students do have wet lab experience, but a lot don't. Even if they've been in research, like the academic labs for credit, you may not get a full wet lab experience. You know, it's pretty common for people to spend their time, which is very important for students, in doing work like data analysis or shadowing a position, which really helps build the competitive mindset but doesn't help build the skills you need in the lab.

So for the team here, we started doing something kind of like a wet lab boot camp early on in the course, where the students spend several days just learning mechanics of pipetting, how to work with the bacteria. That kind of stuff.

JUR: If you could describe iGEM to a first year student, how would you describe what it is?

Dr. Meyer: So, iGEM is fantastic for people who want to develop research skills because the idea of the project is that students develop a new technology using DNA - DNA parts that they build themselves that will have a useful impact on society. So they start the project from scratch, and they brain-

storm ideas, choose their own idea, and decide what their DNA parts are going to be; what their experiments are going to be; what their essays are going to be. And then, they try it out themselves and see how far they can get before the Jamboree happens. They have to do every part of the project, right, so the brainstorming, the planning, the carrying out, the fundraising, the PR.

JUR: Are there ever prompts or themes for project topics, or are you free to choose any topic for a project?

Dr. Meyer: I run my team such that I'm open to any project the students think of that solves a useful problem and seems feasible. Besides that, the only thing to think about is the bioethical and biosecurity concerns. We want to have something that'll be good for society and not have unintended consequences that are negative.

JUR: What does a typical competition look like for students?

Dr. Meyer: So you would spend two months working together with your teammates, but mostly independently, around what problems you think that we can solve using new DNA technologies. Then as a team you would narrow down the list, pick your final project, and get more input from the advisors. We have a certain set of goals for this project. What do we have to design? What experiments? What new parts? It is going to be in a living cell, is it going to be in a test tube? Do we have to work together with hardware and modeling? You have to flesh out the whole project, order and design everything you. That point is more at the end of the spring semester. Then at the start of the summer, that's when there's the boot camp to learn your wet lab skills and hardware skills. Then, you do the project. All your components have come in. You make sure of all the DNA parts; you're testing if it works; you check the DNA; you model the behavior; you'd be doing education and outreach events in the community, talking to various stakeholders or end users to see how our design would impact them, and can our project be designed differently to be more useful or more positive in some way, more acceptable.

Then typically, by the time the semester starts in the fall, things are just starting to work in lab, so it's kind of a scramble in the first one or two months in the fall to kind of finish up your experiments. At the same time classes have started again. Also at the same time you write a description of what you've been doing, all the data you've got, and your ideas for the future that goes on this website that every team gets — a wiki page that's a record of their project going forward forever. Everything is summated in the wiki. And then, now after the COVID pandemic, things have been more manageable. We pack up and go to Boston. It used to be Boston, now it's Paris for the championship, the grand jamboree, and that's always an amazing experience. We meet thousands of students from all over the world, all of whom are really interested in helping society - developing new technology. You learn about their projects, they learn about your projects. It's a really amazing experience.

JUR: Post-competition, then, do students decide to go further

with their projects, or is it a thing where once it's done with competition, people don't really go back to it?

Dr. Meyer: It depends a lot on the project. Once the project is over, the team members are usually desperate to catch up on their classes and finish their degrees. We've definitely had projects where we've gone one way or another. For the 2020 project, we're writing out the modeling component for that course to be a research publication. Some of [...] those projects ended up being a published paper in a research journal. A lot of the iGEM in my lab and other labs live on in the labs of the advisors, departments. Such amazing concepts, many people I know want to continue the work once they have graduated.

JUR: Of the projects you've seen, what have been some of your favorites?

Dr. Meyer: There's so many projects that have been exciting. The one I know that was really interesting was the team I advised back in 2015. This team was really ambitious. They had the idea, "Can we do 3-D printing, where the material of printing is bacteria?", so they were the first people to ever 3-D print bacteria, kind of an editing manufacturing style, reading it from a printing nozzle. It was just amazing because the idea was revolutionary. They built the 3-D printer from scratch using connect toys. From the ground up they engineered the whole printer, developed the biochemistry of the ink to allow for stable structures and the bacteria to survive, and they had the idea for what would be an application of this fundamental advance. Their idea basically was, can we model biofilm, reproducible biofilm, which could be useful for medical biotesting. And that project ended up winning the top prize that year, the iGEM students were authors on eventual publication. And the technique was so popular that now people are doing it all over the world, inspired by these students.

JUR: For students looking to get involved, could you explain how they would do so and how they would know they are ready for something like this?

Dr. Meyer: We're past the application season now, but in early September and October, I'll start recruiting students, send emails, put it in the bulletin, and have an interview time for people to ask questions. Then I accept applications in October and make a decision in early November. We time it so students know before they register for their spring classes. In February, students first start meeting and kicking off the project.

JUR: Would you recommend any class year to join, or to have certain experience?

Dr. Meyer: Anyone can apply. We do tend to pick sophomores and juniors just because they have fulfilling experiences if they have a fundamental knowledge of the science classes we offer here, and wet lab skills. That makes the experience more rewarding — more enjoyable for the students. The kind of ideal candidate would be someone who has worked in one or two research labs, in high school or here. We

want people who are thoughtful about why they want to join iGEM. We have the applicants for the application write a cover letter for themselves, why they want to join, what projects have inspired them, what projects they want to work on, and if they've already taken on an engineering mindset and think about what they want to work on.

JUR: So one last question I wanted to ask you was, what do you think is the most rewarding part of joining iGEM?

Dr. Meyer: So there's so many different parts that the team does. Everyone gets a leadership job, there's 12 or more, and they all work on a teamwork job. The team works on raising money, learning more about the systems, hardware, wet lab, outreach, education. There's so many different components. If a group of students all put their efforts together over so many months for a project with so many components, it's really inspiring.

JUR: Is there anything additional you want to add?

Dr. Meyer: iGEM's purpose is to design something with DNA technology, but it's not something only for scientists. There's other things, like a business model, the bioethics and biosecurity, outreach and education, so people from all different backgrounds can find a niche for themselves.

Maria Schapfel ('23) and Daniel Nakamura ('23) were members of the 2021 iGEM team, creating a wearable device to help diagnose sepsis.

JUR: How did you first find out about iGEM and what motivated you to apply?

Maria: I looked over the flyer that was sent in an email, thought it was interesting and wanted to learn more, so I went to the information session. iGEM is very multidisciplinary and interesting, and it's not something that I've ever had the opportunity to do. There are also many different types of research within one large project that you can do.

Daniel: I first heard about iGEM when our genetics professor, Dr. Stein, made an announcement about it in class and I thought it sounded interesting, so I looked into it. I was trying to decide at the time if I wanted to do chemistry or biology research and I liked the idea of synthesis, so I wanted to try synthetic biology, which is why I ended up applying to try to get that experience.

JUR: How would you describe iGEM to a first-year student who's interested in joining?

Maria: It's very team based and collaborative, so you'll work very closely with about 11 other people on a project and you will get to know them very well. You choose a topic and go with it, and you do a lot of different types of research within that one specific topic. There's a lot of room to figure out what

aspect of research you like because it is a very modular project that you end up doing.

Daniel: It is the most freedom that you will ever get as an undergraduate researcher because you decide the project and the experiments. Everything was ultimately a student decision and it's an amazing amount of freedom. You want to pick something and stick with it because it's so easy to get pulled in a million different directions. Overall, it's a very unique experience not just because of how much independence you get but because you're going for a competition and so your goals and approach are a little bit different than a normal research lab.

JUR: Could you describe one of the projects that you worked on?

Maria: Every team chooses a global problem that they are trying to solve. In our case, we were trying to create a diagnostic device for sepsis and our final product was a prototype that could use sweat to monitor biomarker concentrations, and then use those concentrations to help determine whether a patient has sepsis. We ran a lot of analyses and tested the binding ability of biomarkers. We also had a hardware component. We made a sleeve with a microfluidic channel to filter sweat through a circuit breaker board that would then take the results and send it to a computer to be used for analysis.

Daniel: I engineered bacteria to turn graphene oxide into reduced graphene oxide which is the material that we used to build our machine. My roles ranged from making the graphene oxide to building a metabolic model of the bacteria. I also correlated the electrical activity of the material to how much oxygen was still attached to it. There was also community outreach that we did, so it was a little bit of everything.

JUR: How did you manage to narrow down the specific focus?

Maria: When we initially started meeting it was all of us just sitting together and attempting to brainstorm. We were all surprised with how hard that was initially to find something that we could use synthetic biology to try to solve. I think we ended up coming up with 40 potential ideas and everybody was tasked with research on their own ideas. We each brought two to three ideas to the table and presented them for five minutes and gauged if people were interested. We used lots of spreadsheets and voting mechanisms to then narrow down these 40. Then we made it to our top ten through voting and further research and many presentations within the group. For our top ten we made a more specific, flushed out presentation that we presented to our advisors.

Daniel: We got our advisors to give us feedback on our top ten projects and then we took our top ten and voted down to our top three after some discussion among the team. Again, the advisors gave feedback, but it was the students who decided on what the top three would be. After that, we split the team up into three different groups and each group did a lot of research into one of our top three ideas. Then we presented again to our advisors, where they gave us feedback, and then we had one final meeting where we decided on our final project.

JUR: What is the most interesting project that you've seen?

Maria: Australia did a cool project about saving coral reefs. They also created a lab kit that could test the fresh milk from a cow to see if they had a disease.

Daniel: One of the graduate school ones that won one of the main awards involved using bacteria as model organisms for plant biology. The problem with plant biology is how long it takes to get your results because plants take so long to grow. They had a new approach to plant-based biology using bacteria. There was one group who used bacteria to turn methane into methanol which you can use as a biofuel for many chemical purposes.

JUR: What was the competition like at the annual Jamboree?

Maria: It was a little bit sad since we were supposed to go to Paris but then because of COVID we had it virtually. It was interesting because part of doing iGEM and doing the competition is to collaborate with another team and learn about their project and help them in some way. We collaborated with a team from Brazil and that was interesting just to learn about their project while they were doing all their research and then have them learn about our project. We swapped some skills and knowledge.

Daniel: I think that was the most interesting part was that it never really felt like a competition. Intellectually I knew that we were trying to score points but it never really felt like a competition. There was a team in the Netherlands and we wanted to use their particular gene they expressed four years ago — that was useful for one of our projects — and asked them if they could send it to us. They said yes and were very supportive and nice. They even dug through all their material from previous years, and they shipped it to us. Throughout the event, it never felt like we really hoped we beat this particular team.

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In The Main: Exploring The Interdisciplinary Anthropological Work Of Zora Neale Hurston

Sasha Nakita Murray '23, Dance Studies Advised by Anne Wilcox, Department of Dance and Movement

Abstract

Harlem Renaissance writer Zora Neale Hurston, popular for her 1937 novel, Their Eyes Were Watching God, is lesser known for the folk concerts she produced. The most notable of these concerts, "The Great Day," ran on January 10th, 1932 after Hurston had conducted anthropological research on folk dance and song traditions in the U.S. and Bahamas. Hurston aimed to portray these traditions as authentically as she could, and in particular, accurately represent black dance forms on stage. This study aims to contribute to existing conversations surrounding Hurston's impact on American dance and explore the connection between literature and dance in her work. The work at hand consists of a creative dance project that reflects the findings of Hurston's dance contributions. The study revealed that Zora Neale Hurston's work was grounded in both her commitment to accurately representing black identity and in her interest in hoodoo: "African-derived magic practices or beliefs" (Hurston 22). By choreographically exploring Hurston's work, I was able to develop my understanding of the themes guiding her work.

Introduction

This project began as an investigation into the connection between literature and dance, the two fields central to my interdisciplinary study. I was guided by my interest in black dance. The questions that initiated my research were: What is dance to the African American identity? What do black choreographers' choices tell us about movement as it relates to African American identity? And finally, what do black authors' choices tell us about African American identity?

I approached these questions by reading literature about black dance. I found EmBODYing Liberation: The Black Body in American Dance, which spanned several centuries of American history through the discussion of a wide range of topics following the evolution of black American dance forms, in my campus library. In the chapter, "Re-scripting Origins: Zora Neale Hurston's Staging of Black Vernacular Dance," I learned that the writer had also been an anthropologist and had studied folk dance and songs in the U.S. and Caribbean. She had a connection to both literature and dance that I wanted to learn more about. I knew that fellow anthropologist Kathrine Dunham had done similar work to that of Hurston. The work of both women and the pool of knowledge between them are both interesting but too large to undertake in a single paper. I decided to focus on just Zora Neale Hurston as the impact of her dance work is still being uncovered and her literature is so

broadly discussed. Hurston uniquely fits the parameters of my questions surrounding the choices that black authors/choreographers make toward communicating ideas about African American identity. The research process consisted of reading literature on Hurston's work. From the findings of the literature, I choreographed a dance piece entitled, "In the Main."

Literature Review

My interest in Zora Neale Hurston's work started with a desire to understand black dance and identity as told through the art form. The landscape of American dance history is irremovable from the experiences of African Americans. The story of black dance in America shares its beginning with the story of black people in America and the trans-Atlantic slave trade. Dance was an integral part of the lives of the West Africans who were captured for labor in the Western Hemisphere. Dancing served many functions, including commemorating both the celebratory and the sacred. Dance had been "associated with religion, with farming, with births and deaths, and weddings and other ceremonies" (Haskins 5). Dancing, as the enslaved Africans knew it, transformed on the slave ships they were packed onto for their journey. A routine part of their daily experience while on these ships was being brought up to the deck to be exercised. The way the slave captains did this was through a method called "dancing the slaves" (Haskins 4). This forced dancing was prompted with whips and its function was to maintain their physical appearance, so they'd fare better at auctions (Haskins 3). When the enslaved arrived in the Western Hemisphere, they brought their dances and their cultural histories with them. Depending on their destination, enslaved people's dances encountered varied reception. The West Indies and the Caribbean were colonized by Catholic countries like France and Spain. Slaves of these colonies were allowed to retain many elements of their cultures though there were serious attempts to Christianize them. Slaves who converted to Catholicism worshiped African Gods alongside the Catholic Saints, and they were allowed to worship in their unique fashion, which included singing and dancing (Haskins 5). The significance of dance for the enslaved in North America was very different. The Protestant English colonists who settled there saw slaves as property and as less human than themselves. The efforts by the protestant settlers to convert slaves to Christianity required that they give up their "heathen beliefs and practices" (Haskins 8). For them, there was no room in the Protestant faith for saints and other gods, and dancing was seen as sinful by many Protestant sects. The dances that developed within these strictly ruled enslaved communities reflected the restrictions on their movement. For example, the ring shout, a dance accompanying worship, consisted of a group shuffle that moved counterclockwise in a line with an emphasis on not lifting the feet (Haskins 8). Another restriction, The Slave Codes of 1740, banned large drums. These drums were used by the enslaved in the same way they were used in Africa, to create music and communicate (Haskins 9). Due to this ban, new instruments were made by the enslaved, including the use of their bodies. Pattin' Juba was a percussive form that consisted of patting and clapping on the thigh and hip in a syncopated rhythm (Haskins 10). New arrivals of enslaved persons from the West Indies and other parts of the developing states meant varying African-based traditions intermingled. This cultural mixing occurring between Africans brought the tradition of African dancing to North America (Haskins 13).

White people were interested in the dance forms of enslaved people. They presented these dances on stage in mocking portrayals. White entertainers wanted to appear more authentic in their portrayals, so they began darkening their faces with birth cork, and with that, minstrelsy was born (Haskins 15). Minstrel shows would become popular in the 1820s. They depended on black dances, music, and dialect. These performances were put on by almost exclusively white entertainers for an exclusively white audience. Minstrel shows established many of the stereotypes about black people that were popularized and persist even today. By the time slavery was abolished with the end of the Civil war in 1865, black performers were allowed into minstrel shows. Though they had to perform in blackface, the inclusion of black performers allowed black people to in some way represent themselves. The interest from white audiences in black life sustained minstrelsy into the 20th century as minstrel acts became a part of vaudeville and a part of many other shows (Haskins 20).

At the turn of the century, W.E.B Du Bois published "The Souls of Black Folk," a collection of essays and sketches that sought to communicate the reality and beauty of being black in America to American society. W.E.B Du Bois sought to approach race from many angles. In the chapter, "The Sorrow Songs," Du Bois discusses the importance of music, specifically "slave songs," to American identity. Du Bois wrote that many of these slave songs had been largely forgotten while "others were caricatured on the "minstrel" stage and their memory died away" (Du Bois 253). Du Bois argued that these songs held the very history of America, stating, "The first is African music, the second Afro-American, while the third is a blending of Negro music with the music heard in the foster land. The result is still distinctively Negro and the method of blending original, but the elements are both Negro and Caucasian" (Du Bois 258). An example of the third step in the blending of African traditions is jazz music and dancing. Du Bois' interrogation of America and black identity would go on to influence the works of intellectuals during the Harlem Renaissance.

In the early years of the twentieth century, even the best black performers had trouble gaining acceptance in white theaters, and there weren't many opportunities to perform on white

stages. There were few black theaters, and most were in big northern cities where there were large black populations to support them (Haskins 31). In cities like New York, some black performers could perform at downtown theaters and on Broadway, but this ended when anti-black riots in the city pushed many of the black population to flee northward to Harlem. With that, a new black community formed, and talented black performers created theaters and music companies (Haskins 31). A major part of the shows put on by these companies was the dancing. The Darktown Follies was an original black musical comedy produced in 1913. It was filled with dance and was one of the first shows to feature tap dancing, which was relatively new at the time (Haskins 32). The act, "At The Ball," featured a dance craze called "Ballin' The Jack" and the show became popular in Harlem because of it (Haskins 34). This popularity drew white audiences to Harlem and with that, it became fashionable for white people to visit for entertainment (Haskins 34). Florenz Ziegfeld, the curator of The Ziegfeld Follies, the most popular white downtown show at the time, purchased the rights to "At The Ball"; the act became one of the greatest hits the Follies ever had (Haskins 34). In 1919, after World War 1 had come to an end, the Volstead Act was passed, prohibiting the sale and manufacture of liquor. This opened a way for organized crime to make millions selling it illegally and nightlife in big cities flourished as a result (Haskins 35).

Shuffle Along, the first all-black show on Broadway would be the musical production that changed Harlem for years to come. The show was the love child of two successful black musical comedy teams, Sissle and Blake and Miller and Tiles. When it opened in 1921, it became known for its high-energy dancing and music. The dancing consisted of current dance steps and was heavy on tap - something the creators intentionally sought to legitimize (Haskins 38). The show was also revolutionary for the portrayal of black love on stage. During the number, "Love Will Find a Way," a black couple kissed onstage for the first time and the idea of romantic love between blacks was legitimized (Haskins 38). The show played on Broadway for a full year and toured across the country. It created a craze for jazz dancing that attracted white audiences to Harlem in greater numbers than before.

The craze for jazz dancing that came following Shuffle Along changed the popular narratives about black dance. Before its production, black social dancing was associated with "lowlife dancing" and was far from the dancing that occurred in ballrooms (Haskins 26). After Shuffle Along, colored dance studios opened on and off-Broadway to teach black dance styles (Haskins 39). The Harlem Renaissance was in full swing. Black artists and writers received unprecedented attention from whites. Black musicians and singers received most of the attention, evidenced by the 40 musicals that opened between 1921 and 1939 (Haskins 41). Though the cultural perception of black dance had changed for white audiences, within the black community, the merit of black dance as a socially impactful art form was a debated topic. According to Wendy Perron in her essay, "Dance in the Harlem Renaissance: Sowing Seeds," dance was central to Harlem life and culture, as evidenced by the poetry, novels, and paintings of the era. However, it failed to get recognition by the intellectual consciousness of Harlem (Perron 23). The New Negro, written by intellectual Alian Locke and inspired the new negro movement, set the tone of the Renaissance.. Dance was not included in the framework of the movement, specifically, the dancing performed in black musicals. He communicated that Josephine Baker and Bill "Bojangles" Robinson needed a more "dignified medium" (Locke, as cited in Kraut, 62). This was due to the stereotypical representations of black people he expressed had "passed on" by this point in history in The New Negro (Locke). For Harlem intellectuals like Locke, according to Perron, the dancing in these musicals "undermined black achievements in literature and scholarship" (37). Locke did not see dance as central to black life the way Du Bois saw negro spirituals, which Locke recognized as "artistically precious" and heroic (Locke, as cited in Perron, 25). Younger writers like Claude McKay and Langston Hughes felt differently about dance. Both men wrote about black dancers in their poems and Langston Hughes was so moved by Shuffle Along that he moved to Harlem (Perron 23). Claude Mckay felt that dancing, when lifted to the level of artistry, can be edifying to the race (Perron 26).

As I have mentioned, many Harlem Renaissance writers gained popularity for their portrayals of black life. Of these innovative writers was Zora Neale Hurston, who was also interested in dance. She traveled to the southern U.S. and Bahamas to "collect folklore, work songs, dances, sermons and spirituals" for her anthropological research (Biers 67). She organized a folklore concert to showcase what she collected. came to the stage of the John Golden Theatre in New York on January 10th, 1932. Anthea Kraut's investigations into the dance works of Zora Neale Hurston have been published in many books and articles including in her book on the topic. In a chapter of EmBODYing Liberation, "Rescripting Origins," Kraut says that Hurston actively sought to distinguish her concerts from the popular Broadway productions of the time. This is reflected in the "note" on the program for "The Great Day" written by Alaine Locke. The note reads, "It is a rare sample of the pure and unvarnished materials from which the stage and concert tradition has been derived; and ought to show how much more unique and powerful and spirit-compelling the genuine Negro Folk things are" (Program 3). This statement by Locke should not be interpreted as support for dance as a part of the movement as Locke initially opposed Hurston's plans to produce a concert (Kraut 63). In his statement on the program, he also says "These Folk have always had two arts,--- one for themselves and one for the amusement and beguilement of their masters" (3). The presentation of art for and by black people was something Locke could get behind. It is clear though that Locke's perception of Hurston's work was only in an artistic fashion. Many scholars have unpacked the social and cultural impact of Hurston's work beyond her artistic contributions.

Katherine Biers' discussion of Hurston's work in "Practices of Enchantment: The Theatre of Zora Neale Hurston" reflects the show's impact. "Hurston proffered to New York audiences an illustration of the richness and complexity of black folk culture, one designed to undermine perceptions of both innate racial difference and inferiority" (Biers 68). Hurston's dance contribution is often disregarded when people discuss her work. In Kraut's article, "Between Primitivism and Diaspora: The Dance Performances of Josephine Baker, Zora Neale Hurston, and Katherine Dunham," she argues Hurston's staging of West Indian dance and that of Southern Black folk within one program helped reorient an understanding of black dancing away from the mocking legacy of blackface and minstrelsy and toward an understanding of how practices rooted in African tradition arrived on American shores (Kraut 434). Kraut argues Hurston's work "recast black dance practices in a broader transnational framework" (441); something that Josephine Baker's work may have laid the foundation for, and something Katherine Dunham would also do. Kraut's conversations around Hurston also suggest that Hurston should be recognized as a dance artist similar to Katherine Dunham, particularly recasting Dunham as her contemporary (Kraut 60). John Perpener's review of Kraut's book on Hurston, "The Dance Legacy of Zora Neale Hurston," reflects his conflict with that idea. He feels she pushes too hard to get readers to accept Hurston as a dance artist and that her unique position in theater "falls between the cracks of categorization" (Perepener 164).

Other scholars have examined her impact, specifically on the establishment of black dance in America. It has been determined that Katherine Dunham had a major impact on situating black dance through her anthological research. In "Dunham Possessed: Ethnographic Bodies, Movement and Transnational Constructions of Blackness," Stephanie L. Batiste discusses Dunham's work and impact. She made the point that though Dunham participated in the imperial process of appropriating native culture through ethnographic studies, she reinvigorated US black national identity by forging a transnational black identity. Batiste communicates that the science of anthropology stands on defining "those racially and culturally different from the white West as inferior, thus providing a justification for imperial economic, military, and political power around the world" (Batiste 9). She communicates that Dunham has reproduced but also dismantled this framework through her work. Dunham's anthropological work was very similar to Hurston's and while both women have contributed to a transnational relationship between black populations, I think it's worth recognizing the way Hurston avoided reproducing some hierarchical structures in the way she presented the findings of her study. Within anthropology, there is an ethnographic perspective that positions native cultures as primitive. This is something Batiste discusses. Primitivist thought is reflected in how Dunham described her relationship to diasporic culture, she saw it as an opportunity to "restore lost traditions" to "the negro" (11). Batiste communicates that this implies the primitivist view of the Caribbean is "closer" to a native Africa, which is untrue when you consider that the Caribbean has also been affected by "the distance of time, space," and the impact of the transatlantic slave trade (11). This reproduces hierarchy by insisting on the nativity of the object of the study. Primitivist thought concerning diasporic communities was not unpopular at the time; however, Hurston avoided contributing to the hierarchy of primitivist thought in her work. Instead, she embodied Afrocentricity with her staging choices. The program of "The Great Day" begins at a Floridian black work camp and ends with the "Fire Dance," an "exceedingly African folk dance" Hurston came upon in the Bahamas (Hurston 294). This created what Anthea Kraut describes as "a line of descent back toward the Caribbean," establishing a non-hierarchical connection between African American culture and that of the Caribbean (Kraut 442). This "recast[s] black dance practices in a broader, transnational framework" (Kraut 441). Black dance forms were demonstrated to possess an embodied connection.

Considering the vast difference between Hurston's work and the popular black theatrical productions of the day, communicating this embodied connection was a bold choice. In "Black Dance and Dancers and the White Public: A Prolegomenon to Problems of Definition.", Brenda Dixon discusses how the white public may approach the work of black choreographers and the difficulties that arise when defining black dance. Black dance is difficult to define because of assumptions from white audiences that "black dance is what black dancers do"(Dixon 118). Dixon suggests that instead of labeling what black dancers do as "black dance," audiences should interrogate the black-rooted aspects of the works, especially considering that the works of many black choreographers like Alvin Ailey and Katherine Dunham were truly fusions of many dance forms. Only certain aspects of their works can be described as black dance. Brenda Dixon communicated that "any serious attempt to study Black dance demands a study of African and newworld cultures" (120). Black aesthetics and choices would be understood by white audiences with this knowledge. By staging different folk dances together, Hurston challenged her audience to investigate the connection between them for themselves. Whether or not they would understand what Hurston was suggesting would be to the degree of their understanding of these African-derived aspects, something Hurston may have been prepared for. Kraut communicated this in "Between Primitivism," asking, "might Hurston have designed The Great Day with two audiences in mind: a largely white audience with certain inveterate assumptions about black performance and a smaller but nonetheless significant audience of discerning viewers who might be more responsive to the heterogeneity of black expressive styles" (Kraut 445)?

As previously discussed, Hurston did effect social change by contributing to transnational constructions of blackness. Though it could be argued that Hurston's work is not truly representative of dance because of how she compares to choreographers who "established dance companies, created repertories of works that bore their individual stylistic stamp, and contributed to their choreographic process..." (Perpener 164). Her staging of the diasporic connection between folk

culture was innovative and may have called for a specific interrogation from her audience that was ahead of her time. Hurston's work reflects the problems of the definition Dixon discusses regarding black dance. By Dixon's definition, Hurston's work can be wholly interpreted as "black dance" due to the representation of the dance traditions of Africanderived people. Hurston's work requires a more nuanced discussion of black dance because even though a diasporic understanding unifies the performances in "The Great Day," the program is specific to the way folk traditions developed in the southern US and the Bahamas. This work represents only a small aspect of the diaspora. A white audience member may be inclined to "Africanise" Hurston's work upon seeing black cultural dancing on stage. This erases the specific cultural moments at work. Hurston's production, however intentional on her part, calls for a more nuanced understanding of black dance, one that transcends the categorization itself.

Hurston's Study Results

Zora Neale Hurston innovated many things with "The Great Day" and the several plays she wrote but never staged. Of the many aspects of Hurston's work informing her staging choices, what affected my choreographic process the most were her choices to stage diasporic similarities and differences and her relationship to representing hoodoo on the theatrical stage. These reflect two of Hurston's passions, black people and hoodoo. Though she was successfully able to bring her anthropological work about folk songs and dances in the U.S. and Bahamas to the stage, her interest in staging hoodoo practices never made it. Nonetheless, Hurston's approach to staging these areas pushed my choreographic approach. In "The Great Day," Hurston used the staging of her program to draw attention to the similarities and differences between the folk cultures in the U.S. and Bahamas, communicating an underlying, deeper connection between the cultures. Staging similarities and differences were, in this case, a powerful tool to draw lines between seemingly different things. When a movement is changed in some way (time, space, effort, etc.) and reorganized on stage and distributed amongst dancers, the product becomes an exchange of movement between them. The vision of black identity Hurston had was presented on stage via the exchange of different black dance forms. Hurston sought to bring the core ideas of the hoodoo practice to the forefront in the unstaged performances and plays revolving around rituals. Using "How It Feels To Be Colored Me" as a reference gave me the imagery needed to explore these themes through the way I staged my dancers. Seeing that the movement potential of my dancers could be representative of more than human beings or even a human experience lent itself to more creative choreography.

Purpose of Research and General Hypothesis

My research had four goals:

To unpack and contribute to existing conversations surrounding Zora Neale Hurston's contributions to dance.

d an c e s t u d i e s

To understand, in some measure, the connection between literature and dance in Hurston's life and work.

To understand the impact of Hurston's work on situating black dance in America.

To create a choreographed dance work that reflects the discoveries of this research.

I hypothesized that there is a connection between Hurston's literature and dance works, grounded in her desire to reflect black life.

Limitations

Creating choreography as a part of this research project came with certain limitations. The abundance of text surrounding Hurston's dance work made it considerably easier to gather information for my literary research. However, the lack of footage of any of Hurston's dance stagings made it difficult to initiate a choreographic relationship with Hurston. I developed choreography based on my literary discoveries and while I found this to be successful, I could have benefited from being able to explore Hurston's movement as the basis of my choreography. The time frame for my choreography was also a limitation as it aligned strangely with my research timeline. I started my choreography before I had cemented my research and I was nearly finished with my piece when I'd finally collected my thoughts. Because of the timing, my choreography could only develop alongside my research, and as a result, it is not a full-fledged reflection of Hurston's dance contributions.

Research Methodology

My movement-based research occurred over 4 months. I held my first rehearsal in late January and, then, biweekly rehearsals until SEED (Spring Explorations and Experimental Dances), the event where my piece was showcased. Throughout the process, I received and incorporated faculty feedback from two showings that occurred at the start of my process and toward the end. I selected 5 dancers for my piece to gather an ethnically diverse group with black dancers represented. I also wanted diversity in the levels of dance experience and in the dance styles each person had. I knew each dancer would bring their own presence to the piece.

This piece developed alongside my research, so, as I found material I could incorporate, I added it to my choreography. I was not sure of my direction at the start; however, I knew I wanted to incorporate Hurston's literature in some way. I started by exploring what I knew about narrative structure. In the dance journal I kept to record my progress, I wrote, "setting up the choreographic process as you would a play or novel, in a series of acts." This was one of my general ideas toward choreography. I was also guided initially by questions like, What does world-building look like in dance? How can I establish a beginning, middle, and end? How can I incorporate Hurston's ability to use African American Vernacular English in contrast with Standard English in my piece? My very first rehearsal with my dancers was held on Zoom due to the rising COVID-19 cases on campus. In this rehearsal, I had my dancers read the first page of Zora Neale Hurston's Their Eyes Were Watching God. I had each dancer, and myself, create a phrase of movement from what they read. I also had us discuss what helped us choreograph movement from the text. This helped me get a feel of each of my dancers' relationship to dance while helping me think about the ways I could incorporate literature into the piece. The dancers said utilizing the imagery and descriptive words in the text to inform their movement qualities helped them translate text to movement. Specifically, they used words in the text correlating to locations in the body to create phrases. My intention for the piece was not to directly translate words to movement so I did not incorporate that idea, but the idea of correlating movement qualities to ideas and imagery was interesting.

We officially began rehearsals the first week of February. The first showing of student choreography was on February 12th and at that point, I had around 90 seconds of movement to share. The piece began with my dancers facing downstage, performing a phrase in rows between each other in the group. Then 4/5 of my dancers "peeled off" into different parts of the stage to create some visual and texture changes. I had one dancer stay in an audience-facing position improvising a solo. The other dancers entered at different moments and traveled across the stage in a restricted walk. I also had two dancers come in and perform a 10-second duet. Having the dancers begin the piece "together" and then leave the center of the stage to do other choreography while one dancer remained was meant to mirror the way Their Eyes Were Watching God began. The first paragraph describes the physical landscape of the location we are placed in: "Ships at a distance have every man's wish on board. For some they come in with the tide" (Hurston 1). While this paragraph cues us into the ideas the text may deal with, the second paragraph leans into this more with lines like, "Now, women forget all those things they don't want to remember, and remember everything they don't want to forget" (Hurston 1). Finally, we are introduced to our main character in the third paragraph. It begins with the line, "So the beginning of this was a woman and she had come back from burying the dead. Not the dead of sick and ailing with friends at the pillow and the feet" (Hurston 1). In this text, the world was built, both thematically and with imagery, before we are introduced to the main character. This was my movement exploration into the concept of structuring my dance piece like a story since the "main character" is introduced after the scene is set. My first choice of music was "What's New -Live At The Perishing, Chicago, 1958" by the Ahmad Jamal Trio.

The dance faculty gave me much-needed feedback that helped me incorporate necessary changes to my choreographic approach at the showing. I received feedback that my movement was stylized and went along with the peaks and valleys of the music. This was not a negative response. However, what I intended to explore was not clear on stage. Following the showing, I wanted to move my choreography away from the stylized way it had materialized. I also wanted to change my relationship with the music. Though I loved this first choice, I struggled to choreograph much movement material to explore with my dancers by rehearsal time. I also found that my movement choices were tied too closely to the rhythm of the music, as evidenced by the faculty feedback, and I wanted my piece to take a different direction. I started by changing my music. I chose "Angels and Demons at Play" by Sun Ra. This music selection had more rhythmic changes throughout and it gave me room to explore. In the rehearsal following the showing, I made many changes. I shifted the starting position from the center of the stage to a diagonal and I explored having my dancers improvise using different movement qualities and isolations before the phrase I initially choreographed began. I removed the segment where dancers walked across the stage at different times and instead had dancers doing various phrases at the same time instead. At this point in my choreographic process, I shifted away from exploring story structure and instead began leaning into the rhythmic changes and polyrhythms that my new music selection gave me. I began opening up my perspective to include the idea of many stories being told at once. In Hurston's literary work, her use of vernacular stood sharply against the background of Standard English. In this phase of my movement exploration, I contemplated using flowy, stylized contemporary dance movement as my Standard English backdrop and I interpreted the use of improvisation as a vernacular contrast to this.

In the rehearsals that followed, I began to source my inspiration more from the scholarly texts I was reading about Hurston. In "Between Primitivism and Diaspora," Anthea Kraut mentions that "Hurston staged both the similarities and the differences between movement practices of the African Diaspora" and that she had a "variegated vision of diaspora" (443). This is about her including folk dances from the U.S. and Bahamas to suggest a connection between them. Though I was not exploring diasporic movement for my choreography, I found the concept of staging similarities and differences very interesting. With that in mind, I began to explore and change the ways I had my dancers move in space, levels, time, and strength in my piece. I also began to more intentionally consider how I would approach the moments where they moved in unison or not. I incorporated a group improvisation segment at the start of the piece, and I choreographed moments where each dancer did a different phrase simultaneously. I started many of my rehearsals with a warmup where dancers improvised together to get comfortable with each other's styles of movement. For the phrases where dancers would be doing various phrases simultaneously, I kept their dance styles in mind.

By the first week of March, I had choreographed the segment of "Angels and Demons at Play" that I wanted to incorporate into my piece. My choreography at this point had been influenced by two texts: "Practices of Enchantment" by Katherine Biers and Hurston's essay, and "How It Feels To Be Colored Me." Katherine Biers' scholarly article discussed Hurston's goal to present to the world an "uncommercialized and thus undiluted" showcase of black vernacular dance (Biers 68).

Biers discussed a certain deleted scene from "The Great Day," that was omitted by the request of Hurston's close friend and fellow scholar Charlotte Mason. Biers unpacks the significance of leaving this particular scene out due to its subject matter, hoodoo ritual. Bier discusses Hurston's affinity for the practices of hoodoo/conjure, which she has written about many times in her ethnographic and literary works. I found Bier's discussion of how Hurston used the theatrical stage to tell stories surrounding hoodoo particularly interesting. Biers says, "She found narration, with its conventional reliance on a single author or character's perspective, inadequate for describing it, so she turned to the theatre – a form of assemblage, in which people and objects are agents" (73). Biers' understanding of Hurston's work made me consider my dancers' relationship with the audience. Hurston's plans to stage these rituals in a theatrical setting would mean many perspectives could be represented, something that doesn't come as naturally in a narrative form. I began thinking about how I could choreographically position my dancers as agents in this format. I had been working with the idea of my dancers telling multiple stories at once, instead of one narrative, so the theatrical element of multiple perspectives was something I could add to my exploration. Considering my dancers as agents inspired what I would choreograph to end the first section of my piece. To end the first section, I had four dancers face upstage with their backs toward the audience. They did a phrase of movements that resembled the way that an audience member would engage with a dance performance. The phrase included clapping and a shifting motion that resembled a person shifting in one's chair. At the same time, I had one dancer positioned in the center of the stage upstage. His movements mimicked that of a person trapped behind what I instructed him was "an invisible ball surrounding him." The simultaneous claps of the other dancers interrupted his movement. When he heard this sound, he would position his hands in a V shape above his head. After clapping, the four dancers shifted to one side, turned their heads, and looked directly ahead to what would be the audience. For that moment I wanted the dancers to show the audience that they weren't "just" characters. The collective image was choreographed to represent the dancer-audience member relationship. This portion of my choreography was my movement exploration into imaging my dancers with some agency. The incorporation of improvisation throughout the piece can also be read as a product of the dancers' agency.

Biers also discussed the elements of the hoodoo ritual present in Hurston's work, saying Hurston's plays "aspired to represent, and in many ways, recreate, the animistic natural and material world of the conjure or 'hoodoo' rituals" she encountered in her anthropological fieldwork (Biers 70). She also communicates that these rituals "mobilized the boundaries between animate and inanimate." The idea that dancers could be representing inanimate objects or nonhuman entities got me thinking. Who/what could my dancers represent if not solely human? What inspired me to make some material changes to follow this train of thought was Biers' reference to Hurston's essay "How It Feels To Be Colored Me," an essay I had been familiar with. Biers notes that the brown bag of miscellany, "the hoodoo mojo bag or hand," Hurston refers to herself as in the text is representative of something larger (74). She explains, "The self appears here as dispersed across the material domain; in it, the interior is not a soul but an assemblage of objects" (Biers 75). The brown bag of miscellany or "mojo bag" is a metaphor for humans but the "priceless and worthless" items therein are significant in their own right (Hurston). This is an example of the ideas behind "new materialism" that Biers highlighted in the text. "Material things... [are] understood to have life and agency independently of human beings, especially when they join in with other things in the form of 'assemblage'" (72). Though I was unsure where it could take me at this point in my process, I reimagined my work so far through this lens. This entailed thinking about what I had done and changing certain things to incorporate nonhuman things and "assemblage." The improvisation segment I directed had new weight when I imagined my dancers as nonhuman objects colliding with each other in a mojo bag. I liked the idea and to highlight it, I had my dancers improvise diagonally across the stage, from center upstage to downstage left, at the very beginning of the choreography. This less choreographed moment contrasted interestingly with the more choreographed phrases of the piece. Continuing with the idea, if the improvised moments were representative of nonhuman objects, the choreographed moments could be read as human. Even more interesting to me were the phrases where dancers were doing various (choreographed) phrases simultaneously. Perhaps they were flowing along a spectrum of representing the human and non-human. Though much hadn't changed choreographically with this lens attributed to my piece, I had found a way to approach the context in which my dancers were moving. My choreography was loosely informed by Hurston's work up to this point and this lens felt like a way to bridge my interdisciplinary interests and understand hers. I gave my dancers new movement qualities to consider. I specified moments where I wanted them to embody nonhuman things. This is most evident in the very beginning where the improvisation starts with dancers moving through and past each other at a staccato pace. As they traveled across the space, they slowly shifted towards flowing through and past each other, signaling a shift from the nonhuman to the human.

At the second showing on March 20th, I showed the first section of my piece to the faculty and received feedback that I should set an intention for my dancers, something that would make the piece ready for an audience. I would work on this in the following rehearsals while I finished the piece. For the audio of the section following the song by Sun Ra, since I was inspired by the closing paragraph, I used an anonymous reading of the excerpt from Hurston's essay. If in the first section my dancers were dancing along the spectrum of human and nonhuman representation, in this section I wanted the only thing connecting my dancers to "the human" to be the fact that they were humans. For this part, my dancers were the contents of the mojo bag that had been poured out that couldn't be "refilled without altering the content of any greatly" (Hurston). The first section had ended with my dancers bent at the hip with the crowns of their heads toward the floor. Queued by the

voice of the narrator of the excerpt, the dancers began walking along different paths on the stage. The accompanying excerpt used the metaphor of a mojo bag to discuss identity and for this section, it felt inappropriate not to consider the identities of my dancers. I discussed this with my dancers and incorporated their shared identities between them to pair them up. I also created the choreography for their groupings based on what they shared. For example, two of my dancers were Christian women. I paired them together for a short duet. Their phrase included a moment where they were kneeling with their palms to the sky. I ended the piece with each dancer improvising, in similar ways to how they did at the very beginning. Each dancer improvised until they were offstage behind the wings and it closed with the final dancer exiting the stage. I titled the piece "In The Main," from the first line of the excerpt, "But in the main, I feel like a brown bag of miscellany..." (Hurston). These words introduced the collection of Hurston's thoughts in the essay. Similarly, my piece was a collection of my thoughts, ideas, and findings from Hurston's work and impact.

The additions made by the lighting and costume designers added to the themes at work in my piece. For the lighting, the piece began with a blue backlight fading in from black, when the music started. The lighting is only the backlight for a few seconds showing the silhouettes of the dancers as they improvised around each other; a warm light fades in, lighting the dancers from stage left, some lights keep dancers lit from above, then the left light fades away as dancers move to a different part of the stage. The next most notable lighting shift happens when the audio excerpt from "How It Feels To Be Colored Me" begins. When the excerpt starts, all the lighting on stage dims and instead of being lit from above, they are lit with a straightforward light that highlights the shadows on stage. It remains this way until the piece ends and all lighting slowly fades to black. For the costumes, the dancers' outfits were loosely based on the Harlem Renaissance social dance scene. I wanted my dancers to appear pedestrian, so the clothing reflected that. The pallet was colorful and was inspired by visual art that portrays the atmosphere at these Harlem clubs like in the work of Archibald Motley. These lighting and costume additions specifically added to the exploration of materialism in this piece. The lighting choice at the very beginning only showcases the dancers' silhouettes. This starts the piece with a focus on the shapes the dancers were making. The direction I gave my dancers was to move through space with angular movements at a staccato pace. This was meant to represent the mojo bag in reference. The lighting added to the abstract feeling. The lighting changed to show the dancers' bodies and costumes slowly and this lined up with the shift in movement qualities occurring at the same time as the dancers' movement became more fluid and uninterrupted. This contributed to the concept of my dancers shifting, at this moment from nonhuman entities to humans. The dimly lit stage in the last section of the piece contributes to this concept as well. With the lighting at a low point, the dancers' faces and costumes are no longer in focus and their movement is the main thing we can see. This also abstracts the feeling that the dancers are human. The choice to have the dancers in pedestrian costumes makes them feel like humans, or characters, throughout the piece. This highlights movement as the plane where the spectrum is explored. Though their bodies are representative of humans, their movement explores a spectrum of human and nonhuman embodiment. This makes use of dance as a tool in and of itself to communicate abstract ideas. We can see the dancers on stage as representing objects while they are dressed in pedestrian clothes; this contradiction illustrates that objects can have agency and experiences of their own. In this way, the costume choice adds to the ideas of materialism at work in the piece. Also, each dancer had a different color outfit that worked well as a collective. This made it easy to differentiate between dancers on stage, thus making it that much easier to conceptualize the dancers as embodying different perspectives.

Research Methodology

Understanding the development of black dance in America from African enslavement to the Harlem Renaissance is important to understanding the impact of Zora Neale Hurston's dance work. Hurston's most notable folklore concert took the stage on January 10th, 1932. Hurston's goal for the concert was to contrast the popular Broadway productions of the time by presenting an authentic representation of the materials she found in her anthropological research. This has been a source of interest for scholars who aim to uncover the impact of Hurston's dance contributions. Some have communicated that her staging of various black folk traditions in one program had helped reorient the understanding of black dancing away from minstrelsy and blackface toward the actual African traditions brought to America and that her work recast black dance traditions in a broader transnational framework. In my choreographic exploration of the literature surrounding Hurston's work, I explored her staging of similarities and differences, hoodoo ritual, and her use of the theatrical stage. I incorporated these in my choreography, and this gave me a lens through which to explore the connection between literature and dance in Hurston's work and further understand my interdisciplinary interests. The lighting and costume design in the final stages added to the themes of the piece. I found that the connection between literature and dance in Hurston's work was grounded in her goal to accurately represent black life and in her spiritual interest in hoodoo. I concluded that Hurston's impact is in her contribution to a transnational understanding of blackness and that this is only compounded by her Afrocentric approach. Hurston's work was ahead of its time and "The Great Day" was a true representation of black dance. Studying her work today calls for more conversations around whether black dance is a broad enough category.

Works Cited

Locke, Alain. "African American Poetry: 'The New Negro' (Essay by Alain Locke) (1925)." African American Poetry: A Digital Anthology, https://scalar.lehigh.edu/african-americanpoetry-a-digital-anthology/the-new-negro-essay-by-alainlocke-1925. Batiste, Stephanie L. "Dunham Possessed: Ethnographic Bodies, Movement, and Transnational Constructions of Blackness." Journal of Haitian Studies, vol. 13, no. 2, Center for Black Studies Research, 2007, pp. 8–22, http://www.jstor.org/ stable/41715354.

Biers, Katherine. "Practices of Enchantment: The Theatre of Zora Neale Hurston." TDR (1988-), vol. 59, no. 4, The MIT Press, 2015, pp. 67–82, http://www.jstor.org/stable/24585031.

Dixon, Brenda. "Black Dance and Dancers and the White Public: A Prolegomenon to Problems of Definition." Black American Literature Forum, vol. 24, no. 1, 1990, p. 117-123.

Du Bois, William E. B. "The Sorrow Songs." The Souls of Black Folk, Oxford University Press, Oxford, 2008.

Haskins, James. Black Dance in America, Welcome Rain Publishers, New York, NY, 1999.

Hurston, Zora Neale. "Excerpt from Their Eyes Were Watching God", The Official Website of Zora Neale Hurston, https:/ /www.zoranealehurston.com/resource/excerpt-from-theireyes-were-watching-god/, Accessed 04 Mar. 2022

---. "How It Feels to Be Colored Me," Encyclopedia of African-American Writing, edited by Shari Dorantes Hatch, Grey House Publishing, 3rd edition, 2018. Credo Reference, https://login.ezp.lib.rochester.edu/login?url=https://search.credoreference.com/content/entry/ghaaw/how_it_feels_to_be_colored_me/0?institutionId=1154. Accessed 03 Mar. 2022.

---. "Hoodoo in America." The Journal of American Folklore, vol. 44, no. 174, 1931, pp. 317–417, https://doi.org/ 10.2307/535394. Accessed 4 May 2022.

---. "Dance Songs and Tales from the Bahamas." The Journal of American Folklore, vol. 43, no. 169, University of Illinois Press, 1930, pp. 294–312, https://doi.org/10.2307/534942.

Kraut, Anthea. "Between Primitivism and Diaspora: The Dance Performances of Josephine Baker, Zora Neale Hurston, and Katherine Dunham." Theatre Journal, vol. 55, no. 3, Johns Hopkins University Press, 2003, pp. 433–50, http://www.jstor.org/stable/25069279.

Kraut. Anthea. "Re-scripting Origins: Zora Neale Hurston's Staging of Black Vernacular Dance." EmBODYing Liberation: The Black Body in American Dance: A History through Its People, edited by Dorothea Fischer-Hornung and Alison D. Goeller, Lit, Mun[°]ster, 2003, p. 59-77

Perpener, John O. "The Dance Legacy Of Zora Neale Hurston." Dance Chronicle, vol. 33, no. 1, Taylor & Francis, Ltd., 2010, pp. 159–64, http://www.jstor.org/stable/20749319.

Perron, Wendy. "Dance in the Harlem Renaissance: Sowing Seeds." EmBODYing Liberation: The Black Body in American Dance: A History through Its People, edited by Dorothea Fischer-Hornung and Alison D. Goeller, Lit, Mun⁻ster, 2003,

p. 23-39

Program, "The Great Day", 10 January 1932, John Golden Theatre, Prentiss Taylor Papers, Archives of American Art, Smithsonian Institution; also reprinted in Hill, Social Rituals, 17-18.

About the Author

Born and raised in Brooklyn, New York, Sasha Murray is a University of Rochester student pursuing a Bachelor of Arts in Dance. Within the field of dance research, Sasha is most interested in investigating connections between dance, language, identity, and more. These were all guiding ideas behind this research project. For undergrads interested in research Sasha's advice is, "No question is too big. Go for it. In the worst case is you'll have a lot of reading to do, in the best case, you'll discover something cool."

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Drinking Water as a Source of PFAS Exposure in Pregnant Women: Well versus Municipal Water Sources in Monroe County and the City of Rochester

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1. Background

An ever-growing body of research points to the serious adverse health effects that perfluoroalkyl and polyfluoroalkyl substances (PFAS) present to humans, especially pregnant women, their fetuses, and infants. Drinking water has been a large and consistent source of PFAS exposure and has been shown to lead to increases in blood serum PFAS concentration. Data from the UPSIDE (2021) study found that in Monroe County and the City of Rochester, the estimated percent difference in PFAS exposure was lower in private well water sources than in the municipal water supply. It was also found that PFAS concentrations differ significantly in Rochester's and Monroe County's municipal water sources, with Lake Ontario having more than Hemlock Lake. This study suggests that further research regarding PFAS and drinking water is needed.

PFAS are a large family of synthetic organic chemicals used for various purposes. They are a specific group of fluorinated compounds, classified by having at least one carbon on which all the hydrogen atoms have been replaced by fluorenes (Buck et al., 2011). Thousands of different PFAS have been produced since the 1940s, with some more studied and widely used than others (US EPA, 2021). Two of the most used PFAS are Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS), which are banned from use in the United States and most western nations (US EPA, 2021). These and other PFAS are of great concern given that they are widespread throughout the environment, are extremely persistent, and bioaccumulate in tissue (Post, 2021). There is significant evidence of their detrimental effects on human health, including heightened cholesterol levels, decrease vaccine response in children, effect liver enzymes, greater risk of preeclampsia or high blood pressure in pregnant women, decreases in infant birth weights, and increased potential for kidney or testicular cancer(Potential Health Effects of PFAS Chemicals | ATSDR, 2020). Although there are many sources of these harmful chemicals,

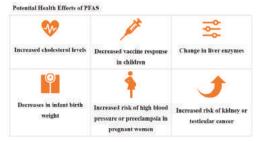
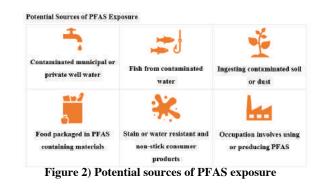


Figure 1) Potential health effects of PFAS

some of the most common places that PFAS can be found are in water repellent coatings, flooring materials, protective coatings in consumer and industrial products, adhesives, electrical wire insulation, fire-fighting foams, and nonstick cookware (Callan et al., 2016). Wide use of PFAS and dumping into the environment by various manufacturers and other sources has led to significant human exposure.



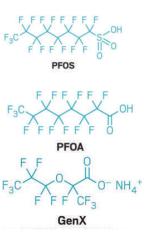
Primarily, humans are exposed to PFAS through drinking contaminated water, eating seafood from polluted water, ingesting soil or dust containing PFAS, consuming food packaged in PFAS containing material, working in industries that use or produce PFAS, and using domestic products that contain PFAS, such as non-stick cookware and stain and water resistant products (PFAS Chemical Exposure | ATSDR, 2021). PFAS primarily enters the body orally but can also enter by inhalation or through dermal exposure (ATSDR, 2021). There is little research on PFAS that are inhaled or absorbed through the skin. Using animal models, it has been estimated that there is a wide range of fractional absorption of orally administered PFAS, ranging from greater than 50 percent to greater than 95 percent, depending on the specific molecule (ATSDR, 2021). Once PFAS have entered the body, they become widely distributed primarily ending up in the liver, kidneys, and blood (ATSDR, 2021). PFAS then bind to various proteins found throughout different bodily systems, such as albumin in the blood (ATSDR, 2021). Thus, the transfer of PFAS to the fetus during pregnancy or to nursing infants through breast milk may occur. PFAS are not metabolized, do not undergo any chemical reactions in the body, and excretion can occur through a few different routes (ATSDR, 2021).

Rates of excretion, the half-lives of PFAS, do not appear to depend on the route of exposure (ATSDR, 2021). PFAS leave

the body primarily through urine and to a lesser extent through breast milk and feces. Excretion rates vary substantially between chemical species and animal species; sex and age-based differences have also been observed with certain PFAS (ATSDR, 2021). In humans, half-life estimates range from hours 72 to 81, for PFBA, to several years (ATSDR, 2021). PFOA's half-life in the body may range from 2.1 to 8.5 years, 3.1 to 7.4 years for PFOS, and 4.7 to 15.5 years for PFHxS (ATSDR, 2021). Differences in excretion rates between genders were more significant in rats than in humans (ATSDR, 2021). Women under 50, however, do appear to eliminate PFOS more rapidly due to menstruation compared to older women and men (ATSDR, 2021). Generally, elimination rate increases as branching increases, and decreases as chemical chain length increases (ATSDR, 2021).

1.1 Chain Length

It is important to differentiate between long and short-chain PFAS as these chemical differences lead to varying effects on exposure and possible health effects. There are two categories of long-chain PFAS which differ based on the amount of perfluorinate carbons they contain (i.e. perfluoroalkyl carboxylic acids (PFCAs) and perlfuoroalkane sulfonates (PFSAs)). PFCAs are composed of seven or more perfluorinated carbons, while perlfuoroalkane sulfonates (PFSAs)



have six or more perfluorinated carbons, such as PFOA (Buck et al., 2011). The different definitions for long-chain PFCAs and PFSAs are necessary as PFSAs with the same number of carbons as a PFCA have a greater tendency to bioconcentrate and bioaccumulate (Buck et al., 2011). Short-chain PFAS have much shorter half-lives in humans since they are excreted much more rapidly as compared to long-chain PFAS (Post, 2021). This difference has led to drinking water concentration guidelines for short-chain PFAS being generally one to two orders of magnitude greater than for long-chain PFAS (Post, 2021). Differences in drinking water guidelines not only exist between short and long-chain PFAS, but also between the states and federal government. Many states have their own guidelines for PFOA, PFOS, and other long-chain PFAS that are lower than those of the federal government. Additionally, new

Generation X (GenX) PFAS have been developed in recent years, something manufacturers claim to be less harmful than older legacy PFAS such as PFOS and PFOA (Buck et al., 2011). However, little is known about the health effects of GenX PFAS and they are rarely tested for.

There are currently no federal regulations aimed at limiting general exposure to PFAS. The U.S. Environmental Protec-

tion Agency has implemented a lifetime health advisory of 70 ng/L in drinking water (Guelfo & Adamson, 2018). Despite this, at least six million people across the country ingest drinking water contaminated with PFOA and PFOS that exceeds the EPA's lifetime health advisory, and up to 110 million Americans drink water contaminated with PFAS (Guelfo et al., 2018; Hu et al., 2016; EPA, 2018; Kuehn, 2019). It has been demonstrated through multiple studies that even relatively low concentrations of PFAS can lead to significant increases in human exposure (Post, 2021). Drinking water is a major source of exposure to PFAS because water with concentrations of 10, 40, 100, or 400 ng/L could cause mean blood serum levels to increase by 25%, 100%, 250% and 1,000% respectively, as compared to the general population's background serum concentrations of around 4 ng/L (Daly et al., 2018).

2. Sources and Routes of Exposure

There are many sources of exposure to PFAS. Drinking water is one of the most common and significant sources of exposure, both from public water systems and private well water (PFAS Chemical Exposure | ATSDR, 2021). Soil and effluent from disposal sites, landfills, hazardous waste sites and water and sewage treatment plants contribute to exposure and contamination in drinking water sources (Nakayama et al., 2019). Another common source of water contamination are foams used in firefighting, specifically aqueous film-forming foams (AFFFs) used to extinguish liquid-based fires during emergency training exercises at military bases, shipyards, chemical plants, refineries, airports and firefighting training centers (Nakayama et al., 2019). Apart from the direct manufacturing of PFAS, their use in the production of paper, some textiles, chrome plating and electronics contribute to exposure (Nakayama et al., 2019).

Food is another key element in PFAS exposure. Many foods are contaminated with PFAS, especially sea food sourced from PFAS contaminated water and dairy products from polluted areas (PFAS Chemical Exposure | ATSDR, 2021). Many types of food packaging contain PFAS, such as microwave popcorn bags, grease-resistant paper, fast food packaging, candy wrappers and pizza boxes (US EPA, 2021). Additionally, many different household items and dust serve as sources of exposure (PFAS Chemical Exposure | ATSDR, 2021). Examples include products used to protect various textiles, clothing, paints, sealants and varnishes, along with non-stick cookware and various cleaning products (US EPA, 2021). Some personal care products, such as cosmetics, dental floss and some shampoos contain PFAS (US EPA, 2021). Finally, biosolids are the original source of exposure to many PFAS by contaminating other goods. Fertilizer from wastewater treatment plants used on farm land leads to contamination of surface and groundwater as well as the animals that may graze on that land (US EPA, 2021). These sources lead to several routes of exposure.

Those exposed to the highest concentrations of PFAS are typically employed in chemical manufacturing and processing, factories that produce PFAS containing products and fire fighting at which PFAS can enter the body through inhalation and ingestion (US EPA, 2021). The primary sources of exposure are drinking water, foods packaged with PFAS containing materials, seafood from PFAS polluted waters, inhalation or ingestion of dust or soil tainted with PFAS, and the use of products containing PFAS or those that are packaged in PFAS containing material (PFAS Chemical Exposure | ATSDR, 2021). Occupations involving the manufacturing or use of PFAS containing materials, such as firefighting, are the largest contributors to individual body burdens of exposure (US EPA, 2021). For the general population, contaminated drinking water is the most common source of exposure and contributes most significantly to body burdens of exposure (US EPA, 2021). The ingestion of foods containing PFAS is the next largest contributor to PFAS exposure and body burden. The following greatest percentage of contribution to exposure is contaminated soil or dust, then PFAS in the air, and then products that are made with or packaged in materials containing PFAS (US EPA, 2021). Studying the sources and routes of PFAS exposure is vital given the varying adverse effects of PFAS on human health.

3. Health Effects

There is a significant and growing body of peer-reviewed scientific studies that show the relationship between PFAS and adverse health effects. The most well studied outcomes to various PFAS exposure include detrimental effects to the immune system, growth and developmental issues in children, and reproductive issues relating to pregnant women. Effects on the immune system include reduced ability of the immune system to fight infections and a reduction in vaccine response (US EPA, 2021). Negative effects on development in children such as accelerated puberty, low birth weight, behavioral changes, and bone variations have also been observed (Callan et al., 2016; US EPA, 2021). Furthermore, pregnant women are faced with several detrimental effects, especially increased high blood pressure and reduced fertility. General adverse health effects linked to PFAS exposure also include the increased risk of certain cancers, including testicular, kidney, and prostate cancer. Interference with typical hormone levels and greater cholesterol levels in conjunction with increased risk of obesity have been observed (US EPA, 2021). The effects of PFAS on pregnant women and lactating women are of particular concern given they are both more sensitive and susceptible than the general population.

3.1 Pregnant Women and Mothers

Women who are pregnant or lactating often drink more water per pound of body weight than normal leading to a possible increase in PFAS exposure and therefore PFAS in their bodies (US EPA, 2021). Not only does this lead to a greater risk of the aforementioned health effects, but it has also been demonstrated that PFAS can cross the placenta and expose a fetus (Callan et al., 2016). This is shown by the correlation between umbilical cord and maternal blood PFAS concentrations (Callan et al., 2016). Fetal exposure to PFOS has been shown to be associated with decreased head circumference, birth weight, and ponderal index (Callan et al., 2016). Greater PFOA concentrations during pregnancy have been linked to decreased birth weight (Callan et al., 2016). There have been few studies focused on examining the effects of other PFAS on pregnant women and newborns. PFOS, PFOA, PFNA, and PFHxS have been specifically looked at by Keto et al. (2014) for how they pass between maternal and cord sera.

It was found that PFOS was found at the highest concentration in maternal and cord sera, followed by PFOA and then PFHxS and PFNA at around the same level. These PFASs were found at significantly higher concentrations in maternal serum at 16 weeks of pregnancy when compared with their concentrations at birth in the mother and infant, further supporting transplacental transfer of PFAS (Kato et al., 2014). These different PFAS were also found to cross the placenta at varying amounts. The ratios of PFOS and PFOA between maternal and infant concentrations varied significantly. At birth, PFOA, on average, was found at the same ratio between maternal and cord serum samples, while PFOS was found at around twice the concentration in maternal samples compared to cord serum (Kato et al., 2014). Apart from differences in concentration between different PFAS between maternal and cord samples, several other factors seem to contribute to maternal concentrations.

A positive correlation between PFAS concentrations and maternal age during pregnancy has been observed (Kato et al., 2014). Especially in women younger than 25 years old, PFOS, PFOA, PFNA, and PFHxS were found at lesser amounts than compared to older women (Kato et al., 2014). There are two likely explanations for this finding. One being that since PFAS bioaccumulate in tissue, concentrations would continue to increase with age as total lifetime exposure grows. The other likely cause of the difference is the production of PFOS and its derivatives and precursors were halted by 3M, the primary manufacturer of PFOS, in the early 2000s (Kato et al., 2014). PFAS production also peaked during the 1980s through 1990s, exposing older women to greater concentrations (Kato et al., 2014). Age was not the only factor that seemed to predict PFAS serum concentrations; race and wealth also appear to play a role.

3.2 Adults

Women who reported the lowest household income and non-Hispanic Black women had lower concentrations of PFOS, PFOA, PFNA, and PFHxS as compared to women in wealthier households or non-Hispanic white women (Kato et al., 2014). This finding was in agreement with other studies and the U.S. population in general (Kato et al., 2014). These differences in concentration are likely explained by differences in lifestyle. This includes diet, use of products containing PFAS, physiology along with a mixture of other factors (Kato et al., 2014). Not only do PFAS impact pregnant women and mothers, associations between exposures and ovarian health have been found.

PFAS have been detected in follicular fluid in ovaries demonstrating their ability to pass through the blood-follicle barrier (Ding et al., 2020). Several cross-sectional epidemiological studies have found associations between increased PFAS exposure and delayed first menstruation, irregular menstrual cycles, longer cycles, reduced levels of estrogens and androgens, and earlier onset of menopause (Ding et al., 2020). Additionally, experimental findings have found that ovarian folliculogenesis and steroidogenesis are adversely affected by PFAS. A possible mechanism by which PFAS act on ovary function is by reducing proper hormone synthesis through the activation of peroxisome proliferator-activated receptors, leading to disruptions of intercellular communication through gap junctions. This lack of communication further disrupts hormone production and interferes with proper enzyme synthesis, inhibiting normal signaling in the hypothalamus related to ovarian processes (Ding et al., 2020).

3.3 Children

Children are also especially vulnerable to PFAS exposure. Since children are still developing, they are more sensitive to the harmful effects of PFAS. Additionally, children breath more air, drink more water, and eat more food per pound of body weight than adults which increases their exposure to PFAS (US EPA, 2021). Younger and smaller children are more heavily affected, not only because of greater proportional consumption of food, water, and air, but also because they crawl along the floor and exhibit greater hand to mouth behavior (US EPA, 2021). This behavior exposes them to more dust, carpets, and cleaning products that contain PFAS. Infants may have greater exposure to PFAS through breast milk from mothers with PFAS in their blood or from formula made with PFAS contaminated water (Callan et al., 2016).

Breastfeeding has been found to be inversely associated with PFOS and PFOA concentrations in maternal serum (Kato et al., 2014). Therefore, while breastfeeding may reduce the burden of PFOS and PFOA on the mother, they lead to increased exposure of the breastfeeding child to these and likely other PFAS. One example of this effect is when 6-8 year old girls in the greater Cincinnati and San Francisco area were studied for PFAS concentrations, those who were breast fed for longer had greater serum levels of PFAS (Kato et al., 2014).

3.4 Mechanism of Toxicity

Once in the body, PFAS become widespread throughout the body, but primarily accumulate in the liver, kidneys, and bloodstream (ATSDR, 2021). PFAS affect a wide range of bodily systems by binding to various proteins. Studies using human populations have found that PFAS interact with thyroid hormone binding proteins (Fenton et al., 2021).

PFAS can also disturb the feedback relationships between free thyroid hormone and the hypothalamic-pituitary-thyroid axis through interference with thyroid peroxidase (Fenton et al., 2021). Animal toxicology and histology along with human population data points to PFAS disrupting hepatic metabolism, which leads to lipid accumulation in the liver (Fenton et al., 2021). The mechanism behind this effect is still unknown, but may relate to acid metabolism, mitochondria perturbations, and processes affecting cholesterol levels (Fenton et al., 2021). In blood and other tissues, PFAS mechanisms of toxicity are based on their interactions with various proteins, such as serum albumin in blood (Fenton et al., 2021).

The half-lives of PFAS appear to largely be based on their interactions with specific proteins and receptors. It has been shown in mammals that renal reabsorption of PFAS is affected by PFAS interactions with transporters such as organic anion transporting polypeptides (Fenton et al., 2021). Saturation, or inactivation, of these receptors at increased doses of PFOA and PFOS, leads to significant reductions in the halflives of these PFAS (Weaver et al., 2010). PFAS have not been shown to be directly mutagenic (Fenton et al., 2021). There is evidence, however, that PFAS can cause oxidative stress and DNA damage, such as strand breaks, and other genotoxic effects (Fenton et al., 2021). These effects have been observed at concentrations that are relatively high compared to the amounts of PFAS humans are generally exposed to in the environment (Fenton et al., 2021). It is important to continue research into the mechanisms by which PFAS affects the body as their ability to bind to various proteins and hormones could lead to a host of negative effects.

4. Testing

As concern and regulation regarding legacy PFAS has continued to grow and some PFAS have become banned, PFOS and PFOA, a new class of PFAS are continuing to be developed. Research has continued to shift away from older PFAS to new fluorinated compounds possessing one or more perfluoroalkyl (-CnF2n-) portions (Nakayama et al., 2019). These new PFAS have begun to appear in the environment. These new PFAS have exacerbated the existing challenges in PFAS testing and presented new problems as well.

There are currently three especially large challenges to PFAS testing today. First, assessing the risk of PFAS classes is difficult since there is a lack of information on mixture effects, individual hazards, total burden, mechanisms of toxicity, and the presence of numerous known and unknown PFAS (Nakayama et al., 2019). Second, there is a lack of techniques to detect the continuous discharge of PFAS from various sources or to detect the decrease in levels of PFAS already in the environment (Nakayama et al., 2019). Third, new alternatives with similar structures to existing PFAS will continue to be developed and released into the environment (Nakayama et al., 2019). Various techniques are employed to best test for PFAS in different mediums. These varying mediums present their own unique set of challenges as well.

<u>4.1 Testing in the Environment: Air, Soil, and Drinking Water</u>

There are several different methods for collecting airborne PFAS such as glass fiber filters and resin sandwiches in conjunction with polyurethane foam (Nakayama et al., 2019). Collected samples are then analyzed using gas chromatography-mass spectrometry or by high performance liquid chromatography-tandem mass spectrometry (Nakayama et al., 2019). The lack of a standardized sample collection technique is the main challenge when looking to monitor PFAS in the air (Nakayama et al., 2019). A standardized method is needed for JŬR

easier comparison of various studies and testing. Using bioindicators, such as tree bark and leaves, could provide a new path for detecting atmospheric PFAS (Nakayama et al., 2019). There has been progress in improving testing techniques for aqueous environments.

Miniaturization of extractions procedures has reduced the sample size and amount of solvent needed for aqueous samples (Nakayama et al., 2019). Testing for short-chain PFAS, which has been used significantly more, poses specific challenges as they are more susceptible to matrix effects that cause ionization suppression, making them more difficult to detect accurately (Nakayama et al., 2019). Since short-chain PFAS are more volatile than long-chain PFAS, causing short-chain PFAS to persist longer in the environment and diffuse more widely, increasing the accuracy in detecting them is key.

Abiotic solids, such as sediment, sludge, dust, and soil, often contain PFAS originating from another source, such as aqueous film-forming foams used for firefighting. Many PFAS, especially new cationic and zwitterionic species, have been shown to be strongly attracted to abiotic solids (Nakayama et al., 2019). Additionally, dust is one of the primary routes of PFAS exposure for humans so finding a means to extract a broad range of PFAS from abiotic solids is key to long term monitoring and future research on environmental and health implications.

4.2 Testing in People: Blood and Urine

Testing in wildlife and humans presents a unique set of challenges since samples are drawn from both aqueous and solid sources (Nakayama et al., 2019). Development of efficient extraction and cleanup methods, especially using green chemistry, has been the focus of recent research (Nakayama et al., 2019). Taking care to use containers that do not contaminate samples is key to ongoing research. Increasing the use of noninvasive human sample collection, such as hair, nail, and urine samples, will make future studies easier to conduct. The vast majority of studies (around 90%) looking at human PFAS exposure over the past twenty years have used blood samples from which serum is extracted (Nakayama et al., 2019).

5. Drinking Water

Currently, there are nine states with their own guidelines for PFOA and PFOS in drinking water that are more stringent than the 70 ng/L health advisories created by the EPA in 2016 (Post, 2021). No state has created less demanding guidelines and ten have created additional guidelines for other PFAS (Post, 2021). Individual state guidelines for PFOA in drinking water range from 8 to 35 ng/L and 10 to 40 ng/L for PFOS (Post, 2021).

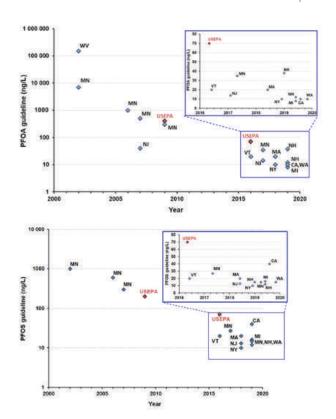


Figure 4) State and US Environmental Protection Agency drinking water guidelines for perfluorooctanoic acid (PFOA), left, and perfluorooctane sulfonate (PFOS), right 2000 to 2020. (Post, 2021)

These more stringent guidelines developed by numerous states reflect the emergence of new information on health effects from PFAS and recent reviews of older information used when guidelines were first created (Post, 2021). The EPA health advisory of 70 ng/L, at an average rate of drinking water consumption, would increase serum PFOA levels by around 5-fold and 4-fold for PFOS from the current US general population means (Post, 2021). This is a significant increase in serum concentration of PFOA, PFOS, and other PFAS from just drinking water alone. To accurately look at the effect of PFAS contaminated drinking water on a population, it is important to also examine the original source of the PFAS which contaminated the water source .

Data from the City of Rochester's Bureau of Water reported that PFOS was detected in Lake Ontario water at about 2.55 ng/L and PFOA at 2.10 ng/L which is acceptable under the EPA's guidelines of 70 ng/L in drinking water, but both PFOS and PFOA were not detected at any level in Hemlock Lake sourced water (City of Rochester Bureau of Water, 2020b) (Post, 2021). Many states have their own drinking water limits on PFAS that are significantly lower than the EPA's, including New York States (Post, 2021). New York State currently has a health advisory limit of 10 ng/L for both PFOS and PFOA. While the water from Lake Ontario still falls below this, it is much closer to the New York State limit.

5.1 Sources of Contamination in Drinking Water Sources

In general, there are two types of sources of PFAS that contaminate drinking water sources, point sources and diffuse sources. Point sources describe a particular place from which PFAS that contaminate water can be traced from. Effluent contaminates drinking water sources by flowing from point sources containing PFAS. Common point sources are wastewater treatment plants, industrial and manufacturing sites, airfields and places where firefighting foams are often used, and landfills (Banzhaf et al., 2017). In Germany it was found that the majority of PFAS enter rivers from point sources, especially wastewater treatment plants (Banzhaf et al., 2017). In the United States, industrial emissions often from sites using organic solvents, specifically from PFAS producing and using sites, had a significant impact on PFAS contamination of surface water used as a source for tap water (Banzhaf et al., 2017). Military and commercial airfields are also notable point sources given they often use aviation firefighting foams which contain PFAS and their precursors (Banzhaf et al., 2017). Rain can wash residue from the use of these chemicals into nearby streams which flow into larger bodies of water. Firefighting training centers hold the same risk for the same reasons. Finally, landfills are a major source of PFAS contamination in ground water especially (Banzhaf et al., 2017). This ground water is often then used as a source for drinking water. While point sources can be easy to trace as sources of PFAS pollution, diffuse sources contribute even more to pollution and are nearly impossible to trace back to one specific location.

Diffuse sources of PFAS contamination are caused by many different urban and rural industries and land uses. Atmospheric deposition, in which precipitation captures atmospheric pollutants and brings them to the ground and bodies of water, and contaminated upstream water sources contribute to PFAS contamination of larger bodies of water and drinking water sources (Banzhaf et al., 2017). The contribution of different pathways of PFAS into various larger bodies of waters was looked at using mass balancing models across large geographical areas. For Lake Ontario, it was found that discharge from wastewater treatment plants (WWTPs) into the lake had a minor role in PFOA levels in Lake Ontario as compared to the contribution of the inflow from the other Great Lakes (Boulanger et al., 2005). A mass balance of PFASs in the Baltic Sea found that west deposition and discharges from rivers were the primary contributors to PFAS in the Baltic Sea and that discharge from WWTPs into the sea played only a minor role (Filipovic et al., 2013). While effluent from WWTPs may not be the primary contributor to PFAS pollution in larger bodies of water, they still play a significant role.

Filipovic (2013) found that WWTPs still contribute considerably, greater than 5 ng/L, to PFAS in aquatic environments. PFAS levels in wastewater treatment plants' effluent can be increased by pollutants in the atmosphere and from used tap water (Banzhaf et al., 2017). Contaminated groundwater used as a source for municipal tap water can end up being discharged by WWTPs back into the environment (Banzhaf et al., 2017). Groundwater contamination from PFAS has not been investigated to as full of an extent as contamination from surface water sources has. Underground drinking water sources are much harder to study since drawn samples are only representative of the area immediately around the well from which the sample was taken (Banzhaf et al., 2017). Additionally, knowing how underground water flows and its other characteristics are much more difficult to study than surface level sources. No matter the source of drinking water, PFAS concentrations in the water significantly impact bodily PFAS levels.

.5.2 Exposure from Drinking Water and Blood Serum Levels

Numerous studies have found positive connections between PFAS levels in tap water and blood plasma PFAS levels (Post, 2021). Drinking water is a significant source of exposure to PFAS as water with concentrations of 10, 40, 100, or 400 ng/L could cause mean serum levels to increase by 25%, 100%, 250% and 1,000% respectively, as compared to the general population's background serum concentrations of around 4 ng/L (Daly et al., 2018).

In a study conducted from 1989-1990 among women in the United States with no occupational exposure to PFAS, a significant positive association between tap water and plasma PFOA and PFNA concentrations was found (Hu et al., 2019). Individuals who consumed eight or more cups of water per day had the strongest relationship (Hu et al., 2019). There was also an increase in various PFAS in tap water samples from 1989-1990 to 2016 (Hu et al., 2019). This likely reflects trends in the growing number of new PFAS that continue to be developed as well as degradation of PFAS.

It was estimated that 2.2% to 34% of PFAS found in plasma were from tap water PFAS from 1989-1990 (Hu et al., 2019). The continuing presence of PFOS and PFOA in drinking water samples obtained between 2013-2016 highlights the fact that legacy PFAS continue to persist in the environment and contribute to human exposure long after their production has ceased (Hu et al., 2019). Therefore, even if the use of PFAS in consumer products and packaging decreases, the presence and longevity of PFAS in the environment will continue to drive exposure. Since the existence of PFAS in the environment, and therefore drinking water, plays such a key role in human exposure to these chemicals, looking at the differences between drinking water sources is essential.

When looking at differences in PFAS exposure from drinking water, considering well versus municipal tap water is crucial. Under the Third Unregulated Contaminant Monitoring Rule (UCMR3) the U.S. Environmental Protection Agency carried out a nationwide study of six different PFAS from 2013-2015. Data from this screening covered all large public water systems, systems serving 10,000 or more customers, in the United States and 800 small public water systems, systems serving less than 10,000 customers (Guelfo & Adamson, 2018). When the water systems to be studied were selected in

2010, they served an estimated 248 million Americans or around 79% of the population at the time (Guelfo & Adamson, 2018). It was also estimated that in 2010, 44.5 million U.S. residents got the majority of their drinking water from private wells (Guelfo & Adamson, 2018). While data from UCMR3 did not collect data from private well water sources, data from small public water systems can be assumed to be representative of private wells (Guelfo & Adamson, 2018). This allowed for an accurate comparison of private well water versus larger municipal tap water systems.

Important differences were seen between large systems and well water or small systems. For small public water systems using ground water, the detection rate for at least one PFAS being present was 0.4% compared to 1.9% in all groundwater samples. Since small groundwater systems serve as an accurate proxy for private wells, it is believed that private wells are 4.5 times less likely to detect at least one PFAS as compared to other systems (Guelfo & Adamson, 2018). Small groundwater public water systems were also discovered to have higher PFAS concentrations, median of 0.18 µg/L, compared to all other detections with a median of 0.053 μ g/L (Guelfo & Adamson, 2018). Around half of samples that contained PFAS contained two or more types of PFAS with 72% of detections occurring in groundwater (Guelfo & Adamson, 2018). Large public water systems were 5.6 times more likely to contain PFAS than small water systems, yet when PFAS were detected, median concentrations were higher in small public water systems, at 0.12 μ g/L, and 0.053 μ g/L in larger systems (Guelfo & Adamson, 2018). This difference in concentration is even more drastic when small water systems use groundwater sources, which is the closest approximation to private wells. Based on these findings, per capita risk of PFAS exposures are likely greater at contaminated small public water systems, especially those using ground water, and private wells compared to contaminated large public water systems (Guelfo & Adamson, 2018). There are a few possible explanations for this difference.

Small water system wells have less dilution than a larger system (Guelfo & Adamson, 2018) leading to higher concentrations of PFAS. Water in these systems also sits for longer due to slower pumping rates and the capture of water from a smaller area (Guelfo & Adamson, 2018). These factors allow for PFAS levels to increase as the water sits in contact with contaminated material that is concentrated in a smaller area. Using past cases in which PFAS contaminated a specific area provides key insights into how PFAS in drinking water affects human exposure and differences between water sources.

A chemical spill in 2007 led to increased levels of PFOA, and other PFAS to a lesser extent, in drinking water in the Mid-Ohio Valley. After the spill, PFOA concentrations in the region's public water supply where 3.55 ng/L. Private wells in the area were found to have concentrations ranging from undetectable (0.010 ng/L) to 14.0 ng/mL (Emmett et al., 2006). To try and address this problem, two districts began filtering their water supplies to decrease PFOA concentrations. After filtration began, PFOA levels in both the drinking water and serum decreased (Herrick et al., 2017). PFOA concentration in blood serum decreased at around 26% per year (Domingo & Nadal, 2019). PFOA serum concentration remained elevated in the region compared to the general population, but no other PFAS were found to be increased. This established that the concentration of PFAS in drinking water directly affects the level of PFAS in humans (Domingo & Nadal, 2019). Amounts of PFOA in serum decreased after filtration began removing it from the water supply further supporting the direct connection between PFAS concentrations in drinking water and blood serum. This case, and others like it, have provided data on how concentrations of PFAS in drinking water lead to increases in human exposure and concentration, but there is little data regarding how typical background levels in municipal water supplies affect human PFAS concentrations.

Learning how background concentrations of PFAS affect human blood concentrations is key, which is why these effects will be studied using data from the Understanding Pregnancy Signals and Infant Development (UPSIDE), conducted in 2021. Specifically, maternal serum PFAS, geographic, demographic, and other questionnaire data from UPSIDE will be examined.

5.3 Rochester and Monroe County Municipal Water

Rochester's municipal drinking water supply is supplied by the City of Rochester Water Bureau which provides water to 210,000 people and numerous businesses in Rochester (City of Rochester Bureau of Water, 2020b). Over 40 of the Water Bureau's employees are NYSDOH (New York State Department of Health) certified as water system operators (City of Rochester Bureau of Water, 2020b). In 2020, Rochester became involved in the Partnership for Safe Water which is the American Water Works Association and EPA's joint program to assist water providers in exceeding current water quality regulations (City of Rochester Bureau of Water, 2020b). The City of Rochester and Monroe County, through the Water Bureau and Monroe County Water Authority, receive water from two sources.

The primary source of water for Rochester, since 1876, is Hemlock Lake in the Finger Lakes south of the city. Hemlock Lake water treatment plant is operated by the City of Rochester Bureau of Water (City of Rochester Bureau of Water, 2020b). The plant regularly tests water at the entrance and exit points of the plant. After uptake from the lake, the water undergoes processing such as pH adjustment, coagulation, filtration, disinfection, and fluorination (City of Rochester Bureau of Water, 2020b). Every day, 48 million gallons of water from Hemlock Lake is transported to the city through pipes to Cobs Hill and Highland Reservoirs from which it is then distributed.

One part of the city receives its water from Lake Ontario and the other part receives a mix of water from both Lake Ontario and Hemlock Lake (Monroe County Water Authority, 2020). The Monroe County Water Authority run the Shoremont water treatment plant which sources its water from Lake Ontario. Most of Monroe County receives this water from Lake Ontario with parts receiving water from Hemlock Lake.



Figure 5) Drinking water source map of Monroe County and the City of Rochester

Rochester purchases some water from Monroe County to fulfill needs in the western and northwestern areas of the city (Monroe County Water Authority, 2020).

5.4 Research Aims

The goal of this thesis is to shed light on how much different sources of drinking water contribute to an individual's exposure to PFAS, specifically pregnant women in the City of Rochester and Monroe County. Using data from Understanding Pregnancy Signals and Infant Development (UPSIDE) and water quality testing reports from the City of Rochester and Monroe County, two questions will be investigated. First, what is the association between maternal concentrations of PFAS and the use of municipal (public) water as compared to private well water? Second, are there differences in maternal PFAS concentrations across municipal water sources — Hemlock Lake versus Lake Ontario?

6. Methods 6.1 UPSIDE

The purpose of the Understanding Pregnancy Signals and Infant Development (UPSIDE), a pregnancy cohort study on prenatal exposure mechanisms for child health, was to study causes of maternal stress. Through extensive research, maternal prenatal distress is likely associated with perinatal and child health outcomes which may persist into adulthood (O'Connor et al., 2021). To better understand the mechanism behind this connection, UPSIDE included analysis of multiple biological pathways of interest, repeated assessments across trimesters, and incorporation of placental structure and function as mediators of child health outcomes (O'Connor et al., 2021).

Participants of UPSIDE consisted of women with normal risk pregnancies who were recruited at less than fourteen weeks gestation. Sociodemographic, psychological, health behavior, and biospecimen collections occurred at study visits in each trimester (O'Connor et al., 2021). At birth, placental and cord blood were collected. Behavioral, clinical, neuroimaging, an-

thropometric, and biological assessments were used to evaluate child physical and neurodevelopment at visits from birth and at 1, 6, 12, 24, 36, and 48 months old (O'Connor et al., 2021). A questionnaire regarding where the mothers sourced their water was collected during the first trimester.

As of April 2021, 326 women have been recruited with 294, 90%, remaining in the study until they gave birth (O'Connor et al., 2021). Of the 326 enrolled, three were lost to screening failures, five to pregnancy losses, eight became ineligible to continue during pregnancy, two lost for follow up, and four-teen dropped out (O'Connor et al., 2021). The remaining mothers who gave birth during the study had the following statistics.

On average, the women were 28.9±4.7 years when recruited (O'Connor et al., 2021). The majority of the women were White (61.2%) with 25.5% of participants being Black and 9.9% being of Hispanic ethnicity (O'Connor et al., 2021). The study population was socioeconomically diverse, 34.2% having obtained a high school degree or less and 25.7% having a post-college degree (O'Connor et al., 2021). 74.9% were employed and 60.3% were married or living as married at the time of recruitment (O'Connor et al., 2021). Alcohol use and smoking during early pregnancy were self-reported and relatively uncommon at 3.4% and 7.8% respectively (O'Connor et al., 2021).

Across all trimesters the success rates for prenatal biospecimen collection were high, 94-97% for urine, 96-99% for saliva, 96-99% for blood, 96% for placentas, 88% for cord blood, and 93% for buccal swab (O'Connor et al., 2021). Of the 277 eligible babies 94% participated in a birth examination and ongoing postnatal visits (O'Connor et al., 2021). Each biospecimen was collected in a specific manner.

For blood, 40 mL was collected every trimester and divided into aliquots of serum, cells, plasma, and whole blood (O'Connor et al., 2021) for use in various tests. These samples were used in analysis of sex steroid hormones, specifically estradiol, estrone, testosterone, and free testosterone using liquid chromatography with tandem mass spectrometry (O'Connor et al., 2021). Using radioimmunoassays, placental corticotropin releasing hormone was analyzed. Finally, immune and related markers such as C reactive protein, angiogenic markers, Mullerian inhibiting factor, and high sensitivity cytokines were evaluated (O'Connor et al., 2021). Upon admission for delivery maternal blood was also collected.

Cord blood was collected by two different means: mixed cord blood, up to 25 mL, was collected from the delivery room and fetal venous and arterial blood, up to 30 mL, was collected from placental vasculature immediately following delivery (O'Connor et al., 2021). These blood samples were stored in specific tubes depending on the amount collected.

There are several key strengths and limitations of the UPSIDE study. Most importantly for the collection of data related to PFAS concentrations was the rigorous collection of biospecimens throughout the pregnancy period and just after birth. This was possible due to recruitment of participants from obstetric clinics near the labs where processing and storage of the biospecimens occurred (O'Connor et al., 2021). A key part of the ability to collect the placenta and cord blood immediately after birth and process the placenta within three hours, preferably one hour, after birth, was a large team that was available around the clock(O'Connor et al., 2021). Due to the successful implementation of these strategies, UPSIDE obtained 96% of placentae (O'Connor et al., 2021). This obtainment of placentae allowed for rich data collection on morphology and molecular biology which help address questions regarding maternal exposures and child outcomes (O'Connor et al., 2021). There are additional strengths of UPSIDE, such as continued follow up of participants and their children, but this is not important for the questions asked in this analysis. Some of the limitations of UPSIDE are relevant to measurement of PFAS exposure.

One limitation of UPSIDE is that purposeful recruitment of low-medical risk pregnancies prevents using this data to answer questions addressing complications or outcomes. Furthermore, the relatively small sample size and generally healthy newborns prevents addressing hypotheses investigating exposures during pregnancy in relation to pediatric clinical outcomes such as autism spectrum disorders, developmental delays, and birth defects (O'Connor et al., 2021). Preterm infants were also not included in follow ups due to differing developmental time tables relative to term infants; thus, the same exposure related inquiries will not be applicable for that population (O'Connor et al., 2021).

6.2 Estimated Percent Difference PFAS Analysis

Whole maternal blood was collected, centrifuged, and up to 3 mL of plasma was removed and stored at -80°C at the University of Rochester Medical Center. Samples were then shipped to the Organic Analytical Chemistry Laboratory at Wadsworth Center, NYS Department of Health, in Albany, NY on dry ice for chemical analysis by Dr. K. Kannan. After

thawing, clean-up, and extraction, plasma samples were analyzed for five prevalent PFAS: perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS), and Perfluorodecanoic acid (PFDA). The analytic method consists of a combination of high-performance liquid chromatography interfaced with tandem mass spectrometry techniques. The Wadsworth laboratory has published quality assurance/control procedures for PFAS and participates in yearly Arctic Monitoring and Assessment Program inter-lab studies to validate their methods (AMAP; Institut National de Santé Publique du Québec, Québec, Canada). Dr. Kannan's laboratory has analyzed tens of thousands of blood samples for PFAS concentrations and was the first laboratory to quantify PFAS in human blood specimens throughout the world.

6.3 Rochester Municipal Water

Testing for PFAS in municipal water supplies was required in New York for the first time in 2020 (Albany Dep. of Water, 2020). Both Albany and Buffalo PFAS levels in municipal water sources were compared to PFAS levels found in Rochester's municipal water sources. However, neither city had tested for PFAS prior to 2020 and when testing occurred in 2020 levels for both were undetectable so further testing was not required (Albany Dep. of Water, 2020; Buffalo Water, 2018, 2019, 2020).

As part of running the Hemlock Lake water treatment plant, the City of Rochester Water Bureau conducts regular testing of a wide range of potential drinking water contaminants. Between one and five have been tested annually since 2015 (City of Rochester Bureau of Water, 2020b). In 2020 PFOS and PFOA were tested for five times while PFHxS and PFNA were tested for once (City of Rochester Bureau of Water, 2020b). Samples are taken at the entry point before any treatment has taken place (City of Rochester Bureau of Water, 2020b). Samples are then sent to Eurofins Scientific to be analyzed at their lab in South Bend, Indiana.

All the water testing performed by Eurofins, including testing for perfluorinated compounds, are carried out following EPA approved methods (D. Rowley, personal communication, April 4, 2022). Case method 537.1 was used to analyze samples for PFAS (Eurofins Scientific Analytical Report, 2022). First, surrogates are added to 250-mL water samples which are then passed through an SPE cartridge containing polystyrenedivinylbenzene which extracts the analytes and surrogates. Next, a small amount of methanol is used to elute the compounds from the solid phase sorbent. Then, nitrogen in a heated water bath concentrates the extract to dryness, and the samples are adjusted to a 1-mL volume with 96 (methanol): 4 (water) (vol/vol). Internal standards are then added to confirm the accuracy of detection. This is followed by 10 µL being injected into a liquid chromatograph interfaced with a tandem mass spectrometer. This procedure isolates and then identifies the analytes by comparing the obtained retention times and mass spectra of calibrated standardsusing the same procedure with pure samples of each substance (Shoemaker & Tettenhorst, 2018). Results are then tabulated and reported in ng/L or μ g/L. When multiple samples are taken the minimum, maximum, and average concentrations are reported. If a sample is not detected it is indicated as 'ND' (City of Rochester Bureau of Water, 2020b).

For case method 537.1, the minimum reporting level (MRL) ranges from 0.5 to 6.5 ng/L (ppt) (Shoemaker & Tettenhorst, 2018). MRL is the lowest analyte concentration that meets data quality objectives that are determined based on the specific use of method 537.1 (Shoemaker & Tettenhorst, 2018). Each laboratory must establish their own lowest concentration MRL (LCMRL), which is the lowest concentration that can be detected with 99 percent confidence (Shoemaker & Tettenhorst, 2018). While concentrations that fall below the MRL are considered not detected (ND) in terms of reporting that does not mean there are no PFAS present at all, just that their levels are too low to accurately confirm their exact concentration. The Eurofins laboratory that analyzes samples for the Rochester Water Bureau has a MRL or LCMRL of 1.8 ng/L (D. Rowley, personal communication, April 19, 2022). Testing sensitivity for PFAS has greatly increased from 2015 to 2020, with the MRL in 2015 being around 20 ng/L compared to the 0.5 to 6.5 ng/L MRL of today (D. Rowley, personal communication, April 19, 2022).

The Monroe County Water Authority performs similar tests at the entry point of their Shoremont pumping facility before any treatment has occurred (Monroe County Water Authority, 2020). Between one and four samples are collected annually (Monroe County Water Authority, 2020). The Water Authority tested for several PFAS in 2015 and then did not do so again until 2019. When multiple samples are taken the minimum, maximum, and average concentrations are reported. If a sample is not detected it is indicated as 'ND' (Monroe County Water Authority, 2020).

For comparing water sourced from Hemlock Lake and Lake Ontario, data from the City of Rochester Bureau of Water and Monroe County Water Authority concerning concentration of PFOS, PFOA, PFNA, PFHxS, and PFDA were pulled from publicly published annual water quality reports. These PFAS were specifically chosen since they are the same five PFAS for which UPSIDE collected data. This allowed for a comparison between results from UPSIDE and data from the sources of the municipal water consumed by UPSIDE participants. These figures were then tabulated and compared.

7. Results <u>7.1 UPSIDE</u>

To estimate the crude and adjusted associations between water source type and each PFAS homologue, linear regression models were utilized. PFAS concentrations were transformed by the natural logarithm in all regression models and served as the dependent variable and water source (public or private) the independent variable. The regression coefficients were interpretable as the percent difference in exposure concentration comparing public to private water sources. Adjusted regression models included a covariate for maternal parity. Table 1) Estimated percent difference in PFAS exposure for private (well) water versus public water source. 'UA' denotes unadjusted values and 'PA' denotes parity adjusted values.

	PFOS		PFOA		PFNA		PFHxS		PFDA	
	UA	PA	UA	PA	UA	PA	UA	PA	UA	PA
N	199		199		199		199		177	
Estimated % difference	-11	-10	+7	-4	-2	+1	0	0	-40	-40
95% confidence interval	[-30, 8]	[+29, 9]	[-30, 17]	[-25, 18]	[+24, 20]	[-23, 21]	[-16, 16]	[-16, 16]	[-84, 3]	[-84 4]
p-value	0.26	0.3	0.56	0.72	0.85	0.94	0.98	0.97	0.07	0.0

For FFOS, PFOA, PFNA, and PFHxS 199 samples were used for analysis. For PFDA, 177 samples were used for analysis as some samples fell below the lowest detectable limit. The estimated percent difference in PFAS exposure for private (well) water sources versus public water sources for unadjusted exposures differed between the five PFAS. The unadjusted PFOS value was found to be 11% ([-30%, 8%] p= 0.26) lower in subjects whose water came from private wells as compared to those who received public water. PFOA was found to be 7% ([-30%, 17%] p= 0.56) lower, PFNA was found to be 2% ([-24%, 20%] p= 0.85) lower, there was no difference for PFHxS between the two sources ([-16%, 16%] p= 0.98, and PFDA was found to be 40% ([-84%, 3%] p= 0.07) lower.

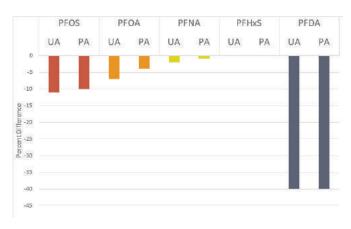


Figure 6) Unadjusted (UA) and parity adjusted (PA) estimated percent difference in PFAS exposure for private (well) water source versus public water source

Percent differences in public and private water source PFAS exposure for parity adjusted exposures differed less widely than the unadjusted values in all the PFAS tested except for PFDA and PFHxS. The parity adjusted value for PFOS was 10% ([-29%, 9%] p= 0.3) lower for those who received their water from the public water supply. PFOA was 4% ([-25%, 18%] p= 0.72) lower, PFNA was 1% ([-23%,21%] p= 0.94) lower, there was no difference for PFHxS between the two sources ([-16%, 16%] p= 0.97), and PFDA was 40% ([-84%, 4%] p= 0.08) lower. Parity adjusted figures were calculated to see if the number of children a mother has had prior to the pregnancy studied in UPSIDE was a possible confounder. Pregnancy can change a woman's normal body fat composition and metabolic rate that may affect how they metabolize and retain PFAS.

7.2 Rochester Municipal Water Supply

For Hemlock Lake from 2015 to 2020, PFOS and PFOA have not been detected above the MRL for each year since testing began in 2015. Both were tested for every year from 2015 to 2020. PFNA and PFHxS were tested for in Hemlock Lake in 2015 and 2020, with tests for both in each year falling below the MRL. PFAS other than PFOS and PFOA appear to be tested for every five years in Hemlock Lake. As of 2020, no PFAS have been detected in Hemlock Lake above the MRL, meaning essentially no PFAS have been detected in Hemlock Lake.

Table 2) Average PFOS, PFOA, PFNA, PFHxS, and PFDA Concentrations in Hemlock Lake (H) versus Lake Ontario (O) 2015-2020. A '<' is followed by the MRL for that PFAS for that year and is equivalent to not detected (ND). A '-' means that PFAS was not tested for in that year.

	PFOS (ng/L)		PFOA (ng/L)		PFNA (ng/L)		PFHxS (ng/L)		PFDA (ng/L)	
Year	Hemloc k (H)	Ontari o (O)	н	0	н	0	н	0	н	0
2015	<90	ND	<20	ND	<20	ND	<30	ND	-	ND
2016	ND	-	ND	-	-	-	-	-	-	-
2017	<1.8	-	<1.8	-	-	-	-	-	-	-
2018	<1.8	-	<1.8	-	-	-	-	-	-	-
2019	<1.8	3.6	<1.8	2.85	-	-	-	2	-	-
2020	<1.8	2.55	<1.8	2.1	< 0.02	ND	< 0.02	1	-	ND

For Lake Ontario, PFOS, PFOA, PFNA, PFHxS, and PFDA were not detected in 2015. No further testing for PFAS was conducted from 2016 to 2018. In 2019 and 2020, which is the most recent year for which the testing report has been published, PFOS, PFOA, and PFHxS were again tested for and all three were detected above the MRL in both years. With concentration decreasing from 2019 to 2020 by 29.2% for PFOS, 26.3% for PFOA, and 50% for PFHxS. PFNA and PFDA were only tested for in 2020 in Lake Ontario and neither were detected.

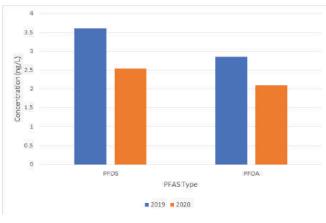


Figure 2) PFOS and PFOA Concentrations for Lake Ontario 2019 and 2020

(City of Rochester Bureau of Water, 2015, 2016, 2017, 2018, 2019, 2020a; Monroe County Water Authority, 2020; City of Rochester Bureau of Water, 2020b)

8. Analysis / Discussion

8.1 Municipal Water versus Private (well) Water Expo sure

The results obtained regarding the first research question (is there an association between maternal PFAS concentrations and their source of drinking water), specifically private well water compared to municipal (public) water sources, were contrary to what was hypothesized. It was theorized that mothers who used private well water as their source of drinking water would have higher concentrations of PFAS in their blood serum as compared to those who used municipal water. Private wells have several characteristics that potentially make them more susceptible to containing increased levels of PFAS (Guelfo & Adamson, 2018). It was found that for four of the five PFAS that were tested for, PFOS, PFOA, PFNA, and PFDA, that the estimated percent differences in exposure were lower for private well users than for those who used the municipal water supply. Therefore, it is estimated that pregnant women who drank municipal tap water were exposed to higher concentrations of PFAS compared to those who drank private well water.

When compared to previous research, these findings are not surprising. Small water systems and private wells are less likely to contain PFAS, but when they do it is often at significantly higher concentrations and includes multiple PFAS (Guelfo & Adamson, 2018). Therefore, it is probable that the ground in which most private wells in Rochester and Monroe County are dug is not contaminated with PFAS, and therefore neither is the water from most wells. Municipal water from Lake Ontario does contain PFAS as of 2020, but in low concentrations, agreeing with previous research (Guelfo & Adamson, 2018).

For PFOS, PFOA, and PFNA there was a small estimated percent difference between private and municipal water sources, with insignificant p-values for the 95 percent confidence intervals. There was no difference between water sources for PFHxS, which also had an insignificant p-value for its 95 percent confidence interval. Based on the insignificant p-values and slight differences in estimated percent difference it can be concluded that there is no significant difference in PFAS exposure for private versus public water sources.

PFOS and PFOA had a 11 and 7 percent lower difference, respectively, between well water sources which indicates that there may be a significant difference in exposure for those two PFAS between private and public water sources. The high p-values obtained for these two PFAS indicates that further research with a larger sample size should be pursued to clarify possible percent differences. The high p-values for all five tested PFAS points to the need for further research in this area. One of the five tested PFAS, PFDA, had by far the largest estimated percent difference.

PFDA was estimated to be 40 percent higher in the public water source than for private wells. This finding also had by far the lowest p-value, which was nearly significant for the 95 percent confidence interval. However, this is likely due to 22

of the samples testing for PFDA falling below the lowest detectable limit (LOD). These data points were therefore removed when the percent differences were calculated. Therefore, if these data points were added back into the calculation as undetected, the results for PDFA would be similar to those of the other four PFAS. These results reveal the difficulty in researching PFAS exposure as there are many possible confounding factors.

One possible confounder that was eliminated was parity. The parity adjusted estimated percent difference in PFAS exposure for private well water sources as compared to the public water source are nearly identical to the unadjusted exposures and corresponding p-values. Parity is a crucial possible cofounder to eliminate. Since these exposures are estimated from pregnant women's plasma, metabolic and fat distribution differences are key to accurately estimating PFAS exposure. Previous pregnancies that may have altered metabolic processes and fat distribution could cause large differences in PFAS levels in plasma even if exposure levels are the same. Eliminating parity as a possible cofounder increases the accuracy of the estimated exposures and is a strength of this study.

Apart from parity there are many other potential confounding factors that were not accounted for which is the largest weakness of this study. Most significantly, exposure to PFAS can come from a wide range of sources (PFAS Chemical Exposure | ATSDR, 2021). From different pieces of clothing to nonstick cookware, household and workplace dust, and any product that contains or has come in extended contact with a PFAS containing substance (US EPA, 2021). This wide range of exposures makes it difficult to precisely estimate exposure from one specific source because the levels of PFAS in subjects' water were not directly measured. Occupational exposure to PFAS, which can be a major route of exposure, was not accounted for either in the UPSIDE study (US EPA, 2021)(O'Connor et al., 2021). Additionally, based on the Rochester Water Bureau's testing history, individuals receiving their water from Hemlock Lake, for example, are likely exposed to different levels of PFAS than those who receive water from Lake Ontario and from those who receive a mixture of both sources for their municipal tap water (City of Rochester Bureau of Water, 2020b).

8.2 Hemlock Lake versus Lake Ontario PFAS Temporal Concentrations

Addressing the second research question, determining whether there are differences in PFAS concentrations between the municipal water sources of Hemlock Lake and Lake Ontario, proved difficult. The fact that the two water sources are governed by different municipalities and government agencies led to differences in what PFAS were tested for, how often, and how long ago testing for PFAS began (City of Rochester Bureau of Water, 2020). The most recent water quality report available for both was 2020.

No PFAS have been detected above the MRL in Hemlock Lake from 2015 to 2020, so it is unlikely that those who receive municipal tap water from Hemlock Lake are exposed to PFAS through their drinking water (City of Rochester Bureau of Water, 2015, 2016, 2017, 2018, 2019, 2020). This does not mean that no PFAS have ever been detected in Hemlock Lake, but rather that none have exceeded the MRL to date. From 2015 to 2020, 2016 was the only year for which no PFAS were detected in either lake. However, at levels below the current day MRL of 1.8 ng/L, exposure from this water would be tiny, and likely barely contribute to increases in blood serum concentration (Daly et al., 2018). It is difficult to track trends in PFNA and PFHxS concentration in Hemlock Lake at greater resolution because the Water Bureau tests for PFAS other than PFOS and PFOA every five years.

Since the data provided by the Rochester Water Bureau and Monroe County Water Authority measures water quality from samples collected at the entry point (City of Rochester Bureau of Water, 2020a), the municipal water provided by these agencies may become contaminated with PFAS during or after treatment. While possible, this is unlikely, and any small contamination the water may encounter along its journey would likely barely contribute to the overall concentrations of PFAS in the water. This small chance of contamination is primarily due to municipal water supplies being in almost constant flow, not contacting potentially contaminated objects or areas for long, and involving huge volumes of water (Guelfo & Adamson, 2018).

Those who receive their water from Lake Ontario are exposed to significant levels of PFAS, specifically PFOS, PFOA, and PFHxS (City of Rochester Bureau of Water, 2019; Daly et al., 2018; Monroe County Water Authority, 2020). A possible explanation as to why exposure estimates point to greater exposure from municipal water sources compared to private wells across Monroe County and Rochester is that many of the participants from UPSIDE receive their water from Lake Ontario (O'Connor et al., 2021). Additionally, it is unjust that some Rochester residents receive water that has PFAS contamination while others do not. Residents all pay the same rates no matter the source of their water and expect to receive the same quality of water.

For water quality data collected by the Monroe County Water Authority for Lake Ontario at their Shoremont pumping station, PFAS testing has not been consistent. There were PFAS detected in 2015 followed by no testing until 2019. The decrease in PFOS, PFOA, and PFHxS from 2019 to 2020 in Lake Ontario (Monroe County Water Authority, 2020) is a good reflection of the growth of the awareness of the harmful effects of PFAS in recent years and many PFAS being phased out of use. The drop of PFOS and PFOA is most likely due to ban on these two PFAS in 2000 (Kannan et al., 2004). The settling out of PFAS from the water is also a likely reason for decreasing concentrations. However, only two years of data greatly inhibits the ability to confirm any longer term and general trends.

There is little data available on surface water PFAS concentrations for Lake Ontario. The data that does exist is either over a decade old or is not a measure of the water itself but of fish in the lake, which is not relevant to this research since PFAS bioaccumulate and have different residence times in tissue compared to water.

The decrease in concentration for PFOS, PFOA, and PFHxS in Lake Ontario from 2019 to 2020 is a positive sign. These concentrations, however, are still significant enough to increase exposure enough to affect blood serum levels (Daly et al., 2018). Implementing filtration at the Shoremont plant would help to reduce these contaminants in the drinking water supply. Testing for PFAS must continue, and more PFAS should be tested for, especially those from the new Generation X family of PFAS, for which little is known, in both Hemlock and Ontario.

Most significantly, the analysis of the water quality data from Hemlock Lake and Lake Ontario points to the importance of the environment for drinking water sources (Scott, 2015). Hemlock Lake is 32 miles south of the closest large population center, Rochester. The Lake is also surrounded by forests with no industry around it. In contrast, all the Great Lakes drain into Ontario since their water moves toward the Atlantic Ocean, along with many other rivers and streams. Large population centers, such as Toronto and Rochester sit on or near the lake, dumping large amounts of contaminants into it. These factors make it essential to increase testing and consider implementing additional filtration, in Lake Ontario especially, as hundreds of thousands of people's drinking water comes from the lake (Monroe County Water Authority, 2020).

Other cities in upstate New York further point to the importance of a water source's surroundings. Syracuse detected no PFAS in its water when tested for in 2020 (Walsh & Awald, 2020). Syracuse sources its drinking water from Skaneateles Lake, a finger lake about 20 miles south of the city (City of Syracuse, 2009). This lake is also surrounded by forest with no industry near it. Albany receives its water from the Alcove Reservoir which is around 20 miles south-west of the city and surrounded by forest with no industry near it (Albany Dep. of Water, 2020). PFOS and PFOA were tested for in 2020, with possible detections falling well below the MRL (Albany Dep. of Water, 2020). The City of Buffalo gets its water from Lake Erie on which the city sits (Buffalo Water, 2020). Testing in 2020 showed low levels of PFOS and PFOA (Buffalo Water, 2020). Lake Erie is drained into by all the Great Lakes other than Lake Ontario. There are also several large population centers, Buffalo, Erie, Cleveland, and Toledo that sit directly on Lake Erie. The City of Detroit is located along water that directly drains into Lake Erie. All these water districts tested for PFAS for the first time in 2020, which is the most recent year data is available.

The comparison between Rochester, other cities, their water sources, and PFAS contamination further supports the importance of a water source's surroundings. Water sources that are surrounded by forests and nature and distant from a population or industrial center contain less contamination than water sources that have population or industrial centers are on their shores. Large bodies of water may also contain greater PFAS contamination due to the number of streams and rivers or other large bodies of water that feed into them. This makes tracing the source of PFAS difficult for these larger bodies of water. PFAS almost certainly enter Lake Ontario from many sources.

8. Conclusion

The ever-growing body of research points to the serious adverse health effects that PFAS present, and their ubiquity throughout the environment and household products, makes further research regarding these chemicals crucial. Pregnant women, their fetuses, and infants are especially vulnerable to the harmful effects of PFAS that can damage their health and impact their development. A large and consistent source of PFAS exposure is drinking water. PFAS present in drinking water have been shown to lead to increases in blood serum concentration of PFAS above average background levels of 4 ng/mL in the body (Daly et al., 2018). This means that PFAS levels in drinking water pose a serious threat to pregnant women and their children.

Using data from the UPSIDE study, it was found that in Monroe County, which includes the City of Rochester, that the estimated percent difference in PFAS exposure for PFOS, PFOA, PFNA and PFDA were lower in private well water sources than in the municipal water supply, while PFHxS concentrations were equal. The high p-values for the obtained values indicate the need for further research into PFAS exposure from different drinking water sources.

When compared, both Hemlock Lake and Lake Ontario, from which Rochester and Monroe County source their municipal drinking water, greatly differ in the concentration of PFAS found in each water source. Hemlock Lake did not have levels of PFAS above each year's MRL from 2015 to 2020. Lake Ontario had significant, but low, levels of PFOS, PFOA, and PFHxS for 2019 and 2020. PFDA and PFNA have not been detected in either source above the MRL. This difference in PFAS concentrations between the two sources means some residents of Rochester are receiving safer municipal drinking water than others. The PFAS present in Lake Ontario's water may help to explain why those who used municipal tap water were estimated to receive higher exposures than those who used private well water sources based on the data from UP-SIDE.

Comparing the characteristics of Hemlock Lake, which is surrounded by forests, and Lake Ontario, which has many industrial and population centers on its shores and water from many other sources draining into it, the importance of a water source's surroundings was highlighted. Looking at the water sources and PFAS concentrations of the municipal water sources for Syracuse, Albany, and Buffalo reinforced the importance of a water source's surroundings when it comes to PFAS concentrations. Water sources surrounded by a natural environment and more distant from population and industrial centers appear to have less PFAS contamination compared to water sources on which a population or industrial center sit, or that have many other bodies of water draining into them.

Further research into the impact of PFAS on human health,

especially on pregnant women and their infants, and on drinking water as a source of exposure is greatly needed. With so many PFAS in existence and new varieties frequently being made, research and testing frequency must also improve and increase. Additionally, municipalities must consider the surroundings of their water sources and what flows into them as these aspects appear important for the likelihood of municipal drinking water being contaminated with PFAS, and no doubt other pollutants as well.

References

Albany Dep. of Water. (2020). Annual Drinking Water Quality Report for 2020. DEPARTMENT OF WATER & WATER SUPPLY.

ATSDR. (2021). Toxicological Profile for Perfluoroalkyls. 993.

Banzhaf, S., Filipovic, M., Lewis, J., Sparrenbom, C. J., & Barthel, R. (2017). A review of contamination of surface-, ground-, and drinking water in Sweden by perfluoroalkyl and polyfluoroalkyl substances (PFASs). Ambio, 46(3), 335–346. https://doi.org/10.1007/s13280-016-0848-8

Boulanger, B., Peck, A. M., Schnoor, J. L., & Hornbuckle, K. C. (2005). Mass budget of perfluorooctane surfactants in Lake Ontario. Environmental Science & Technology, 39(1), 74–79.

Buck, R. C., Franklin, J., Berger, U., Conder, J. M., Cousins, I. T., de Voogt, P., Jensen, A. A., Kannan, K., Mabury, S. A., & van Leeuwen, S. P. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. Integrated Environmental Assessment and Management, 7(4), 513–541. https://doi.org/10.1002/ ieam.258

Buffalo Water. (2018). Annual Drinking Water Quality Report For Calendar Year 2018. Buffalo Water/Managed by Veolia NA, LLC.

Buffalo Water. (2019). Annual Drinking Water Quality Report For Calendar Year 2019. Buffalo Water/Managed by Veolia NA, LLC.

Buffalo Water. (2020). Annual Drinking Water Quality Report For Calendar Year 2020. Buffalo Water/Managed by Veolia NA, LLC.

Callan, A. C., Rotander, A., Thompson, K., Heyworth, J., Mueller, J. F., Odland, J. Ø., & Hinwood, A. L. (2016). Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. Science of The Total Environment, 569–570, 1107–1113. https://doi.org/10.1016/j. scitotenv.2016.06.177

City of Rochester Bureau of Water. (2015). 2015 Drinking Water Consumer Confidence Report (Supplemental Data). https://www.cityofrochester.gov/article.aspx? id=8589960095 City of Rochester Bureau of Water. (2016). 2016 Drinking Water Consumer Confidence Report (Supplemental Data). https://www.cityofrochester.gov/article.aspx?id=8589960095

City of Rochester Bureau of Water. (2017). 2017 Drinking Water Consumer Confidence Report (Supplemental Data). https://www.cityofrochester.gov/article.aspx?id=8589960095

City of Rochester Bureau of Water. (2018). 2018 Drinking Water Consumer Confidence Report (Supplemental Data). https://www.cityofrochester.gov/article.aspx?id=8589960095

City of Rochester Bureau of Water. (2019). 2019 Drinking Water Consumer Confidence Report (Supplemental Data). https://www.cityofrochester.gov/article.aspx?id=8589960095

City of Rochester Bureau of Water. (2020a). 2020 Drinking Water Consumer Confidence Report (Supplemental Data). https://www.cityofrochester.gov/article.aspx?id=8589960095

City of Rochester Bureau of Water. (2020b). 2020 Water Quality Report (p. 9). file:///Users/evanbushinsky/ OneDrive%20-%20University%20of%20Rochester/Downloads/WEB%20Water%20Quality%20Report%2020.pdf

City of Syracuse. (2009). http://www.syrgov.net/Water_FAQs.aspx

Daly, E., Chan, B., Talbot, E., Nassif, J., Bean, C., Cavallo, S., Metcalf, E., Simone, K., & Woolf, A. (2018, April 1). Per- and polyfluoroalkyl substance (PFAS) exposure assessment in a community exposed to contaminated drinking water, New Hampshire, 2015. https://www-clinicalkey-com.ezpminer. urmc.rochester.edu/#!/content/playContent/1-s2.0-S1438463917308040

Ding, N., Harlow, S. D., Randolph Jr, J. F., Loch-Caruso, R., & Park, S. K. (2020). Perfluoroalkyl and polyfluoroalkyl substances (PFAS) and their effects on the ovary. Human Reproduction Update, 26(5), 724–752. https://doi.org/10.1093/ humupd/dmaa018

Domingo, J. L., & Nadal, M. (2019). Human exposure to perand polyfluoroalkyl substances (PFAS) through drinking water: A review of the recent scientific literature. Environmental Research, 177, 108648. https://doi.org/10.1016/j.envres.2019.108648

Emmett, E. A., Shofer, F. S., Zhang, H., Freeman, D., Desai, C., & Shaw, L. M. (2006). Community Exposure to Perfluorooctanoate: Relationships Between Serum Concentrations and Exposure Sources. Journal of Occupational and Environmental Medicine, 48(8), 759–770.

Eurofins Scientific Analytical Report. (2022, January 20). J9216-1 UDS Level 1 Report Rev(2) Final Report.

Fenton, S. E., Ducatman, A., Boobis, A., DeWitt, J. C., Lau, C., Ng, C., Smith, J. S., & Roberts, S. M. (2021). Per- and Polyfluoroalkyl Substance Toxicity and Human Health Review: Current State of Knowledge and Strategies for Informing Future Research. Environmental Toxicology and Chemistry, 40(3), 606–630. https://doi.org/10.1002/etc.4890

Filipovic, M., Berger, U., & McLachlan, M. S. (2013). Mass Balance of Perfluoroalkyl Acids in the Baltic Sea. Environmental Science & Technology, 47(9), 4088–4095. https://doi. org/10.1021/es400174y

Guelfo, J. L., & Adamson, D. T. (2018). Evaluation of a national data set for insights into sources, composition, and concentrations of per- and polyfluoroalkyl substances (PFASs) in U.S. drinking water. Environmental Pollution, 236, 505–513. https://doi.org/10.1016/j.envpol.2018.01.066

Herrick, R. L., Buckholz, J., Biro, F. M., Calafat, A. M., Ye, X., Xie, C., & Pinney, S. M. (2017). Polyfluoroalkyl substance exposure in the Mid-Ohio River Valley, 1991–2012. Environmental Pollution, 228, 50–60. https://doi.org/10.1016/ j.envpol.2017.04.092

Hu, X. C., Tokranov, A. K., Liddie, J., Zhang, X., Grandjean, P., Hart, J. E., Laden, F., Sun, Q., Yeung, L. W. Y., & Sunderland, E. M. (2019). Tap Water Contributions to Plasma Concentrations of Poly- and Perfluoroalkyl Substances (PFAS) in a Nationwide Prospective Cohort of U.S. Women. Environmental Health Perspectives, 127(6), 067006. https://doi.org/ 10.1289/EHP4093

Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K. S., Loganathan, B. G., Mohd, M. A., Olivero, J., Wouwe, N. V., Yang, J. H., & Aldous, K. M. (2004). Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. Environmental Science & Technology, 38(17), 4489–4495. https://doi.org/10.1021/es0493446

Kato, K., Wong, L.-Y., Chen, A., Dunbar, C., Webster, G. M., Lanphear, B. P., & Calafat, A. M. (2014). Changes in serum concentrations of maternal poly- and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003-2006. Environmental Science & Technology, 48(16), 9600–9608. https://doi.org/10.1021/es-501811k

Monroe County Water Authority. (2020). 2020 Monroe County Water Authority Water Quality Report (p. 2). https:// mcwa-wordpress-media.s3.amazonaws.com/wp-content/ uploads/2020-MCWA-AWQR.pdf

Nakayama, S. F., Yoshikane, M., Onoda, Y., Nishihama, Y., Iwai-Shimada, M., Takagi, M., Kobayashi, Y., & Isobe, T. (2019). Worldwide trends in tracing poly- and perfluoroalkyl substances (PFAS) in the environment. TrAC Trends in Analytical Chemistry, 121, 115410. https://doi.org/10.1016/j. trac.2019.02.011

O'Connor, T., Best, M., Brunner, J., Ciesla, A. A., Cunning, A., Kapula, N., Kautz, A., Khoury, L., Macomber, A., Meng, Y., Miller, R. K., Murphy, H., Salafia, C. M., Vallejo Sefair, A., Serrano, J., Barrett, E., & UPSIDE study. (2021). Cohort profile: Understanding Pregnancy Signals and Infant Development (UPSIDE): a pregnancy cohort study on prenatal exposure mechanisms for child health. BMJ Open, 11(4), e044798. https://doi.org/10.1136/bmjopen-2020-044798 PFAS chemical exposure | ATSDR. (2021, November 19). https://www.atsdr.cdc.gov/pfas/health-effects/exposure.html

Post, G. B. (2021). Recent US State and Federal Drinking Water Guidelines for Per- and Polyfluoroalkyl Substances. Environmental Toxicology and Chemistry, 40(3), 550–563. https://doi.org/10.1002/etc.4863

Potential health effects of PFAS chemicals | ATSDR. (2020, June 24). https://www.atsdr.cdc.gov/pfas/health-effects/index .html

Rowley, D. (2022, April 4). PFAS Exposure Research [Personal communication].

Rowley, D. (2022, April 19). PFAS Exposure Research [Personal communication].

Scott, T. (2015). Does collaboration make any difference? Linking collaborative governance to environmental outcome. Journal of Policy Analysis and Management, 34(3), 537–566.

Shoemaker, J., & Tettenhorst, D. (2018). METHOD 537.1 DETERMINATION OF SELECTED PER- AND POLYFLUORINATED ALKYL SUBSTANCES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS). U.S. Environmental Protection Agency, Office of Research and Development, National Ceneter for Environmental Assessment, Washington, DC.

US EPA, O. (2021, October 14). Our Current Understanding of the Human Health and Environmental Risks of PFAS [Overviews and Factsheets]. https://www.epa.gov/pfas/ourcurrent-understanding-human-health-and-environmentalrisks-pfas

Walsh, B., & Awald, J. (2020). CITY OF SYRACUSE DE-PARTMENT OF WATER: Consumer Confidence Report 2020 Newsletter. 40.

Weaver, Y. M., Ehresman, D. J., Butenhoff, J. L., & Hagenbuch, B. (2010). Roles of rat renal organic anion transporters in transporting perfluorinated carboxylates with different chain lengths. Toxicological Sciences: An Official Journal of the Society of Toxicology, 113(2), 305–314. https://doi.org/ 10.1093/toxsci/kfp275

About the Author

I became involved in this research when I entered the honors program for my major. With PFAS being an important issue with global implications it was a unique and interesting opportunity to be able to study them in Rochester, the city I was living in at the time. Toxicology is also a fascinating area that I wanted to get more involved in and explore on my own. Undergrads should just find a way to get involved and go for it, working in a lab at URMC and writing my thesis were very rewarding.

Usefulness of the Reactive Strength Index (RSI) in Assessing Burnout in Division III College Athletes

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Abstract

This study aims to determine if reactive strength index (RSI) scores could be used to assess burnout in Division III college athletes. In accordance with previous research, it was hypothesized that low RSI scores would be associated with high Shirom-Melamed Burnout Measure (SMBM) scores in studentathletes, while high RSI scores would be associated with low SMBM scores in student-athletes. The sample of participants consisted of 100 male and female NCAA Division III studentathletes of the University of Rochester that were between the ages of 18 and 23. Pearson's correlation test was used to examine the statistical relationship between SMBM scores and RSI scores. The results obtained from Pearson's correlation test determined a weak negative correlation between the two variables, indicating that there was not a significant statistical relationship between SMBM scores and RSI scores. However, other statistical methods used in this study suggest that there is the possibility that this study was not able to accurately determine the statistical relationship between RSI scores and SMBM scores. Hence, the study concluded that more research is needed to determine the statistical relationship between RSI scores and SMBM scores.

Keywords: athlete burnout, reactive strength index, Shirom-Melamed burnout measure, drop vertical jump

Introduction

Adolescents, notably, engage in organized sports during their years of formal education. In the United States, around 40,000 students participate in athletic programs yearly (Sorkkila et al., 2020). That said, combining athletics and academics has been proven to be a stressful task that has drawn the attention of international sport psychologists and government entities. The European Commission, for instance, has stressed the need to promote sport development more responsibly. The simultaneous pursuit of sports and academics has been suggested to put student-athletes at risk for both sport and school burnout (Sorkkila et al., 2020). Sport burnout, in particular, has received a lot of attention in the past three decades. In athletes, high burnout symptoms have been associated with decreased performance, reduced motivation, and leaving the sport. Because of the negative effects burnout has on athletes, an indepth understanding of this issue is crucial for coaches, managers, psychologists, and sport organizations (Gerber et al., 2018a).

In the sports literature, burnout has several definitions. However, researchers have reached a consensus that athlete burnout is a "multidimensional syndrome" (Gerber et al.,

2018b, p. 313). Experts have developed multiple methods to assess burnout in athletes. In particular, the Athlete Burnout Questionnaire (ABQ) has become the preferred method to assess athlete burnout symptoms (Gerber et al., 2018a). It is based on the definition that considers burnout a syndrome that consists of three dimensions: emotional and physical exhaustion, sport devaluation, and reduced sense of accomplishment. Although there is evidence for ABQ's reliability and validity, researchers have found several limitations as well. First, the definition of burnout on which ABO is based has not been supported by clinical observation nor theory. Likewise, ABQ's dimensions overlap considerably with other psychological constructs – for example, sense of accomplishment with self-efficacy (Gerber et al., 2018a). Gerber et al. (2018a) examined the psychometric properties of the Athlete Burnout Questionnaire (ABQ), and its usefulness in clinically detecting burnout symptoms. Researchers concluded that even though the factor structure and internal consistency of the ABQ is supported, issues with its psychometric properties and validity suggest that there are more suitable ways to assess burnout.

Initially, burnout started as a work-related syndrome that resulted from experiencing chronic emotion and interpersonal stress at work (Gerber et al., 2018c). This "burnout syndrome" was extensively studied by Shirom (1989) who identified burnout as "the chronic depletion of an individual's energetic resources" (p. 26). Shirom's definition is based on Hobfoll's conservation of resources theory (COR) (Gerber et al., 2018c). COR theory states that people are motivated to "obtain, retain, and protect what they value" (Shirom, 2003, p. 250). Based on this, Shirom conceptualized burnout as relating to energetic assets and physical, emotional, and cognitive energies. Burnout, according to Shirom (2003), consists of three dimensions: physical fatigue, emotional exhaustion, and cognitive weariness. Besides having basis in theory, Shirom's conceptualization of burnout does not overlap with other psychological constructs, such as affective dysfunction or a temporary state of fatigue (Melamed et al., 1992). Based on Shirom's conceptualization of burnout, the Shirom-Melamed Burnout Measure (SMBM) questionnaire was created. The SMBM has been accepted internationally, and its validated cutoff score allows researchers to assess pertinent symptoms of burnout according to the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) criteria for reactions to severe stress (Gerber et al., 2018b). Likewise, the Shirom-Melamed Burnout Measure has been shown to have satisfactory convergent, discriminant and factorial validity in young people from school to working life (Gerber et al, 2018c).

Among its many negative effects, burnout has been linked to musculoskeletal injuries in people that do physically, mentally, and emotionally demanding activities that put considerable stress on the musculoskeletal system, such as firefighters (Vaulerin et al., 2015). Similarly, Ahola et al. (2013) found that each one-unit increase in a burnout score measured by the Maslach Burnout Inventory-General Survey increased by 9% the risk of injury in Finnish forest industry workers. There are several explanations for what leads to risk of injury. For instance, physiological arousal increases muscular tension and diminishes the coordination of movement, which may lead to injuries (Nideffer, 1983). Moreover, Andersen and Williams (1988) found that disruption of attentional processes and concentration narrows the peripheral vision and leads to less vigilance to signs of physical danger.

Since musculoskeletal injuries are common in people that do physically demanding activities, sports medicine professionals and coaches usually carry out functional performance tests (FPT) during offseason and preseason periods to determine which athletes are at greater risk of injury. The drop vertical jump (DVJ) test is an FPT proven to be promising in identifying athletes that may be at risk for initial or subsequent anterior cruciate ligament (ACL) sprains. The DVJ test involves making the athlete drop off a box, land on force plates, and jump vertically (Brumitt et al., 2020). Reactive strength index (RSI) scores are obtained from DVJ tests by dividing jump height by contact time on the ground. RSI scores are used to quantify athletes' lower extremity power, and their ability to transition from an eccentric muscular contraction to a concentric muscular contraction. It has been suggested that low RSI scores indicate that an athlete is not adequately trained for the physical demands of a sport (Brumitt et al., 2020). Furthermore, Brumitt et al. (2020) found that female volleyball players with RSI scores of 0.9125 m/s or less were 4 times more likely to be injured.

Understanding sport burnout in student-athletes is important for coaches, managers, sport organizations, and athletes themselves. The Shirom-Melamed Burnout Measure (SMBM) has been proven to be a more adequate method to assess burnout in athletes than the Athlete Burnout Questionnaire (ABQ). In a similar manner, reactive strength index (RSI) scores have been proven to be promising in assessing athletes at risk of injury. Taking into consideration that RSI scores and burnout symptoms are both associated with an athlete's risk of injury and that there is a need for better methods to assess burnout other than ABQ, the current study aimed to examine the statistical relationship between SMBM scores and RSI scores in student-athletes to determine if burnout can be assessed using RSI scores obtained from a just jump mat. Based on previous research that has suggested that low RSI scores are associated with higher risk of injury, it was theorized that low RSI scores would be associated with high SMBM scores in student-athletes, while high RSI scores would be associated with low SMBM scores in student-athletes.

Methods Participants

The sample of participants consisted of 100 male and female NCAA Division III student-athletes of the University of Rochester that were between the ages of 18 and 23. The study included 20 men's basketball players, 20 women's basketball players, 25 softball players, and 35 women's soccer players. Women's soccer and softball athletes were tested halfway through their season while men's and women's basketball athletes were tested in their off-season. Athletes were tested in the University of Rochester Varsity Athlete fitness center located at the University of Rochester. This study was conducted in collaboration with the University of Rochester Medical Center (URMC) Fitness Science team, directly working with two performance conditioning coaches. The assessments were part of the regularly scheduled programming that their athletic teams take part in on a consistent basis. The Institutional Review Board at the University of Rochester approved this study. Primary data was provided in the form of unidentifiable data with only access to the individual's identification number, which coincided with their sport and their RSI scores. The URMC Fitness Science team documented all participants' Just Jump Mat scores. The RSI scores were calculated by the URMC Fitness Science team. The measurements of the Shirom-Melamed Burnout Measure were presented to participants by the URMC Fitness Science team, and the results were accessed by the URMC Fitness Science team.

Procedure

Before testing, each athlete completed a three-section, tenminute dynamic warm-up and the Shirom-Melamed Burnout Measure Questionnaire. The questionnaire was distributed to the athletes using a QR code, which allowed them to scan and complete the questionnaire using their phones before their session. The first section consisted of a mobility warm-up which involved the athlete performing movements in the fitness center: 90/90 hip stretches, heel sits, toe sits, adductor rocks, spiderman stretches, and toe touch squats. The second section was the activation warm-up: shoulder glides, hip lifts (glute bridges), single-leg hip lifts and leg lowers. The third section was a linear dynamic warm-up that consists of several exercises: leg cradles, reaching quad stretches, backwards lunges with hamstring stretches, backward straight leg deadlift walks, A skips, power skips, high knees to sprint, heel ups to sprint, small step backpedals and long step backpedals. All warm-ups took place in the same facility as testing took place. Then, the rubber Just Jump Mat was placed in front of the 30.48 cm (12 inch) foam box that the participants used. Each athlete was instructed to complete two practice test jumps to aid in their ability to complete the drop jumps properly while testing. The URMC Fitness Science team demonstrated the drop jump to each participant and provided test performance instructions. Athletes were instructed to stand on top of the foam box with feet shoulder-width apart then told to place one foot over the just jump mat, elevating it in the air. They were then instructed to drop off the box, land on the rubber mat, and then jump as fast as they could off the mat. Participants were allowed to use their arms in this jump and move them as desired for optimal performance. Each athlete completed three successful jumps from the 30.48 cm (12 inch) box, and each RSI score was populated to the reader placed in the hand of a URMC Fitness Science team member. Every RSI score was recorded in the spreadsheet under each participant's ID. The height of the box used was the height that is often used in traditional drop vertical jump (DVJ) tests. This height can discriminate anterior cruciate ligament (ACL) injury risk (Brumitt et al., 2020). Once all DVJ trials were completed an average of the RSI scores for each athlete was calculated and used for statistical analysis. SMBM and RSI scores were collected once for every athlete throughout two weeks.

Experimental Design

The research study used a cross-sectional design to assess the statistical relationship between RSI scores and SMBM scores at a particular period of time. Participants' burnout symptoms were assessed using the 14-item Shirom-Melamed Burnout Measure (SMBM) questionnaire. Items were answered on a 7-point Likert scale ranging from 1 (never or almost never) to 7 (always or almost always). SMBM consists of three subscales: physical fatigue, cognitive weariness, and emotional exhaustion. However, because the original SMBM was made to assess adults, a modified version suitable to assess young people from school to working life was used. This version was obtained from Gerber et al. (2018c) research study (see Appendix A). To administer the questionnaire, a Google Form containing the questions was created. A QR code of the form was generated so participants could scan the code and answer the questionnaire using their phones. Accordingly with the work of Lundgren-Nilsson et al. (2012), a score of 4.40 on the Shirom-Melamed Burnout Questionnaire was considered a clinically relevant level of burnout. To determine SMBM scores, the mean score across the 14 items was calculated. Additionally, the SMBM scores of each of the three dimensions were obtained by calculating the mean score of the questions associated with each dimension: physical fatigue (question 1 to 6), cognitive weariness (question 7 to 11), and emotional exhaustion (question 12 to 14). Likewise, the reactive strength index (RSI) scores were calculated from the drop vertical jump (DVJ) tests athletes performed on a Just Jump mat. The reactive strength index (RSI) scores were calculated by dividing jump height over contact time on the ground.

Results

After calculating the athletes' burnout scores, five participants were determined to be clinically burnout according to the cutoff score of 4.40 on the Shirom-Melamed Burnout Measure (SMBM). Four of these participants were female basketball players, and one participant was a female softball player. Further statistical analysis was carried out on these participants to determine if there was a significant statistical relationship between burnout and RSI scores.
 Table 1) Raw data of SMBM scores and RSI scores of burnout participants

Sport	Average RSI (m/s)	Average SMBM Score
	0.93	4.93
Female	1.43	4.42
Basketball	0.67	4.71
	1.37	4 .71
Female Softball	1.63	4.93

Descriptive

First, Cronbach's alpha, a measure of reliability or internal consistency, was calculated for the SMBM scores. The Cronbach's alpha of the SMBM scores was 0.87, which indicates that there is a good internal consistency in the Likert scale used in this study. In other words, the items in the test are considerably correlated. In this study, the coefficient of variance — a measurement of the reliability of the RSI scores — was 3.8%, which indicates reliable measurements (Brumitt et al., 2020).

Then, the mean () and standard deviation (SD) of the RSI scores of non-burnout participants and burnout participants were calculated. The average RSI score of non-burnout participants was 1.28 with a standard deviation of 0.41. Likewise, the average score of burnout participants was 1.21 with a standard deviation of 0.39. Therefore, it can be stated that the average RSI score of burnout participants was lower than the average RSI score of non-burnout participants. In addition, a relatively small standard deviation (SD = 0.39) for burnout participants indicates that the results of these participants were less dispersed from the mean than the RSI scores of non-burnout participants.

Inferential

Since the data collected was continuous, Pearson's Correlation test was used to determine whether there was a relationship between athletes' average scores from the Shirom-Melamed Burnout Measure (SMBM) and their Reaction Strength Index (RSI) scores. Pearson's test score was -0.23, which indicates a weak decreasing correlation between athletes' SMBM scores and their average RSI scores.

In addition, linear regressions were used to further explore the statistical relationship between RSI scores and SMBM scores. For example, from 6 (very frequently) to 7 (always/almost always) — given that all other variables were kept constant – there was an average decrease of 0.116 in athlete's RSI scores. Further linear regressions were carried out on each of the three dimensions of Shirom-Melamed Burnout Measure: physical fatigue, cognitive weariness, and emotional exhaustion (see

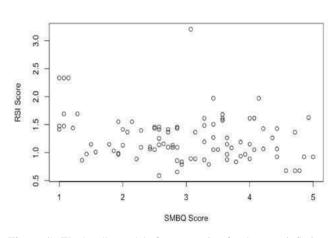


Figure 1) The hurdle model after correcting for the over-inflation of number of zeros in the data.

Appendix B). In the physical fatigue domain, two measures were statistically significant. Athletes who reported lack of energy in the morning were more likely to have higher RSI scores compared to those who reported high energy in the morning. With one level increase in the fed-up question, there was an average decrease of 0.061 in athletes' RSI scores. In general, athletes who reported to be fed up were more likely to have lower RSI scores. In terms of cognitive weariness, there were not any statistically significant correlations. However, in the emotional exhaustion dimension, athletes who reported feeling incapable of investing emotionally in other people were associated with lower RSI scores. With one level increase in the emotional exhaustion dimension, given that all other variables were kept constant, there was a decrease of 0.098 in athletes' RSI scores.

Discussion

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The aim of the study was to determine if RSI scores could be used to assess burnout in Division III college athletes. To do so, Pearson's correlation test was used to examine the statistical relationship between Shirom-Melamed Burnout Measure (SMBM) scores and reactive strength index (RSI) scores. In accordance with previous research that suggested that low RSI scores are associated with a higher risk of injury, it was hypothesized that low RSI scores would be associated with high SMBM scores in student-athletes, while high RSI scores would be associated with low SMBM scores in student-athletes. The results obtained from Pearson's correlation test determined a weak negative correlation, which indicates that there is not a significant statistical relationship between SMBM scores and RSI scores. Although the main inferential statistical test used did not find a significant statistical relationship, the linear regressions that were carried out determined that with one level increase in SMBM – for example, from 6 (very frequently) to 7 (always/almost always) - there was an average decrease of 0.116 in athlete's RSI scores. Furthermore, linear regressions that were carried out on each of the three dimensions of Shirom-Melamed Burnout Measure (SMBM) - physical fatigue, cognitive weariness, and emotional exhaustion - showed that there were some significant

statistical relationships between some of SMBM's dimensions and RSI scores. Likewise, the descriptive statistical methods used to analyze the data indicated that the mean RSI score of the burnout participants was considerably lower than the mean RSI score of non-burnout participants. Based on the results of the descriptive statistical methods used, there could be a statistical relationship between SMBM scores and RSI scores that this study was not able to determine.

Since this research study used questionnaires to assess burnout, there are certain limitations that might have influenced the data collection. Questionnaires are self-reported data. Although questionnaires are a quick and easy way to collect data, they are prone to response biases. Social desirability bias could have influenced the participants' responses. Participants might have inflated their positive traits and underreported their negative traits. Another limitation that the study encountered was the height of the boxes. Brumitt et al. (2020) carried out a similar study where they examined the ability of preseason RSI scores to determine athletes at risk of injury. However, besides the 30.48 cm (12 inches) box, they also used a 60.96 cm (24 inches) and a 76.20 cm (30 inches) box. These box heights were used because they represented the jump heights that college athletes perform in their specific sport activities. The 60.96 cm (24 inches) represented blocking during volleyball, and the 76.20 (30 inches) box represented rebounding in basketball. Using boxes with heights that resemble the jumps athletes perform in their respective sports could increase the ecological validity of the study, which is the level to which the results obtained in a laboratory setting can be generalized to real-life situations. Moreover, a confounding variable that might have affected the results is that some teams were in season while others were off-season. In Brumitt et al. (2020), it was stated that training habits at different points of a season can affect RSI scores. Usually, off-season training differs from in-season training. This could have lowered the RSI scores of some athletes considering that athletes that are not adequately trained for the physical demands of their sport tend to get low RSI scores. In this study, four of the five burnout participants were in the women's basketball team that was off-season.

Even though the aim of the current study and Brumitt et al. (2020) is different, the mentioned study also examined RSI scores. Their participants consisted of male basketball players and female volleyball players. The results showed that there was no association between preseason RSI scores and injury in male collegiate basketball players, but there was a significant association between preseason RSI scores and injury in female volleyball players. In a similar manner, out of 100 male and female NCAA Division III student-athletes that participated in the current study, only female participants showed significant statistical relationships between some of the SMBM questions and RSI scores. This might suggest that RSI scores could be better predictors of burnout in female athletes than in male athletes. Further research is needed to determine if RSI scores are better predictors of burnout in female athletes than male athletes.

If the study were to be carried out again, it would be insightful to collect more demographic information about the participants, such as height, weight, body mass, and body mass index. Likewise, in-season and off-season teams should be studied separately. In addition, using boxes with heights that resemble the jumps athletes do in their respective sports could also provide further insight on this topic. Due to data collection issues, the design of this study was cross-sectional, meaning that the data collected from the athletes was from a single point in time. Carrying out this study with a longitudinal design could provide greater insight and a deeper understanding of the matter. Besides collecting SMBM scores and RSI scores, it is recommended to also collect injury incidence. Particularly, because there is the need to identify a cutoff RSI score associated with increased risk of injury (Brumitt et al., 2020).

In summary, this study concludes that there exists a weak negative correlation between RSI scores and SMBM scores, meaning that burnout cannot be assessed using RSI scores according to the main statistical test used. However, other statistical methods in this study suggest that there is the possibility that this study was not able to accurately determine the statistical relationship between RSI scores and SMBM scores. Therefore, more research is needed to determine the statistical relationship between these two variables.

References

Ahola, K, Salminen, S., Toppinen-Tanner, S., Koskinen, A., & Väänänen, A. (2013). Occupational burnout and severe injuries: An eight-year prospective cohort study among Finnish forest industry workers. Journal of Occupational Health, 55(1), 450-457. https://doi.org/10.1539/joh.13-0021-oa

Andersen, M. B., & Williams, J. M. (1988). A model of stress and athletic injury: Prediction and prevention. Journal of Sport & Exercise Psychology, 10(3), 294–306. https://doi.org/ 10.1123/jsep.10.3.294

Brumitt, J., Dorociack, R., Dunn, S., Critchfield, C., Benner, J., & Cuddeford, T. (2020). Lower preseason reactive strength index scores are associated with injury in female collegiate volleyball players but not male collegiate basketball players. Journal of Science and Medicine in Sport, 24(6), 549-554. https://doi.org/10.1016/j.jsams.2020.11.018

Gerber, M., Gustafsson, H., Seelig, H., Kellmann, M., Ludyga, S., Colledge, Brand, S., Isoard-Gautheur, S., & Bianchi, R. (2018a). Usefulness of the Athlete Burnout Questionnaire (ABQ) as a screening tool for the detection of clinically relevant burnout symptoms among young elite athletes. Psychology of Sport & Exercise, 39 (1), 104-113. https://doi. org/10.1016/j.psychsport.2018.08.005

Gerber, M., Best, S., Meerstetter, F., Isoard-Gautheur, S., Gustafsson, H., Bianchi, R., Madigan, D. J., Colledge, F., Ludyga, S., Holsboer-Trachsler, E., & Brand, S. (2018b). Cross-Sectional and longitudinal associations between athlete burnout, insomnia, and polysomnographic indices in young elite athletes. Journal of Sport and Exercise Psychology, 40(1), 312-324. https://doi.org/10.1123/jsep.2018-0083

Gerber, M., Colledge, F., Mücke, M., Schilling, R., Brand, S., & Ludyga, S. (2018c). Psychometric properties of the Shirom-Melamed Burnout Measure (SMBM) among adolescents: results from three cross-sectional studies. BMC Psychiatry, 18 (266), 1-13. https://doi.org/10.1186/s12888-018-1841-5

Lundgren-Nilsson, Å., Jonsdottir, I. H., Pallant, J., & Ahlborg Jr, G. (2012). Internal construct validity of the Shirom-Melamed Burnout Questionnaire (SMBQ). BMC Public Health, 12(1), 1-8. https://doi.org/10.1186/1471-2458-12-1

Melamed, S., Kushnir, T., & Shirom, A. (1992). Burnout and risk factors for cardiovascular diseases. Behavioral Medicine, 18(2), 53-60. http://dx.doi.org/ 10.1080/08964289.1992.9935172

Nideffer, R. M. (1983). The injured athlete: Psychological factors in treatment. Orthopedic Clinics of North America, 14(2), 373-385. https://doi.org/10.1016/S0030-5898(20)31354-7

Shirom, A. (2003). Job-related burnout: A review. In J. C. Quick, & L. E. Tetrick (Eds.), Handbook of occupational health psychology; Handbook of occupational health psychology (pp. 245-264, Chapter xvii, 475 Pages). American Psychological Association, Washington, DC. http://dx.doi.org/ 10.1037/10474-012

Vaulerin, J., d'Arripe-Longueville, F., Emile, M., & Colson, S. S. (2016). Physical exercise and burnout facets predict injuries in a population-based sample of French career firefighters. Applied Ergonomics, 54(1), 131-135. https://doi.org/ 10.1016/j.apergo.2015.12.007

About the Author

I've been interested in Sport Psychology for a while, but I never had a good reason to dive into the field. While I was taking a psychology class, the opportunity to work with the Fitness Center came up. I thought it was the perfect excuse to learn more about the field. What interested me the most about the topic was the idea of bringing to life plain numbers. Once I paired the measurements from the Fitness Center with Sport Psychology, suddenly there was something to study. The advice I'd like to give to others is give yourself the chance to explore something that you're randomly interested about.

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Appendix A

Additional file 1 Shirom-Melamed Burnout Measure – German Adolescent Version

ge (E	enn Du an den letzten Monat denkst, wie hast Du Dich dann fühlt? nglish: Please indicate how often, in the past 30 days, you ve felt each of the following feelings.)	Nie/fast nie (never/ almost never)	Sehr selten (very infre= guently)	Ziemlich selten (auite infre, auently)	Manch- mal (some- times)	Ziem- lich oft (auite, fre: auentix)	Sehr oft (verx fre- guently)	Immer/ fast immer (always/ almost always)
1.	Ich fühlte mich müde. (English: I feel tired.)							
2.	Ich hatte keine Energie, um morgens zur Schule zu gehen. (English: I have no energy for going to school in the morning.)							
3.	Ich fühlte mich körperlich völlig ausgelaugt. (English: I feel physically drained.)							
4.	Ich hatte die Nase voll. (English: I feel fed up.)							
5.	Ich hatte das Gefühl, dass meine Batterien leer sind. (English: I feel like my "batteries" are "dead".)							
6.	Ich fühlte mich ausgebrannt. (English: I feel burged out.)							
7.	Mein Denken war verlangsamt. (English: <u>My thinking process is slow.</u>)							
8.	Ich hatte Schwierigkeiten, mich zu konzentrieren. (English: I have difficulty concentrating.)							
9.	Ich hatte das Gefühl, nicht klar zu denken. (English: I feel I am not thinking clearly.)							
10.	Ich hatte das Gefühl, beim Denken nicht bei der Sache zu sein. (English: I feel I am not focused in my thinking.)							
11.	Ich hatte Schwierigkeiten, über komplexe Dinge nachzudenken. (English: I have difficulty thinking about complex things.)							
12.	Ich fühlte mich nicht in der Lage, mich auf die Bedürfnisse von anderen Menschen einzustellen. (English: I feel I am unable to be sensitive to the needs of other people.)							
13.	Ich fühlte mich nicht in der Lage, emotional auf andere Menschen einzugehen. (English: I feel I am not capable of investing emotionally in other people.)							
14.	Ich fühlte mich nicht in der Lage, mich in andere Menschen hineinzuversetzen. (English: I feel I am not capable of being sympathetic to other people.)							

Appendix B

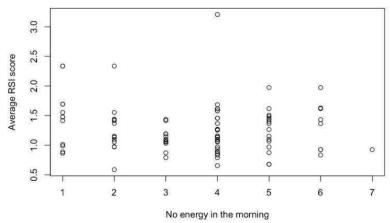


Figure 2) Linear Regression of average RSI scores and scores in question 2 of Shirom-Melamed Burnout Questionnaire

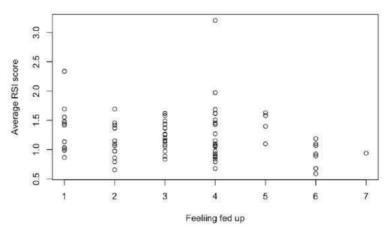


Figure 3) Linear regression of average RSI scores and scores in question 4 of Shirom-Melamed Burnout Questionnaire

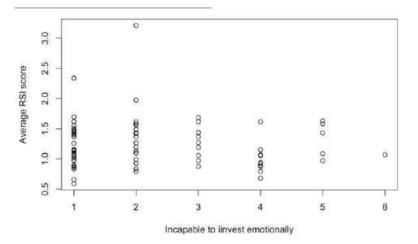


Figure 4) Linear regression of average RSI scores and scores in question 13 of Shirom-Melamed Burnout Questionnaire

p s y c h o l o g y

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Mauritian Creole (Kreol) Kinship Terms of Reference

Jillian Breithaupt '22, Linguistics Advised by Nadine Grimm, Department of Linguistics

Genealogical Referent (GR) Abbreviation Key

F	Father
Μ	Mother
Р	Parent
В	Brother
Z	Sister
С	Cousin
S	Son
D	Daughter
Η	Husband
W	Wife
s-	Step
h-	Half
i-	In-law
0	Older
у	Younger
m	Male
f	Female

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1. Introduction to Kinship Studies

Everyone is born into a family and oftentimes, kinship systems are the first social group that one belongs to. However, kinship systems are much more complex than just a list of who is related to you by blood. Even who is considered to be 'family' or what a 'family unit' is varies between cultures. For example, the 'family unit' of the Na of Yongning County, China is a brother-sister pair who raise the children of a 'genitor' (he lives with his sister, raising her children) who is often unknown to the children (Dousset, 2012). On the other hand, the 'family unit' in the United States is a non-blood-related (often married, but not always) pair who raise their own children, either biological or adopted. This structure also varies depending on one's background and circumstances. In addition to influencing the role people play in child-rearing, kinship systems are also "an important vehicle of social structure, behaviour, and moral order" (Dousset, 2012, p. 210). Kinship relationships dictate things such as inheritance, behavioral rules, and marriage practices and thus are important to study due to the role they play in society.

Linguistically, kinship terms are of interest because they can help us understand the underlying kinship structures, which have social implications. Each language has its own terms for various family relations, but the cultures themselves also have different kinship systems. This prompts the question of what happens to kinship terms and systems when many languages and cultures come into contact, as in the case of Creole languages? Creole languages are those that have "arisen from a language contact situation where speakers of a multitude of languages had to acquire a Means of Interethnic Communication" (Bakker & Matras, 2013, p. 65). Generally, Creoles tend to have a single lexifier language with contributions from several substrate or adstrate languages (Bakker & Matras, 2013). In these situations wherein the contact between languages has led to the formation of a new language, it is interesting to see what is kept and lost from the contributing languages and cultures.

A particularly intriguing language to look at in this context is Mauritian Creole (referred to as 'Kreol' in this paper). Kreol is a French-lexified Creole and is the majority language spoken on the island of Mauritius. Its substrate languages include English, Malagasy, Wolof, and some Bantu languages (Mufwene, 2008). In addition to this, speakers of the language were also in contact with speakers of Hindi and Bhojpuri through Indian traders and speakers of Hakka and Mandarin through merchants from China and Hong Kong (Baker, 1969). These heritage languages continue to be spoken in Mauritius and students often choose to study their heritage language, in school. Mauritians are very aware of their family backgrounds and this impacts their language. In the words of one of my language consultants, Raazi: "In Mauritius, according to your background, it will differ how you call your family members." This is corroborated by the data collected from the four speakers consulted for this project which support my claim that kinship terms serve as a salient marker of ethnic and religious identity in Mauritius.

Section 2 of this paper will provide a brief introduction to Kreol. Subsection 2.1 will detail the language situation in Mauritius including the history and development of Kreol, the other languages spoken on the island, and the special place that French and English hold in society. Section 3 will explain the methodology used for this project including how the recordings and annotations were done in subsection 3.1, and information about the language consultants in subsection 3.2. Within subsection 3.2, there are additional subsections for each speaker. Section 4 will present the kinship systems of interest to the project and introduce the kinship systems of the language consultants. Subsection 4.1 will discuss how there is not necessarily one "standard" system in Kreol and show the similarities between the systems used by different speakers.

Subsection 4.2 will focus on Amelie's kinship system which shows the most influence from French. Subsection 4.3 is about Lin and the kinship system of her heritage language, Hakka. Subsection 4.4 will look at Arnav's kinship system which shows a lot of Hindi influence. Subsection 4.5 highlights speaker Raazi's mixed kinship system that is a result of his mixed background. Finally, Section 5 will discuss the conclusions from the findings of this project.

2. Language Introduction

Mauritian Creole (ISO 639-3: mfe), referred to as Kreol throughout this paper, is a French-lexified Creole spoken mainly on the island of Mauritius located off the eastern coast of Madagascar. The language has approximately 1.03 million speakers including about 976 thousand in Mauritius and another 54 thousand between Australia, France, Madagascar, South Africa, and the United Kingdom (Eberhard et al., 2021). Although its lexifier language is French, Kreol was also heavily influenced by the other languages spoken on the island including English, Malagasy, Wolof, and some Bantu languages (Mufwene, 2008). Additionally, the language has been influenced by contact with other languages brought to the island by various waves of immigration.

2.1 Language Situation in Mauritius

The island of Mauritius was uninhabited until the Dutch made the first attempts to colonize it in 1598, naming it after Prince Maurice of Nassau (Baker, 1969). They brought slaves to the island and ended up leaving some when they withdrew in 1710 after failing to establish sufficiently profitable settlements (Baker, 1969). Five years later, the French East India Company gained control of the island renaming it le de France and bringing with them French nationals, African slaves, and Indian traders (Baker, 1969). The last colonial power to control the island was the British, who introduced English as the language of governmental affairs, but allowed for the continued use of French and the influence of French culture and the Roman Catholic Church (Baker, 1969). After the British abolished slavery in 1833, there was a labor shortage, so large numbers of Indian indentured servants were brought to the island (Baker, 1969). Later, there was a final large wave of immigration of merchants from Hong Kong and mainland China (Baker, 1969).

Each wave of immigrants brought with them their languages and cultures which gave Mauritius the multiethnic landscape that persists to this day. This multitude of languages prompted the need for a lingua franca for the island which led to the development of Kreol. Today, Kreol is the dominant language of the island, but French and English hold important places in education and society. Most written school materials are in English, although the language of instruction is Kreol. Furthermore, French holds a place of prestige and tends to be used in more formal settings. My consultants noted that they often speak a mixture of the three languages and Lin commented that "in a sentence we can use Kreol, English, and French." Arnav also remarked that people will often shift their Kreol toward French in formal situations and away from French in informal situations.

In addition to Kreol, French, and English, there are a few other heritage languages spoken by different ethnic groups. Mauritians have the option to learn these heritage languages (mainly Hindi, Arabic, Mandarin, and Urdu) in school and most choose which language to learn based on their cultural background. Each of the four consultants I worked with studied a different language in school, but they all had the same reasoning: their ethnic background. Therefore, it is clear that these heritage languages, while not necessarily spoken fluently or in the home, serve as markers of ethnic identity. Since these heritage languages-which were in contact with Kreol during its development-are still spoken alongside Kreol, it is hard to differentiate between lexical items that are considered part of Kreol because they were incorporated during its development and those that were later borrowed into the language. For the sake of this project, all of the kinship terms that the language consultants use (with the exception of the English terms used by Raazi for step, half, and in-law relations) will be considered Kreol lexical items that were previously borrowed into the language and have become part of it rather than lexical items that are actively being borrowed in. This decision was made because the Kreol dictionary that I had access to (the LALIT Kreol dictionary²) included most of the kinship terms that the speakers used in it.

3.Methodology

3.1 Recordings and Annotations

Data were collected over the course of four months spanning September 2021 to December 2021. All recordings with Arnav and Amelie were done in the Lattimore Student Workspace of the Linguistics Department at the University of Rochester. Recordings with Lin and Raazi were conducted through Zoom since Lin was in the United Kingdom and Raazi was in Canada. In total, 6 hours 7 minutes and 34 seconds of recordings were collected across the five tasks. The exact amount of data collected from each speaker is shown in Figure 1 below.

Speaker	Arnav	Amelie	Lin	Raazi
Audio Data	1:02:13	38:50	43:21	57:46
Video Data	34:46	26:02	43:31	1:00:55
Total	1:36:59	1:04:52	1:27:02	1:58:41

Figure 1. Amount of Data Collected

For Arnav and Amelie, recordings were done using a H4n Pro Zoom recorder and Panasonic GX85 camera for audio and visual recordings respectively. For Lin and Raazi, audio data was recorded using the Voice Memos app on an iPhone and the video data was recorded using the Screen Record feature on Zoom 5.76. Annotations were completed using ELAN 6.2 and Praat 6.1.03. Additionally, the long video recordings of speakers Lin and Raazi were spliced into separate videos for each task using iMovie 10.0.5. The long audio recording of Raazi was spliced into separate files for each task using Audacity 3.1.2.

3.2 Language Consultants

This project included data from four young-adult (20-22 years-old) Kreol speakers who were born and raised in Mauritius. The speakers have been given pseudonyms to keep their identities anonymous. Each speaker is from a different religious/ ethnic background in an attempt to reflect the diversity of the country itself. The four ethnic/religious categories came out of discussions with the main consultant, Arnav. During the previous spring semester when working with him, he would often divide groups in Mauritius by their ethnic and/or religious backgrounds into four main categories: Hindu, Muslim, Creole/Christian, and Sino-Mauritian. According to Arnav, these categories influence what language one studies in school and the terms one uses to refer to family members. To investigate this further, a speaker from each background group was chosen to consult on this project.

The four languages besides Kreol whose kinship terminology are of interest to this project are Hindi, French, Arabic, and Hakka. These languages were chosen because they are the heritage languages of the speakers from whom data were gathered. Arnav is Hindu and he grew up using Hindi when his family would go to the temple and he also formally learned Hindi at school, Lin's family is originally from Hong Kong and her grandparents spoke the Hakka dialect of Chinese, Amelie is Kreol and the main language she used growing up besides Kreol was French, and Raazi is Muslim and Arabic is the heritage language he uses for religious activities and he formally learned it in school.

3.2.1 Speaker Arnav

Arnav was the primary language consultant for this project. He is a 21-year-old male undergraduate at the University of Rochester in Rochester, NY. He was born in Curepipe, Mauritius and attended primary and secondary school in Port-Louis, Mauritius. His family is Hindu and has lived in Mauritius for many generations. His first language is Kreol, but he is also fluent in English and French, having studied them in school from a young age. He never formally studied Kreol in school or elsewhere. His education was conducted in a mixture of French, English, and Kreol. In addition to French and English, he also studied some Hindi in school and knows a lot of mantras, but does not often use the language in his everyday life. He also knows a very small amount of Bhojpuri because some of the elders in his community use it, but his knowledge is very limited.

3.2.2 Speaker Amelie

Amelie is a 22-year-old female undergraduate at the University of Rochester in Rochester, NY. She was born in Curepipe, Mauritius, but grew up in the capital of Port-Louis and also attended school there. Her family is Catholic and has lived in Mauritius for many generations. Her first languages are Kreol and French, but she is fluent in English as well. She formally learned Kreol at church and studied English and French at school. Her education was also done in a mixture of English, French, and Kreol.

3.2.3 Speaker Lin

Lin is a 22-year-old female university student in the United Kingdom. She was born, raised, and attended school in Port-Louis. She identifies as Sino-Mauritian meaning that she is Mauritian, but her family is from China. Her paternal grandfather was born in Meixian District in China and moved to Mauritius where her father was born. Her parents' generation speak the Hakka dialect of Chinese, but she only knows a few words and phrases. In addition to her first language, English, she is also fluent in French and Kreol. She speaks mainly Kreol at home but uses French and English outside of the house. Because of her background and her parents' encouragement, she chose to study Mandarin for 10 years while in primary and secondary school and outside of school with tutors. However, she does not speak Mandarin and understands just a few words that her grandmother uses with her aunts.

3.2.4 Speaker Raazi

Raazi is a male university student in Canada. He was born, raised, and attended school in Port-Louis. He and his father were born and raised Muslim, but his mother was born Christian and converted to Islam when she married his father. His first language is Kreol and he also speaks English and French fluently. At home he mainly speaks Kreol. Because of his Muslim background, he chose to study Arabic in middle school, though some of his Muslim friends chose to study Urdu.

4. Kinship Systems

Kinship systems and their terms have been the focus of numerous anthropological and linguistic research projects. Each language has its own terms for kinship relations and the systems that underlie the use of those terms can be divided into five main categories with two subcategories. The five main types are Dravidian, Iroquois, Hawaiian, Sudanese, and Eskimo, and the two subtypes are Crow and Omaha (Dousset, 2012). These types can be characterized as being classificatory or descriptive, but no system is completely one or the other. Dravidian, Iroquois, Hawaiian, Crow, and Omaha systems are more classificatory, and Sudanese and Eskimo systems are more descriptive (Dousset, 2012).

4.1 Kreol Kinship Systems

It is hard to say that there is one "standard" Kreol kinship system with "standard" kinship terms. Even within the LALIT dictionary, there are many lexical entries listed that correspond to a single kinship relation. For example, if you were to look up the word 'aunt' in the LALIT dictionary, you would find eight entries: *ate*, *chachi*, *kala*, *mami*, *matant*, *mawsi*,

inguistics

pupu, and *tantinn*. While the terms the language consultants used tended to differ for members of their parents' generation and above, they all shared terms for members of their generation and below. The terms the speakers used for members of the parent generation and above were based on their ethnic/religious background and most came from a heritage substrate language. In addition to the different terms, the structure of the kinship systems themselves were different depending on the speaker's background. However, three of the four speakers shared the same terms and structure for members of their own generation and below. These terms are Kreol cognates of their French counterparts. Chart 1 below is a visualization of the kinship system shared by consultants Arnav, Amelie, and Lin that refers to members of their generation and below.

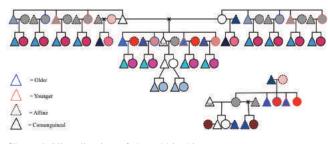


Chart 1. Visualization of shared kinship

In Chart 1 above, the grayed-out members are not technically part of the chart and are only included because they allow for relevant members of the system to be shown. Therefore, they can be ignored when viewing the chart. Family relations that are shaded the same color share the same kinship term. If a person is not shaded, this means that they have their own unique term that is not shared with another member of the tree. This part of the Kreol kinship system is categorized as an Eskimo system. A distinction is made between children of Ego's parents and children of Ego's parent's siblings, between males and females, and between consanguines and affines. Table 1 below shows the shared terms between Arnav, Amelie, and Lin (written in IPA) along with their Genealogical Referent (GR), and corresponding color. The relationships described are from the perspective of Ego.

Kreol	GR	Color	Kreol	GR	Color
papa, pa	F		mama, ma, mamõ	М	1.00
bopejə	s-F, -F		bɛlmejə	s-M, i-M	0
frejə	В	A	sejə	Z	0
demifsejə	s-B, h-B		demisejə	s-Z, h-Z	0
bofsejo	i-B		bɛlsejə	i-Z	٠
gosõ	S		t∫ĭfi	D	
bofis/zən	s-S, i-S	•	belfi	s-D, i-D	•
t∫izõfo	SS, DS, SD, DD	Δ	- 8	(m .)	
neve	BS/ZS	A	nies	BD, ZD	•
kuzē	mC	Δ	kuzin	fC	•

Table 1. Shared kinship chart – POV of 'Ego'

The kinship terms and system shared between the three speakers are very close to those of the Kreol lexifier, French. In fact, the terms are Kreol cognates of French lexical items. For example, the term for BS/ZS is /neve/ from the French /nəvø/. Above Ego's generation, the terms the speakers used diverged based on their ethnicity and background.

4.2 Amelie and the French Kinship System

Amelie's kinship system shows the most influence from French in both terms and structure. The structure is identical to the Eskimo-type French system and the terms are French cognates. Chart 2 below provides a visualization of her kinship system for the two generations above Ego. This system is identical to the French one.

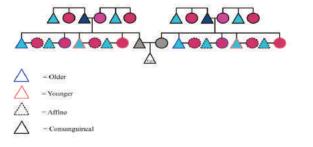


Chart 2. Visualization of Amerlie's kinship system

In Chart 2 above, the parents of Ego are grayed-out and are only included so that their siblings and parents could be shown. Amelie's system does not distinguish between maternal and paternal aunts, uncles, or grandparents. It also does not distinguish between consanguines and affines, although there is a gender difference. Additionally, there is no generational difference for aunts or uncles, meaning that her parents' siblings are called by the same terms as her grandparents' siblings. Table 2 below shows Amelie's kinship terms in IPA along with their French counterpart (written in French orthography), GR, and corresponding color. The relationships described are from the perspective of Ego.

Kreol	French	GR	Color	Kreol	French	GR	Color
grõpejə	grand- père	FF, MF		grõmejə	grand- mère	FM, MM	•
tõtõ	oncle/ tonton	FB, FZH, FFB, FMB, MB, MZH, MFB, MMB	4	matõ/tatfi	tante	FZ, FBW, FFZ, FMZ, MZ, MBW, MFZ, MMZ	•

Table 2. Amelie's kinship chart with French Terms - POV of 'Ego'

One aspect of Amelie's kinship system that is not shown in Chart 2 or Table 2 are her godparents. She is Christian and has a godmother and a godfather. Her godmother is her mother's sister and her godfather was her father's late brother. Her term for godmother is / mauen/ and her term for godfather is /par ϵ ^{-/} which is how she refers to them and how she addresses them. Both of her godparents would refer to her as their /fij ϵ l/, which is the same term her brother's godparents would use to refer to him. It was interesting to note that her brother's godparents are a different aunt and uncle pair than her godparents. Therefore, if she were talking to him about them, she would say the equivalent of 'your godmother/godfather' rather than 'our aunt/uncle'.

4.3 Lin and the Hakka Kinship System

Lin's kinship system is structurally the same as Amelie's and the French system. However, her terms are different. It is unclear what language her terms originated from, but her system is compared to the Hakka one as this is her heritage language and the one that her family speaks. The Hakka kinship system is a Sudanese system in which most kinship relations have their own individual terms. Chart 3 below shows the visualization of the Hakka kinship system. It was very difficult to find information about Hakka kinship terms, so the chart has many familial relations that are grayed out, meaning that I was not able to find a term for that person.

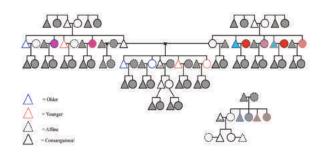


Chart 3. Visualization of Hakka kinship from information from Hashimoto (2010)

In Chart 3 above, the kinship relations that have a shared term are filled in with the same color. If a person is not shaded, this means they are given their own unique term. As can be seen in the chart, almost every person has their own term which marks this as a Sudanese system. On the paternal side in the generation above Ego, there is a distinction made based on age for the males and their wives. There is also a distinction made between consanguines and affines. For the females and on the maternal side, there is no age distinction, but there is one between consanguines and affines. For the children of Ego's parents, there is a differentiation based on age and gender. Table 3 below shows the Hakka kinship terms in IPA with their GR and corresponding color if applicable. The relationships described are from the perspective of Ego.

Hakka	GR	Color	Hakka	GR	Color
aguŋ	FF		ŋoi aguŋ	FM	1542
apo	MF	100	noi apo	MM	722
aba	F		ame	М	
a bak	FoB	0770	a kiu	MB	
bak me	FoBW	-	kiu me	MBW	•
a suk	FyB		227	100	1.22
suk me	FyBW	1	227	8448	1.22
agu	FZ	•	aji	MZ	0
ago	oB	(++)	adzi	οZ	1
lo tai	yВ	1	lo moi	уZ	042
lai ji	S		moi ji	D	
ŋ se	i-S		sim kiu	i-D	

Table 3. Hakka kinship chart	from informatic	on from Hashimoto	
(2010) — POV of "Ego"			

Many of the Hakka terms begin with /a-/ although it is unclear from Hashimoto (2010) why this is. Furthermore, since characters were not included in the book, it is not clear if lexical items that sound similar are actually the same, as in the case of the /a-/ at the beginning of FF, FM, F, FoB, etc. This is partially why it is difficult to trace Lin's kinship system back to Hakka and it remains unclear if her terms come from Hakka.

Lin's kinship system has the same structure as Amelie's and the French Eskimo-type systems. However, her terms are different for members of the two generations above her, but it is unclear from which language they originate. Chart 4 below shows a visualization of Lin's kinship system, which is the same as Amelie's chart.

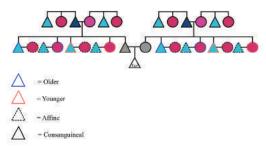


Chart 4. Visualization of Lin's kinship system

Table 4 below shows Lin's kinship terms for the two generations above her written in IPA with their corresponding GR and color, if applicable.

Kreol	GR	Color	Kreol	GR	Color
kõkõ	FF, MF		popo	FM, MM	0
tõtõ	FB, FZH, FFB, FMB, MB, MZH, MFB, MMB	4	jiji	FZ, FBW, FFZ, FMZ, MZ, MBW, MFZ, MMZ	•

Table 4. Lin's kinship chart – POV of 'Ego'

Lin's terms do not have a clear connection to their Hakka counterparts. Furthermore, the structure of her kinship system is not the Sudanese-type that Hakka has, rather it is an Eskimo-type system. I do not have enough information about the contact between Hakka and Kreol to draw further conclusions about whether Lin's kinship terms originate from Hakka or from a different language. If they do come from Hakka, then it would be interesting to look at why the terms made it into Kreol, but not the accompanying structure. However, further research is needed on this topic before conclusions can be drawn.

Something interesting about Lin's family is that each of her family members has two names—their Chinese names and their 'Mauritian' name. Chinese names are used within the family and inside of the home, while 'Mauritian' names are used in public. For example, Lin's parents call her by her Chinese name /IIn/ at home rather than by her Mauritian name. Another interesting aspect of her kinship system is that when addressing her aunts in the grandparent generation, she refers to them by their name plus the honorific suffix /ku/ in order to show respect. To her knowledge, there is no male equivalent of /ku/.

4.4 Arnav and the Hindi Kinship System

Arnav's kinship terms and structure for the two generations above him are very different from those of speakers Amelie and Lin. This part of his system is a Sudanese-type in which there is a distinction between the paternal and maternal sides and between affines and consanguines. In addition to this, there are also alternate terms for grandchildren. The terms for this part of his system are originally from Hindi, although some of the terms have semantically broadened which has slightly simplified the system. Chart 5 below is a visualization of the Hindi kinship system. The people that are shaded the same color share the same kinship terms. I was not able to find information about the terms for the grayed-out people. If a person does not have shading, this means they have their own unique kinship term.

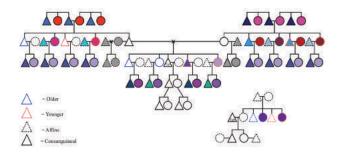


Chart 5. Visualization of the Hindi kinship system

Hindi is a Sudanese-type system in which most members have their own kin term. On the paternal side, there is an age distinction between males of the generation above Ego and their wives. There is also a distinction between consanguines and affines. This is also shown on the maternal side. Table 5 below shows the Hindi terms written in IPA, their GR, and corresponding color, if applicable.

Hindi	Hindi GR Color Hindi		Hindi	GR	Color
dada/dadu	FF, FFB, FMB	A	nana/nanu	MF, MFB, MMB	
dadi	FM, FFZ, FMZ	٠	nani	MM, MFZ, MMZ	•
pita/abba/abbu/ baba/babu	F	1999	mata/amma/ ammi/maa	М	9 1 54
taya/tau	FoB	0.000	mama/mamu	MB	Δ
tai	FoBW	10.505	mami	MBW	•
tʃatʃa/tʃatʃu/kʰaka	FyB	122	622	220	
t∫at∫i	FyBW	3.55	1.75		
b ^h ua/p ^h upi/kaki	FZ	•	maosi	MZ	
phupha	FZH	A	mausa	MZH	Δ
b ^a ai sab/dada	oB	122	didi	οZ	(44)
b*ai	B, mC		behen	Z, fC	0
bhabhi	oBW		dzidza	oZH	**
bhayo	yBW	19442	behnoi	yZH	
sasur	i-F		sa:s/sa:su ma	i-M	
177.1	198	8 5 15	sali	WZ	•
344) (144	848	222	b ^b ai sa:b	WoB	220
**			sa:la	WyB	
beta/putr	S	1.77.	beti/putri	D	277
bahu	i-S	6 98	damad/dʒamai	i-D	(71 1)
pota	SS	(1 44)	nati/nawasa/dohata	DS	
poti	SD	1922	natin/nawasi/dohati	DD	
b ^k atid3a	BS		b ^a nd3a	ZS	Δ
b*atid3i	BD		b*and3i	ZD	0

Table 5. Hindi kinship chart compiled from a few charts fromSuryanarayan & Khalil (2021) and Vatuk (1969) — POV of 'Ego'

In Table 5 above, you can see that most kin relationships have their own kinship term. This is particularly true in the generation above Ego and in Ego's generation. This is similar to the Hakka system, which is also considered a Sudanese-type system. There is no general word for 'cousin', rather they use phrases such as uncle's/aunt's son/daughter to be specific or use the term b^hai/behan ('brother'/'sister') to refer to members of this kin category (Suryanarayan & Khalil, 2021). Further, they seem to distinguish more for the paternal side than the maternal side. For example, there are different words for one's FoBW and FyBW, but there is only one term for one's MBW. Additionally, some of the terms for grandparents are merged such that one's FF and all of the men in that generation on that side (including FMB) are all called by the same term. The women also follow this pattern.

Arnav's kinship system and terms for the two generations above him originate from Hindi. However, some of the terms have been broadened to refer to multiple relationships and some terms have been lost. This system is a modified Sudanese-type system in which general categories of relations have their own terms, but there is no age differentiation (only one term for FB, rather than FoB or FyB). Chart 6 below provides a visualization of Arnav's kinship system for his parents' and grandparents' generation. Ego's parents are included so that their siblings and parents can be shown, but they are grayed out because they are not of interest at this time. The people who have the same shading share a kinship term.

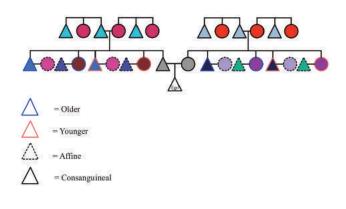


Chart 6. Visualization of the Arnav's kinship system

Arnav's system is similar to that of Hindi but without the distinction between older and younger paternal brothers and their wives. Like in Hindi, the members of his grandparents' generation are merged on each side by gender such that his FF, FFB, and FMB all share a single term. It is interesting that in addition to the Hindi terms, the structure of the kinship system was brought into the language as well. Table 6 below includes Arnav's kinship terms for the two generations above him in IPA, the Hindi terms in IPA, their GR, and their corresponding color.

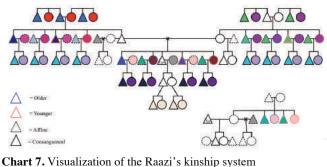
Kreol	Hindi	GR	Color	Kreol	Hindi	GR	Color
dada	dada/dadu	FF, FFB, FMB	4	nana	nana/nanu	MF, MFB, MMB	
dadi	dadi	FM, FFZ, FMZ	•	nani	nani	MM, MFZ, MMZ	•
t∫at∫a	taya/tau/ tʃatʃa/tʃatʃu/ kʰaka	FB		mamu	mama/mamu	MB	
t∫at∫i	tai∕ t∫at∫i	FBW	•	mami	mami	MBW	0
pupu	bhua/phupi/kaki	FZ		mausi	maosi	MZ	•
pupa	phupha	FZH	Δ	mausa	mausa	MZH	

Table 6. Arnav's kinship chart with Hindi terms - POV of 'Ego'

As shown in Chart 6 above, Arnav's kinship system originates from Hindi. However, he does not make a distinction between his father's older brother (and his wife) and his father's younger brother (and his wife). Instead, he uses the Hindi term for FyB /tʃatʃa/ and FyBW /tʃatʃi/ to refer to all of his FB and FBW. Besides this broadening, his system and terms follow the Hindi system from which they originate. In addition to the terms Arnav uses for his parents' and grandparents' generations, he also has special words for SS/DS and SD/DD that come from Hindi. These terms are /beta/ and /beti/, which mean 'son' and 'daughter' in Hindi, respectively. However, for Arnav, these terms are used by consanguineal grandparents to refer to their grandchildren. These two terms are used alongside the terms that are shared by two of the other speakers.

4.5 Raazi and a Mixed Kinship System

Raazi has a different family situation than the other three language consultants in that he is from a mixed family. His mother was born Christian but converted to Islam when she married his father who is Muslim from birth. Since his family is multiethnic, he uses different terms when referring to each side of his family. The system he uses for his paternal side is a Sudanese-type that is almost the same as that of Arnav. This is because Urdu, a language very similar to Hindi, is the claimed heritage language of the Muslim community due to its religious unifying role (Rajah-Carrim, 2004). On the other hand, the system he uses for his maternal side is an Eskimotype that is similar in structure to the system used by Amelie. Although the structure is similar, Raazi is the one speaker who does not use terms that came from French to refer to his step, in-law, and half sibling relationships. Instead, he uses the English terms. Chart 7 below is a visualization of Raazi's mixed system. The people who are shaded the same color share a kinship term and the people who are grayed out are not relevant at this time, they are only included in the chart so that other relations can be shown. If a person is not shaded, then they have their own term that is not shared with another person.



As seen in the above chart, Raazi's system is different for his paternal and maternal sides. His paternal side is a Sudanesetype system in which there is a distinction between consanguines and affines in the generation above Ego. Additionally, the members of the grandparent generation are merged by gender. This is the same structure as Arnav's system. On the other hand, his maternal side is an Eskimo-type system in which all siblings of Ego's mother and grandparents are merged based on gender. This is the same structure as Amelie's and Lin's systems. For children of Ego's parents' siblings, there is no distinction based on whose children they are, but there is a gender difference. The same is true for the children of Ego's siblings. This is the same for all four of the language consultants. Where Raazi again differs from the rest is with regard to his terms for step, half, and in-law relations. Table 7 below shows Raazi's kinship terms in IPA, their GR, and their corresponding color, where applicable. Additionally, the English terms he uses are also included.

Kreol	GR	Color	Kreol	GR	Color
dada	FF, FFB, FMB	A	grõpejo	MF	(3 75
dadi	FM, FFZ, FMZ	•	grõmejo	MM	1.2
pa	F		ma	М	
tfatfa	FB		tõtõ	MB/MZH/ MFB/MMB	
t∬at∬i	FBW	•	matõt	MZ/MBW/ MFZ/MMZ	•
рот	FZ	•			1
pupa	FZH	Δ			
fвеја	В		sejə, ben	Z	
kuzē	mC	Δ	kuzin	fC	0
gəsõ	S	22 ()	ព្រ័ព៍	D	-
t∫izõfo	SS, DS, SD, DD	Δ		-	
neve	BS, ZS		nies	BD, ZD	٠
English	GR	Color	Kreol	GR	Color
brother-in-law	i-B	4	sister-in-law	i-Z	0
stepbrother	s-B		stepsister	s-Z	0.000
half-brother	h-B	225) 225)	half-sister	h-Z	(8 4
father-in-law	i-F		mother-in-law	i-M	1
stepfather	s-F	2211	stepmother	s-M	
son-in-law	i-S	22.1	daughter-in-law	i-D	122

Table 7. Raazi's kinship chart — POV of 'Ego

As shown in Table 7 above, Raazi's kinship terms and system are different from the other speakers. Besides the mixed system from his paternal and maternal side, he also uses English terms for step, half, and in-law relations which none of the other speakers do. He said himself that he likes to use English words in his Kreol. He also noted that if his mother were born Muslim, he would use the maternal-side equivalent of what he uses for his paternal side to refer to her family. The only ones he was able to remember were /nana/ and /nani/ to refer to her father and mother respectively. Unfortunately, I could not investigate this further as he did not remember the other terms because he does not use them himself.

5. Conclusions

Across the four speakers, there were a lot of similarities in kinship system structure and corresponding terms. For referring to consanguines of their own generation, the generation below them, and their parents, all of the speakers used the same terms. These terms are Kreol cognates of French lexical items and are categorized as an Eskimo-type system. Amelie, Arnav, and Lin also used the same terms and system for step, in-law, and half-parent and sibling relationships in which the step and in-law parents are merged based on gender, and the step and half-sibling terms are also merged based on gender. This follows the French system and the terms the speakers used were cognates of their French counterparts. Raazi differed in that he used English terms for step, half, and in-law relationships instead. English does not merge any of these terms like French does, so Raazi has a slightly different system than the other speakers.

It was interesting to see the degree to which kinship terms and their underlying systems were preserved once incorporated into Kreol. In language contact situations, "the borrowing of open-class lexical items... is fairly common" (Winford, 2010, p. 178). Therefore, it is not surprising that lexical items from substrate languages have been incorporated into Kreol. The terms have also been adapted to fit Kreol phonology as in the case of the Hindi word for FZH /p^hup^ha/ becoming /pupa/ in Arnav's system since there is no distinction between /p/ and /p^h/ in Kreol. Additionally, some terms have broadened from their original meaning. For example, the Hindi for FyB / t∫at∫a/ becomes the term for FB (both older and younger) in Arnav's system.

However, in this situation, it is noteworthy that the kinship structures themselves were brought into the language to varying degrees. There is no clear answer as to why both the Hindi terms and structure became part of Kreol when seemingly only the Hakka terms were brought in. One reason could be the size of the communities in Mauritius. Hindus and Muslims combined make up about 66% of the population while Christians and Buddhists constitute 32% and 0.4% respectively (World Population Review). The population of Hindus and Muslims is over double the size of Christians and Buddhists, so the community is much larger. This might be one of the reasons why the kinship structure has been preserved for speakers like Arnav and Raazi.

As expressed by all four speakers, different kinship terms are used based on one's background and they are markers of ethnic identity. Therefore, it is important to study kinship terms and systems as a window into the social realities in Mauritius. Furthermore, Kreol is in the unique situation of being in contact with numerous heritage languages that aren't necessarily spoken fluently, but that serve as important cultural indicators. It is for these reasons that it is pertinent to study Kreol kinship terms and systems in the context of language contact.

6. References

- Baker, P. (1969). The language situation in Mauritius with special reference to Mauritian Creole. *African Language Review*, 8, 73-97.
- Baker, P. & Kriegel, S. (2013). Mauritian Creole. The Survey of Pidgin and Creole Languages, 2, 250-260.
- Bakker, P., & Matras, Y. (Eds.). (2013). *Contact languages : A comprehensive guide*. De Gruyter, Inc.
- Bissoonauth, A. (2021). Language and attitude shift of young mauritians in secondary education. *Journal of Multilingual and Multicultural Development*, 42(1), 64-78.
- Dousset, L. (2012). Understanding Human Relations (Kinship Systems).
- Eberhard, D. M., Simons G. F. & Fennig, C. D. (eds.). 2021. *Ethnologue: Languages of the World*. Twenty-fourth edition. Dallas, Texas: SIL International. Online version: http:// www.ethnologue.com.
- Foley, J. A. (1992). What future for kreol morisyen? Journal of Multilingual and Multicultural Development, 13(4), 359-372.
- Hashimoto, M. J. (2010). *The Hakka dialect: A linguistic study of its phonology, syntax and lexicon* (Vol. 5). Cambridge

University Press.

- Heath, J. G. (1984). Language contact and language change. *Annual review of anthropology*, 13(1), 367-384.
- Hold, J. (2010). Contact and Change: Pidgins and Creoles. *The handbook of language contact*. John Wiley & Sons, Inc.
- Mooneeram, R. (2007). The contribution of creative writing to the standardization of Mauritian Creole. *Language and Literature*, 16(3), 245-261.
- Mufwene, S. S. (2008). Mauritian Creole. Encyclopedia Britannica.
- Rajah-Carrim, A. (2004). The role of Mauritian Creole in the religious practices of Mauritian Muslims. *Journal of Pidgin and Creole languages*, 19(2), 363-376.
- Rajah-Carrim, A. (2007). Mauritian Creole and language attitudes in the education system of multiethnic and multilingual Mauritius. *Journal of Multilingual and Multicultural Development*, 28(1), 51-71.
- Suryanarayan, N. & Khalil, A. (2021). Kinship terms as indicators of identity and social reality: A case study of Syrian Arabic and Hindi. *Russian Journal of Linguistics (Online)*, 25(1), 125–146.
- Tirvassen, R. & Ramasawmy, S. J. (2017). Deconstructing and reinventing the concept of multilingualism: A case study of the Mauritian sociolinguistic landscape. *Stellenbosch Papers in Linguistics Plus*, 51, 41-59.
- Vatuk, S. (1969). A structural analysis of the Hindi kinship terminology. *Contributions to Indian sociology*, 3(1), 94-115.
- Winford, D. (2010). Contact and Borrowing. *The handbook of language contact*. John Wiley & Sons, Inc.
- World Population Review. Mauritius Population 2022 (Demographics, Maps, Graphs). Retrieved Apr 12, 2022, from https://worldpopulationreview.com/countries/mauritiuspopulation

Footnotes

1. 'Heritage language' in this case refers to a language that is spoken or learned because of one's background

2. This dictionary was created by a political party known as LALIT. Information about the party can be found here: <u>https://www.lalitmauritius.org/en/about.html</u>

About the Author

I started working with Mauritian Creole during the senior seminar class for my linguistics major that I took during the spring of my junior year. During the class, our speaker would often categorize Mauritians based on their religious or ethnic backgrounds. I found this really interesting and decided to delve deeper into one way this manifests itself as the topic of my honors thesis. I chose the topic of kinship because family is something that almost everyone can relate to and it's one of the most overt ways these differences are expressed. My best advice for other undergrads interested in research is to reach out to your professors and use them as your soundingboards and resources!

Abolishing the Death Penalty in Pakistan: Public Sentiment, Harm Prevention, and Punishment

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Preface: The Pakistani Context

In March 2021, 27-year-old Noor Mukadam attended the annual Aurat March (Women's March) in my hometown: Islamabad, Pakistan. With her rage at the state of women's rights in the country visible, even behind a mask, she took pictures at the protest with a placard that said, "Hang them! Destroy them! Humiliate them! We won't stay home so they can grow! No more!" (Waheed). The poster referred to the lack of justice and accountability for rapists and perpetrators of genderbased violence (GBV) in Pakistan and the resultant limitations on women's freedoms within their own communities. Four months later, in July 2021, Islamabad woke up the morning before Eid-ul-Azha to the news that Noor Mukadam had been held hostage, tortured, raped, and beheaded by her 27-yearold family friend, Zahir Jaffer, at his house.

The murder sent ripples across the capital and the country, prompting protests and vigils, a #JusticeForNoor online movement, and widespread shock, condemnation, and calls for justice. For most, including Noor's family and friends, justice would entail a public hanging. In February 2022, after a lengthy trial, Jaffer was sentenced to death. However, his parents, the household staff at the scene, and his colleagues at the counseling service TherapyWorks—all of whom allegedly abetted the murder-were acquitted, spurring an unprecedented national and global response. What set Noor's death apart from the hundreds of incidents of GBV in Pakistan was her and Jaffer's socioeconomic positioning as members of the Islamabad elite. However, most victims from socioeconomically-disadvantaged backgrounds could never garner this level of support and do not have access to the legal resources to bring even their abusers to justice, let alone aiders and abettors.

For many Pakistani women, the news of Noor's murder and its aftermath stood out as the most significant event of the summer of 2021. There was a sense of collective grief, trauma, and anger which was, in some ways, comparable to other recent GBV cases, such as the 2018 rape and murder of 7-year-old Zainab Ansari and the 2020 motorway gang rape incident. These incidents also resulted in death sentences for the perpetrators, with then-Prime Minister Imran Khan and other public officials going as far as assuring the public that justice would be served and that future incidents of GBV would be deterred through hangings. However, some abolitionist discourse around Noor's murder questioned the family and the public's calls for capital punishment. Online voices, removed from the situation, wondered whether state violence was the solution to GBV, or whether it was just immediate gratification for a crime embedded in a larger, inherently violent system? However, even those—like myself—who opposed death penalty in princple, were relieved when Jaffer was sentenced to death.

This emotional response highlights a dissonance between primarily Western abolitionist frameworks and the social, cultural, and emotional realities in Pakistan. How can abolitionist theory, which owes much to Black scholars, translate to a Pakistani context, if at all? In Pakistan, unlike the United States, capital punishment remains an overtly moral and emotional issue with higher stakes as opposed to being perceived primarily as an impersonal "legal and procedural cause" (Temkin 6). However, in both countries, the death penalty is inherently rooted in religious and political conceptions of justice and redemption. As with American culture, capital punishment in Pakistan also "[celebrates] the purification of bad violence through good violence" and functions almost like "entertainment," endangering our "ways of relating to one another" due to its preoccupation with, and faith in, violence (Dubler and Lloyd 89). In addition to a plethora of other complications, capital punishment often results in wrongful convictions and rarely helps deter future offenders (FIDH and HRCP 8). Therefore, since capital punishment perpetrates the cycle of crime and punishment without addressing root issues, the state expands its carceral net and calls for tougher, more violent solutions such as chemical castration ("Pakistan Anti-Rape Ordinance"). Along with creating vicious cycles of violence and control, capital punishment can also result in the victimization of vulnerable groups, limiting their mobility and freedoms to purportedly keep them safe without doing the work of resolving their trauma or creating a safer community.

Focus, Goals, and Caveats

My goal is to envision a project that ameliorates a specific facet of carcerality: the death penalty in Pakistan and its connection to public sentiment and pain, particularly for women and their families. It is important to note that myquestions regarding the death penalty are with reference to its viability for what Davis et al. refer to as "really bad people" - rapists, murderers, and the like – as opposed to non-violent offenders, such as drug traffickers and alleged blasphemers in Pakistan. Abolitionist theorists tend to be frustrated by questions about "really bad people" because they make up a small number of the prison population in the US (Davis et al. 149). However, owing to the deep emotional resonance that capital punishment has in Pakistan, it feels like an urgent and appropriate element of carcerality to focus on, especially since the country had the largest death row population in the world as of 2018 ("Death Row Population in Pakistan"). Moreover, the death

penalty in the context of this paper is intrinsically linked to the protection of women, and the INCITE! Critical Resistance Statement notes that abolitionism's "inability to answer the question [of how to hold "really bad people" accountable] is interpreted by many as a lack of concern for the safety of women" (Davis et al. 183).

Therefore, my aim with this project is to address how to develop solutions to build accountability, safety, and healing amongst victims and afflicted families without widening the carceral net and expanding/endorsing a deeply flawed system that cages and murders other humans. How do we address the pain caused by these "really bad" acts in the short-term and the long-term while also working towards eliminating said acts and therefore the need for capital punishment? For the purposes of this paper, I will be narrowing my focus to GBV incidents with "victims" referring to those directly impacted by GBV (including survivors and the deceased), and "the affected" referring to victims' families, friends, and members of the general public that have been indirectly impacted.

By no means does this project, in its skepticism of the death penalty, imply a vindication of abusers such as Zahir Jaffer. Instead, it seeks to understand the circumstances such as those that led to Noor's murder, build healthy avenues of support and healing for GBV victims and the affected, preemptively prevent harm in the future, and redefine how communities think about and demand accountability. Currently, the death penalty is the only option that victims and other affected individuals have, in lieu of homegrown abolitionist imaginations and genuine spaces for healing, prevention, and accountability. Due to this vacuum, the death penalty has understandably come to serve all three purposes: it is touted as a deterrent, a way to heal and find closure, and a mechanism to hold perpetrators accountable. However, I aim to design a model that can start to approach healing and prevention in non-punitive ways within the Pakistani context in the short term. This will eventually engender abolitionist accountability in the long term in ways that are culturally appropriate and relevant.

Intersections with Abolitionist Thought and Theory

Capital punishment is largely driven by public sentiment. Temkin notes that in the US, executions have come to be seen as "community justice" and as an expression of the public's will and the victims' interests, instead of as a "display of governmental power and dominance" (Temkin 9). In "Community Re-Entry," Pedlar et al. also discuss the US prison system's tendency to "operate behind a cloak of invisibility to avoid provoking anger from a vengeful public" (Pedlar 138). After both the 2018 Zainab case and the 2020 motorway case, there were concerns among social media users that the wrong people had been arrested in response to the public's demands for accountability. There are also myriad instances in Pakistan where courts have sentenced the innocent or acquitted the guilty, aligning themselves with public sentiment to avoid backlash-especially in death row sentences for blasphemy cases. Therefore, the state both controls and submits to the

masses by wielding the death penalty. The criminal justice system's lack of transparency subdues the masses by assuring them that what they have been convinced is their will is being carried out through the punishment, isolation, and discipline of criminals. The court of public opinion might be valuable if a society operates under a community-derived social contract that is based on principles such as equity, healing, and justice. However, if the institution—as it currently stands—is steered by public anger, there is the risk that it will cease to live up to its ideals of consistency and impartiality. It may also set a dangerous precedent by allowing sentiment to decide who lives and who dies, thus reproducing both state-sponsored and extrajudicial violence.

Davis et. al., in Abolition. Feminism. Now., also acknowledge a tension between abolition feminism and carceral feminism. Although carceral feminism advocates for the death penalty to put an end to GBV, Davis et al. note that when the state sanctions and encourages violence, this augments harm and leads to more incarceration and violence. Davis et al. frame carceral approaches and solutions such as the creator of sex offender registries-and indeed, the death penalty-as ineffective for a "social justice problem for which the state [is] partially culpable" (113). Instead, they suggest that "centering the experience and analyses" of those affected is important (109). They also criticize the "decontextualized individualistic terms" in which GBV is understood: women and children are framed as vulnerable, while individual perpetrators are thought of as monsters who must be locked away (115). What Dubler and Lloyd have called the pairing of the "innocent victim and the evil perpetrator" creates a dichotomy that results in the marginalization and fetishization of the "sacred body of the innocent" and a "visceral reflex to seek redemption in purifying violence" against the perpetrator (88-89).

This sort of essentialist frame also serves to make the public feel good about itself, delaying any critical self-reflection and improvement. Lone perpetrators are seen as the "beginning and end of these violences": they are considered exceptions to the rule of "good" people or "good" men, and anomalies that are inherently evil and must be arrested, locked away, or hanged (Davis et al. 115). Their crimes cause a moral panic and there is a sense of urgency for the incident to be resolved. However, if justice can simply be served by putting individual criminals in prison or hanging them, the masses never have to confront the reality of a system in which they are complicit. This means that society can conveniently confer the blame to individual offenders and never reflect on or solve the underlying problems that led to the crime being committed in the first place.

Therefore, paternalistic carceral solutions that purport to "protect" vulnerable individuals in fact "consolidate and deepen existing state power" and prevent systematic change, deepening the public's reliance on systematic violence and patriarchal structures (Davis et al. 120). Davis et al. instead ask that, within the framework of abolition feminism, we not isolate individual acts of violence from their larger contexts (183). They demand emphasis on structural violence, social and structural transformation as "the only means to ensure safety for survivors," and organization/advocacy that addresses not only individual care but the root causes of harmful experiences. This will prevent carceral creep, encourage self-reflection and a critical awareness of the "structural and institutional underpinnings" of GBV, and will eventually build accountability and safety outside of the punitive structures of law and order (115).

Contradictions

As outlined by Reverend Jason Lydon, while addressing the pain of victims and the affected within an abolitionist framework, we must ensure that the experiment does not build something that will need to be abolished later. In a similar vein, Mariame Kaba calls for "non-reformist reforms" that don't result in carceral clawback i.e. further strengthening oppressive systems (Kaba 124). Quite fittingly, Kaba provides the example of the abolition of the death penalty as an undesirable reform, if it were to be replaced with life without parole. This is because the alternative simply puts more people in cages and reproduces the system in another form. Instead, Kaba calls for reforms that result in "our relationships [being] transformed over time" so we can "think more clearly about ways to reduce harm" (126).

However, Davis et al. also recognize the "urgent and heartbreaking contradictions" that emerge within abolition feminism-sometimes, abolitionist stances aren't enough or they are unable to respond to ongoing harm or hurt. When thinking about cases such as Noor's, I cannot help but feel torn between the idea of long-term transformative and restorative justice, and the overwhelming feelings of collective grief, trauma, and anger now. If abolitionism seeks to center victims and survivors, what happens when survivors and those affected calls for carceral action and immediate accountability? When one cannot bring a victim back to help them heal and cannot restore what's been lost, does the perpetrator even deserve an opportunity to grow and transform? How do we help survivors and affected individuals find peace in the short-term when they don't have the luxury of waiting for long-term systemic transformation?

At its onset, this experiment will not advocate for the abolition of the death penalty in Pakistan. It is well-established that the death penalty is an ineffective deterrent, "inconsistent and capricious" in its distribution of justice, often subjects the innocent to violence, and is inordinately expensive (Conquergood 360). Therefore, it may seem counterintuitive to not immediately abolish capital punishment, but it is a necessary compromise to make. Within the Pakistani context, the death penalty is adhered to for "emotional and expressive purposes" (Conquergood 360). It is undoubtedly exploited for political gain and the regeneration of violence, but this aspect remains obscured from public consciousness, so it is essential to first find new generative powers within the community to serve as outlets for emotional and expressive energies. This is even more important considering the religious and social implications of opposing the death penalty. One, attempting to abolish it would be seen as an act of "taking away power from the people" and siding with perpetrators (Temkin 9). In addition, since it is rooted in religious conceptions of justice and redemption, with the death penalty seen as a punishment prescribed by Islam for certain offenses, debating its abolition could be perceived as blasphemous. This further complicates any opposition to the death penalty, which ironically also happens to be the punishment for blasphemy. Therefore, in a justice landscape where punitive carcerality has such widespread and deep-rooted support, any moves towards non-violent healing/prevention/accountability must take these factors into account.

Therefore, while the project will aim to not widen the net any further or engender new forms of harm, it will have to function within the existing structure in an effort to shift mindsets and create other outlets of healing, prevention, and accountability. This is because these structures are deep-rooted and difficult to immediately dismantle, especially in the absence of viable alternatives. With this project, I am choosing to prioritize simultaneous healing and immediate accountability instead of pulling the rug out from underneath victims in an already-inadequate justice system, which would create a legal vacuum that might increase violence and pain within the community.

Project Implementation and Outcomes

My project will aim to address the ongoing pain of victims and affected people and introduce non-punitive, non-violent forms of healing and prevention, ultimately leading to accountability as envisioned in abolitionist theory. For my experiment, I will be proposing a two-tiered approach that will result in healing and prevention in the short-term (5-7 years) and will hopefully lead to a gradual reduction in harm and the eventual abolition of the death penalty in the long term (7+ years). That is the future that my experiment would begin to build—addressing trauma and pain now without further carceral creep.

This would be done through a two-pronged approach, with each having both short-term and long-term restorative and transformative outcomes. First, I would like to see the creation of healing circles for anyone impacted by GBV, anywhere in the country. Second, I would like to design an educational campaign to disseminate GBV-awareness in communities, perhaps beginning with mosques and schools.

Since carceral solutions are thought to be community-based and driven by the sentiments of those impacted, healing circles would provide victims and the affected with alternative outlets for their anger, pain, and grief. These discussion-based groups would be victim-centered, not victim-led, thus putting the responsibility of guiding affected individuals through the healing process on licensed professionals. They would also have the space to expand into systems of support and action within communities, thus building "analytical and material spaces to imagine and experiment with more authentic forms of safety" (Davis et al. 117). Especially for women, they could help create structured conversations and mobilization effectively placing power back into victims' hands, allowing them to verbalize and unpack their pain as well as their joy and aspirations, and then imagine new ways forward together.

As mentioned previously, these healing circles could not and would not replace carceral solutions immediately. However, they would provide the public with safe and productive spaces to address their collective trauma, such as that of women after Noor's death. They would have the potential to support survivors, empower women who might find themselves in dangerous scenarios in the future, and help the affected process and heal. However, it would be essential to extend these groups to low-income areas, small cities, and villages, ensuring that people who have the least access to such resources and spaces but are often the most affected can find their way to these conversations and can contribute to negotiating and paving abolitionist futures according to their specific needs.

As previously mentioned, carceral solutions such as the death penalty are frequently framed in a religious light and upheld for religious reasons. However, in his book We Are Our Own Liberators, Jalil Muntaqim introduces the idea of using religion for liberation instead. In the context of mass incarceration, he urges all religious denominations to "aggressively challenge and politically counter" racism, imperialism, and the prison system (259). In addition, he suggests that multiple religious traditions be incorporated to liberate society from "conditions that hinder spiritual growth and evolvement," "deny the basic human quality of life," and exploit and oppress communities (261). He calls for the "transformation of the self-identity" of communities, the creation of solidarity, and the translation of this "enhanced self-image and commitment to solidarity" into political action (261).

Within this framework, using religious education to transform values and mindsets by invoking various religious traditions and bringing together multiple classes/backgrounds of people can be a potent tool to prevent or deter harm before it occurs. For example, GBV could be addressed responsibly and repeatedly, using religious contexts and within religious spaces. Clerics in Pakistan exert a great deal of influence over most communities, and religious spaces are also centers of community interaction. Therefore, clerics' endorsement of liberatory messaging through sermons and madrassahs could prove to be extremely valuable, especially since it has proven to be useful in family planning and COVID-19 vaccination campaigns. It is important to acknowledge that a great deal of GBV occurs within religious institutions. Nevertheless, there is the possibility that the distribution of this knowledge through the pulpit could also help inhibit this sort of violence by raising awareness within religious communities, creating solidarity and defiance, and deterring perpetrators within them.

In addition, disseminating information through schools—via guest visits during morning assemblies, for example, if not through school officials and teachers—can be another way of preventing harm. Through these strategies, which will require careful planning in terms of content and delivery, adults and children alike can unlearn the deep-rooted misogyny and vio-

lent tendencies that fuel GBV. They might also gradually be encouraged to turn to non-violent, non-punitive approaches to justice. Thus, using education—both religious and secular as a way to reshape dominant conversations and cultural consciousness can be more effective at deterring harm than caging or hanging humans.

As Nils Christie would put it, these two avenues—healing circles and education-will allow communities to take back conflicts-or better yet, prevent or intervene in harmful processes instead of reverting to state-sponsored violence such as the death penalty. However, I am skeptical of the "romanticized notion of communities" keeping women and children safe without there being sufficient healing and prevention first (Davis et al. 183). This is because there is the possibility that communities may perpetuate further violence or hurt victims through shame, alienation, ostracism, or physical harm. Indeed, one of the reasons cited by some Pakistani officials and judges in favor of the death penalty is that it can prevent extrajudicial violence, although it is more common to hear of religiously-motivated lynchings than the carrying out of formal death sentences (FIDH and HRCP 19). However, once a sizable percentage of a community has unlocked new ways to process, heal from, and think about violence, wrongful convictions or extrajudicial violence can also be prevented due to the decreased public pressure to hang perpetrators, for example.

There is potential within this experiment for the incorporation of concepts such as Diyat (blood money in Islam as an alternative to equal retaliation) and Jirga (Pashtun community-based conflict resolution councils). However, since both of these concepts require further scrutiny to be implemented in a manner that centers on victims and doesn't perpetuate harm, they are beyond the scope of this essay. Nevertheless, we can only abolish structures such as the death penalty if we adequately address ongoing pain and hurt through healing and prevention techniques, whatever form they might take. Once we have achieved that, there will ideally be less resistance to abolishing the death penalty, because it will no longer be perceived as an essential part of healing, prevention, or accountability.

In conclusion, the use of and need for punitive practices might decrease significantly if Pakistani society addresses its collective pain, grief, and anger. Victims and the affected processing their needs and emotions through healing circles can build sturdy systems of support and facilitate healing in ways that aren't rooted in cycles of violence. Furthermore, educational campaigns can also help create critical conversations around carcerality and GBV, thus dismantling structures of oppression and preventing harm. Both of these branches of my experiment will eventually lead to accountability within an abolitionist framework instead of a carceral framework. A resolution where the root causes of violence are addressed, where there exist ecosystems of safe and productive communitybased conflict resolution, and where capital punishment is never seen as a viable solution.

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Works Cited

- Christie, Nils. "Conflicts as Property*." Restorative Justice, 2017, pp. 37–51., https://doi.org/10.4324/9781351150125-2.
- Conquergood, Lorne Dwight. "Lethal Theatre: Performance, Punishment, and the Death Penalty." Theatre Journal, vol. 54, no. 3, 2002, pp. 339–367., https://doi.org/10.1353/tj.2002.0077.
- Davis, Angela Y., et al. Abolition. Feminism. Now. Hamish Hamilton, an Imprint of Penguin Books, 2022.
- "Death Row Population in Pakistan." Death Row Analysis: Justice Project Pakistan - Death Penalty Database, https://data.jpp.org. pk/page/9pa1cdfd4u.
- Dubler, Joshua, and Vincent W. Lloyd. "The Political Theology of Mass Incarceration." Break Every Yoke, 2020, pp. 65–104., https://doi.org/10.1093/oso/9780190949150.003.0003.
- FIDH, and HRCP. "Mission of Investigation: Slow March to the Gallows Death Penalty in Pakistan." 2007, https://www.refworld. org/pdfid/46f1469f0.pdf.
- Kaba, Mariame. We Do This 'Til We Free Us. Haymarket Books, 2021.
- Lydon, Jason. "Abolitionist Questions to Ask of a Proposed Reform." 2019.
- Muntaqim, Jalil. We Are Our Own Liberators: Selected Prison Writings. Arissa Media Group, 2010.
- "Pakistan Anti-Rape Ordinance Signed into Law by President." BBC News, BBC, 15 Dec. 2020, https://www.bbc.com/news/ world-asia-55314493#: ~:text=Pakistan's%20president%20has%20signed%20a, chemical%20castration%20of%20some%20offenders.
- Pedlar, Alison, et al. "Incarceration and Community Re-Entry for Women." Community Re-Entry, 2018, pp. 1–35., https://doi.org/ 10.4324/9781351204477-1.
- Temkin, Moshik. "The Great Divergence: The Death Penalty in the United States and the Failure of Abolition in Transatlantic Perspective." SSRN Electronic Journal, 2015, https://doi.org/ 10.2139/ssrn.2662109.
- Waheed, Alia. "Pakistan Reckons with Its 'Gender Terrorism Epidemic' after Murder of Noor Mukadam." The Guardian, Guardian News and Media, 2 Aug. 2021, https://www. theguardian.com/global-development/2021/aug/02/pakistanreckons-with-its-gender-terrorism-epidemic-after-of-noormukadam.

About the Author

This research paper was written for "Incarceration Nation," a class that gave me the space and lexicon to begin to unpack difficult concepts. The focus of this paper was a result of the rage and grief felt by myself, millions of Pakistani women, and women worldwide, as well as the nagging feeling that there was a discrepancy between what I was learning about abolitionism and what felt right. I was interested in exploring gender-based violence, transformative justice, and non-carceral solutions within a Pakistani context without discounting abolitionist perspectives. I would encourage other undergraduate students to lean into questions that make them uncomfortable and seek answers, even if they are imperfect.

Investigating the Role of TRMT1L in tRNA Modification

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Introduction

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All transfer RNAs (tRNAs) are subject to post-transcriptional modifications at various positions which contribute to the stability and translational efficiency of the molecule (Pereira *et al.*, 2018). tRNAs are highly modified and have about 13 modifications per molecule. These modifications include methylation at different positions of the tRNA, and a lack of these modifications leads to disease phenotypes such as cancer, mitochondrial dysfunction, and neurological disorders (Pan, 2018). A loss of modification can be caused by the improper folding, loss of aminoacylation, and degradation of the tRNA. The modification that we are interested in studying is methylation on tRNA positions by possible methyltransferase enzymes, in this case, TRMT1L.

tRNAs are RNA molecules that participate in protein synthesis by decoding the message in mRNA into proteins. Through aminoacylation, tRNAs carry the amino acids and bring the correct one to the ribosome based on the mRNA sequence. The important parts of a tRNA include the 3' hydroxyl group and anticodon, which form a bond with the amino acid, along with the D-arm and T-arm, allowing for specificity and efficiency of the tRNA in translation.

In yeast, the TRM1 gene encodes an RNA methyltransferase enzyme that forms dimethylguanosine (m2,2G) by adding two methyl groups on the guanosine. One of the homologs found in humans is TRMT1 and previous research has found that TRMT1 encodes a tRNA methyltransferase enzyme that catalyzes dimethylguanosine (m2,2G) in tRNAs at position 26 (Dewe et al., 2017). When this gene was knocked out, the m2,2G modification is reduced to background levels, which indicates that TRMT1 catalyzes this modification. The features of this TRMT1 include a mitochondrial targeting signal, SAM methyltransferase domain, nuclear localization signal, and zinc finger domain. Previously, it has been found that frameshift variants in this gene are linked to intellectual disability disorders and developmental delay (Zhang et al., 2020). These results suggest that TRMT1 plays a role in proper neurodevelopment.

Vertebrates also encode TRMT1L, another homolog of yeast *TRM1* that could catalyze modification. TRMT1L also has a zinc finger domain, SAM methyltransferase domain, and nuclear localization signal (Dewe *et al.*, 2017). Unlike TRMT1, TRMT1L lacks a mitochondrial targeting signal. Moreover, TRMT1 and TRMT1L exhibit distinct localization patterns in the nucleus (Dewe *et al.*, 2017). It has been found that mice lacking TRMT1L exhibit altered motor coordination and ab-

normal behavior (Vauti *et al.*, 2007). However, we do not know the biological role or substrates of TRMT1L. This paper reports the cloning and expression of human TRMT1L for eventual characterization. Determining if and how TRMT1L modifies RNA would provide us with more information about tRNA modifications and their role in human diseases such as neurological disorders.

Results

Generation and Testing of Lentiviral Constructs Expressing TRMT1L Protein

Here, we aim to express TRMT1L in cells using a lentivirus as the vector encoding TRMT1L. Lentivirus is a retrovirus that integrates into the genome of the cell type and expresses the gene of interest. We designed primers for PCR to amplify N-terminally Strep-tagged TRMT1L and then used T5 exonuclease assembly cloning methods to assemble the insert into the pLenti vector. Both untagged and tagged TRMT1L were inserted into the vector. The Strep-tag is an affinity tag that can be fused to proteins and easily detected to purify the protein of interest, TRMT1L.

To confirm whether this cloning was successful, whole plasmid sequencing was performed to compare the predicted sequence with the actual sequence of the plasmid. The sequencing results were aligned with full-length Strep-TRMT1L and TRMT1L plasmids in lentiviral vectors. For the Strep-TRMT1L plasmid, no mismatches, gaps, or insertions were found with 10,195 bases aligned (Figure 1). This sequencing confirmed the Strep-TRMT1L was correctly cloned into the lentiviral vector. For the untagged TRMT1L plasmid, one gap was found in the WPRE sequence missing a T with 10,077 bases aligned (Figure 2). This sequence is a woodchuck hepatitis virus post-transcriptional regulatory element, and it can increase expression of viral vectors (Higashimoto *et al.*, 2007).

Next, we transfected this plasmid construct along with the virus packaging and envelope constructs into 293T human embryonic cells. After this, we harvested protein extracts and probed them for TRMT1L using Western blotting procedures. The expected protein size for TRMT1L is 81.7 kDa. In Figure 3, the blot was probed with anti-TRMT1L and untagged TRMT1L is being expressed as evidenced by the band found at the expected size. It can also be seen that the Strep-tagged TRMT1L expressed the protein of interest at 85.1kDa, a higher band than the expected size range. There are also two lower green bands that indicate possible degradation of the Strep-tagged protein. When the blot was probed with anti-

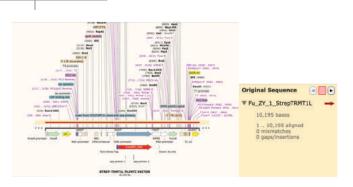


Figure 1) Strep TRMT1L sequencing alignment results. The red indicates the sequencing results aligning with the vector and all its features.

Strep, a red band was seen in the Strep TRMT1L at the expected size range. Another red band was seen, which could indicate degradation of the protein at the C-terminus (Figure 4). In Figures 3 and 4, the empty lane and the pLenti vector had no expression of TRMT1L or the Strep-tag, which was expected for the negative controls. These results validate that the TRMT1L protein was expressed.

Validation of Lentiviral Infection

The media was also collected from transfected cells to test for viral production. Lentivirus was used as the vector to carry the TRMT1L gene, infect the cells and integrate the gene into the genome of the cells. To determine whether lentivirus was produced, the selection process was used to determine which cells integrated our plasmid with puromycin resistance. Human 293T cells were infected with empty virus, pLenti vector, and Strep-TRMT1L virus. The cells were infected with the produced virus and polybrene reagent to increase the efficiency of gene transfer. After infecting the human 293T cells with the pLenti vector, the cells were examined by microscopy, one day post-infection. The pLenti vector expresses GFP, so efficient infection of the vector would show a GFP signal. From Figure 5A, the images taken from the microscope, a strong

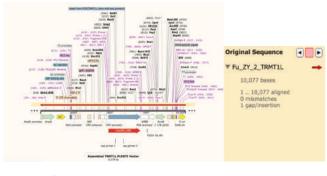
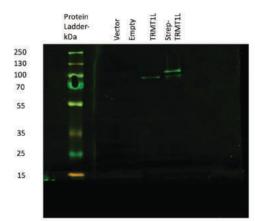


Figure 2) Sequencing alignment results of TRMT1L. The red indicates the sequencing results aligning with the vector and all its features. The orange indicates a gap in the sequence missing from the alignment.

GFP signal can be seen in the vector infected cells. The green in the cell indicates that the cells are producing GFP from the plasmid that was integrated into the cells. This validates the efficient infection of the lentivirus and integration of the vector plasmid into these cells. Figures 5B and 5C show no GFP signal since the empty and Strep-TRMT1L plasmids do not contain a GFP tag.

Since the plasmid contained puromycin, puromycin could be used as the selective media. Since the 293T human cells that were mock-infected should not express the puromycin resistance gene, they should not survive and thus served as a negative control in this experiment. The human 293T cells were examined by microscopy after 2 rounds of selection with puromycin. From Figure 6, it seems that nearly all the cells that were mock-infected were shriveled up and not spread out like a living cell. The microscopy figures of the living 293T cells are not shown for the infected cells, but this experiment will be repeated to obtain this data. With this information, the cells in the experimental plates should have integrated the TRMT1L gene, and protein could then be extracted and analyzed to determine whether there was any expression of the protein.



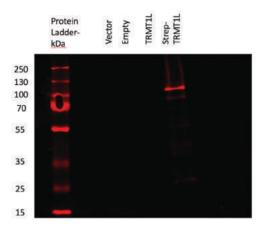


Figure 3. Western Blot probed with MαTRMT1L for primary and GαM for secondary under 800 nm channel. Lanes are pLenti CMV Puro Vector, Empty with no transfer plasmid, TRMT1L, and Strep-TRMT1L.

Figure 4. Western Blot probed with M α Strep for primary and G α M for secondary under 700 nm channel. Lanes are pLenti CMV Puro Vector, Empty with no transfer plasmid, TRMT1L, and Strep-TRMT1L.



Figure 5. (A) Human 293T cells infected with pLenti vector under the GFP channel. (B) Human 293T cells infected with Strep-TRMT1L virus under the GFP channel. (C) Human 293T cells infected with empty virus under the GFP channel

Discussion

In this study, the aim was to express TRMT1L using the pLenti virus as a vector. First, the cloning of TRMT1L into a pLenti virul vector had to be confirmed. For the pLenti-Strep-TRMT1L plasmid, the whole plasmid sequencing results confirmed that it was correctly cloned into the lentiviral vector. Through the alignment of the DNA sequence sent in, it was found to be an exact match. For the TRMT1L plasmid, there was one gap in the WPRE sequence which could possibly influence expression of the gene. Since a base was missing, it is unknown whether this would have a downstream effect on protein expression of this plasmid. This could be determined by looking at the expression levels of the protein through probing of the protein extract from the cells.

Since our constructs were confirmed to be correct, indication of successful infection and integration of the lentivirus produced had to also be confirmed. The optimized viral infection procedure promoted increased efficiency of the virus. From the GFP channel in Figure 5, it could be seen that there is a strong GFP signal indicating that the pLenti vector plasmid was integrated into the genome of human 293T cells. The successful integration of the lentivirus possibly indicates that the viral DNA could also integrate into the genome of the cells and express the TRMT1L protein. In Figure 6, the control plates show all dead cells, indicating that all the cells on the experimental plates have the viral DNA integrated into them and could be further analyzed. Due to this success, this protocol for using lentivirus to rescue protein expression can be used for further experiments done in the lab.

In the future, we hope to identify the function and substrates of TRMT1L. This can be done through mass spectroscopy experiments to identify protein interactions of the TRMT1L protein and whether it has similarities to TRMT1 interactions. Lastly, another future experiment would be to perform RNAsequencing of the interacting RNAs. This information will help to determine both the substrates of TRMT1L and if it catalyzes the m2,2G modification. With knowledge of the TRMT1L substrates and catalysis, we could compare its role to TRMT1 and see if TRMT1L's function is independent. We can then determine a mechanism by which TRMT1L's function is independent of TRMT1 and analyze what happens when there are variants in TRMT1L in relation to human disease.

Materials and Methods

Designing Oligos for PCR Amplification of TRMT1L

The target sequence was obtained from the N-terminally tagged TRMT1L SnapGene sequence file from the Fu lab. Oligos with 21 bases homologous to the vector along with primers homologous to the target sequence were constructed to be used for TEDA Cloning. Forward primers for Streptagged N-TRMT1L and untagged TRMT1L were designed along with the same reverse primer for TRMT1L. In Table 1, the lowercase sequence refers to the region homologous to the vector and the uppercase refers to the region from N-TRMT1L.

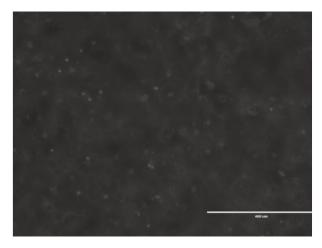


Figure 6. Human 293T cells after 2 rounds of Puromycin selection. Human 293T cells after 2 rounds of Puromycin selection.

TEDA Cloning

The 3rd generation pLenti CMV GFP Puro Vector was received from Addgene and Stbl3 bacteria were streaked out on lysogeny broth (LB) and ampicillin (AMP) plates to be grown overnight at 30°C. The pLenti Vectors were then inoculated into 5mL LB and 5 μ L AMP (100 μ g/ml). DNA was then extracted using the QIAGEN Miniprep Kit Protocol with an extra step of washing the spin column with Buffer PB to remove nuclease activity with endA+ strains. The vector was then digested with BamHI and SaII and the bands were extracted from a 1% agarose gel with TBE.

Oligos	Sequence
Strep-TEDA-F	tagaagacaccgactctagag-ATGGCCAGCGCATGGA
TRMT1L-TEDA-F	tagaagacaccgactctagag-ATGGAGAATATGGCGGAGGAG
TRMT1L-TEDA-R	ttgtaatccagaggttgattg-TTA CGCTTACCATCTTCTGCAGCCA

 Table 1. Table of oligos for PCR Amplification of TRMT1L.

Two PCR reactions for the untagged and Strep-tagged TRMT1L were carried out with the Vector, 1x Herculase enzyme, dNTPs (200 μ M), forward primer for STREP or untagged, reverse primer, N-TRMT1L template, Herc II Buffer, and H₂O. The Thermocycler protocol was a 2 stage PCR with the first stage of 95 degrees for 30 seconds (denaturing), 55 degrees for 30 seconds (annealing), and 72 degrees for 2 minutes (extension) and this was repeated 5 times. In the second stage, the protocol was 95 degrees for 30 seconds (denaturing), 60 degrees for 30 seconds (annealing), and 72 degrees for 2 minutes (extension) and this was repeated 30 times. There was a hold at 72 degrees to make sure everything extended to the end. The PCR product was run on a gel and the bands were extracted.

The TEDA reaction was performed with the pLenti vector, inserts from PCR (Strep and untagged), 5x TEDA enzyme mix, and H_2O for a total volume of 20 µl. The vector and inserts were at a ratio of 1:4 using 70 ng of the vector DNA. The negative control included an elution buffer instead of a PCR insert. The plasmid was assembled at 30 degrees for 40 minutes and then put on ice to stop the reaction. The plasmid was then transformed into NEB Stbl bacteria. Confirmation of insertion was determined through a diagnostic digest with EcoRI and SalI.

Whole Plasmid Sequencing

To confirm that the cloning process was successful, the pLenti-Strep and pLenti-TRMT1L were sent to SNPsaurus for Whole Plasmid sequencing. 300 ng of DNA at a concentration of 30 ng/ μ L for a total volume of 10 μ L was used for both constructs.

Cell Culture

293T human embryonic cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 1% penicillin and streptomycin (P/S), 1% glutaMAX, and 10% fetal bovine serum (FBS). The cells were passaged using the standard procedures with 0.25% trypsin (Invitrogen).

Calcium Phosphate Tranfection

300,000 293T cells were plated into four wells in a six-well plate. The first well was transfected with the pLenti vector as a negative control, the next well contained a virus without the transfer plasmid, the third well contained the TRMT1L, and the last well contained Strep-TRMT1L. Each plasmid was

transfected into the cells with 900 ng of Pax packaging plasmid DNA and 500 ng of MD2G envelope plasmid DNA for a total of 2.5 μ g of DNA. These cells were incubated for 24 hours before the media was changed following the standard cell culture protocols (Fu Lab). After 24 hours, the virus media was collected using a syringe and 0.45 μ M filter tip. The cells were harvested using the Hypotonic Lysis Buffer following Cell Lysis and Protein Extraction standard procedure, though HLB and Np40 were used in equal volumes (Fu Lab).

Infection of Cells with Lentivirus

300,000 cells were plated into each four wells in a six-well plate on Day 1. The first well contained media and cells, the second well contained an empty virus, the third well contained a virus with the TRMT1L gene, and the fourth well contained a virus with the Strep-TRMT1L gene. On day 2, the 1mL virus aliquot was added to 2mL of media with polybrene at a concentration of 10µg/mL. Polybrene is hexadimethrine bromide and it is a catanionic polymer used to increase the efficiency of transduction of cells with lentivirus (Abe et al., 1998). After letting the viral media sit in the cells for 24 hours, fresh media was added to the cells, and then the selection process began. Two rounds of selection were completed with 2µg/mL Puromycin (Gibco). After selection, cells without the fully constructed lentiviral vector in control plates should be dead. The negative controls were the well with the empty vector virus and the well with only media and cells. The living cells were then moved to a 10cm dish and continued to grow. To optimize our procedure, for further infection procedures, only polybrene and the 1mL virus were added on day 1 to increase the efficiency of viral production. To detect if the cells were infected, the cells were inspected under the microscope with the GFP channel to see if the cells with the vector were growing.

Western Blot

The cell extracts were run on a 10% SDS Page Gel and transferred to a PVDF membrane using standard procedures (Fu Lab). The membrane was probed with a primary mouse TRMT1L antibody at a 1:1000 dilution, with a secondary goat antibody at a 1:10,000 dilution and imaged using an 800 nm channel. After this, the membrane was probed with a primary mouse Strep antibody at a 1:1000 dilution, with a secondary goat antibody at a 1:10,000 dilution and imaged using a 700 nm channel.

References

Dewe, J. M., Fuller, B. L., Lentini, J. M., Kellner, S. M., & Fu, D. (2017). TRMT1-Catalyzed tRNA Modifications Are Required for Redox Homeostasis To Ensure Proper Cellular Proliferation and Oxidative Stress Survival. Mol Cell Biol, 37(21). https://doi.org/10.1128/MCB.00214-17

Higashimoto, T., Urbinati, F., Perumbeti, A., Jiang, G., Zarzuela, A., Chang, L. J., Kohn, D. B., & Malik, P. (2007). The woodchuck hepatitis virus post-transcriptional regulatory element reduces readthrough transcription from retroviral vectors. Gene Ther, 14(17), 1298-1304. https://doi.org/ 10.1038/sj.gt.3302979

Pan, T. (2018). Modifications and functional genomics of human transfer RNA. Cell Res, 28(4), 395-404. https://doi.org/10.1038/s41422-018-0013-y

Pereira, M., Francisco, S., Varanda, A. S., Santos, M., Santos, M. A. S., & Soares, A. R. (2018). Impact of tRNA Modifications and tRNA-Modifying Enzymes on Proteostasis and Human Disease. Int J Mol Sci, 19(12). https://doi.org/10.3390/ ijms19123738

Vauti, F., Goller, T., Beine, R., Becker, L., Klopstock, T., Holter, S. M., Wurst, W., Fuchs, H., Gailus-Durner, V., de Angelis, M. H., & Arnold, H. H. (2007). The mouse Trm1like gene is expressed in neural tissues and plays a role in motor coordination and exploratory behaviour. Gene, 389(2), 174-185. https://doi.org/10.1016/j.gene.2006.11.004

Zhang, K., Lentini, J. M., Prevost, C. T., Hashem, M. O., Alkuraya, F. S., & Fu, D. (2020). An intellectual disabilityassociated missense variant in TRMT1 impairs tRNA modification and reconstitution of enzymatic activity. Hum Mutat, 41(3), 600-607. https://doi.org/10.1002/humu.23976

About the Author

After my sophomore year, I completed a Research Experience summer with Dr. Dragony Fu, studying tRNA modifications for ten weeks. My research project is an extension of the summer project studying tRNA methyltransferases as independent research courses. I am interested in RNA biology and RNA modifications because of their connection to human disease. My advice to other undergrads interested in research is to take time to understand how the methods connect to the bigger picture in what your research is trying to find!

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Evolution and Evil-ution: Revealing the Assertion of National and Cultural Identities Through the Twentieth-Century Transformation of la Calavera in Mexico and Bawon Samdi in Haiti

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American and European onlookers tend to marvel at Day of the Dead celebrations, simultaneously fascinated and perplexed by what they perceive as an unusually lighthearted conception of death. While such celebrations occur throughout Latin America, Mexico in particular has conflated Day of the Dead imagery, especially skulls and skeletons, into symbols of national identity. These same onlookers, however, misinterpret the extent to which Haitian Vodou functions as a religion, and associate it and its characteristic figures with sorcery, black magic, and revenge. Thus the rituals that transpire on Fèt Gede, a Haitian function, do not inspire the same amount of international zeal and intrigue as those that characterize Day of the Dead.

Nevertheless, both of these celebrations have become associated with specific icons: la calavera in Mexico and Bawon Samdi in Haiti. While it is clear that these icons are symbolic of the holidays themselves, how they represent national and cultural identity remains underexplored, especially considering the extent to which both have drastically changed since their inception. To understand the modern-day depiction of these icons, it is essential to start at their origin as uniquely identifiable entities at the turn of the twentieth century. It is essential to consider authenticity and utility-how these characteristic symbols operate within and for a community. This examination thus poses the following questions: from the beginning of the twentieth century to the present day, how has the modern portrayal of la calavera in Mexico and Bawon Samdi in Haiti emerged, and what do their transformations reveal about the respective communities interacting with them?

In tracing the trajectory of cultural, economic, and political phenomena over the roughly century-long lifespan of these icons, this comparative project will attempt to show how their fundamental association shifted according to local, national, and international needs. In the first part of this paper, the evolution of la calavera from the turn of the twentieth century will be explored. The second part will follow suit with the representation of Bawon Samdi in Haiti and a comparison of the two icons. With each figure originating at the turn of the twentieth century, I intend to articulate how both of them underwent two fundamental contextual transformations —first in the 1920s-1940s and subsequently in the 1970s-1980s. While the Mexican calavera maintained a threshold monetary association, I will argue that the second transformation of the 1970s-1980s officially rendered it into cultural capital—introducing it as a national symbol for the purpose of economic development and international exploitation. While only a single version of the modern-day calavera exists in Mexican popular culture, it is shared by both Mexico and the world at large. The evolution of Bawon Samdi proves more challenging to analyze, as Vodou has been periodically suppressed and reinvigorated throughout Haitian history. Nevertheless, following the 1970s metamorphosis, a rendition of Bawon Samdi emerged as an evil, vengeful spirit for the international onlooker. For Haitians, however, a liminal Bawon Samdi arose, interacting with the people themselves—traversing the crossroads between life and death, human and god, body and soul. While la calavera has made the interaction with Mexican national identity wholly accessible, only those within the Haitian community can fully appreciate Bawon Samdi as a crossroads figure between life and death. While seemingly different in function, la calavera in Mexico and Bawon Samdi in Haiti adequately fulfill the needs of their respective communities as manifestations of cultural and national identity with the most striking difference between the two being who can associate with these authentic identities.

Part I: La Calavera

Before analyzing the modern-day calavera icon and how it has become associated with Mexican national identity and a unique view of death, it is essential to briefly elucidate its origins. According to Stanley Brandes, the Mexican people interpret death as an "inevitable, social equalizer,"-an attitude which has manifested verbally and artistically in Day of the Dead celebrations. In this milieu, death and its associated icons, especially skulls and skeletons, do not represent morbidity, but humor; yet the skeletal imagery associated with the Day of the Dead has not always been so playful. Elizabeth Carmichael and Chloe" Sayer speculate about the potential pre-Hispanic or Catholic origins of Day of the Dead, assessing two alternative cosmological interpretations responsible for the skull imagery. Scenes of sacrifice, gods of death, and skull racks (tzompantli) dominate the imagery of the Mesoamerican post-Classic period (c. AD 900-1520), while European memento mori became ubiquitous after the Spanish conquest in the New World. Nevertheless, Carmichael and Sayer fail to establish a connection between these two religious traditions. In Todos Santos in Rural Tlaxcala, Hugo Nutini fills this gap by discussing the syncretic nature of skull imagery in a hybrid religious context, noting how the original Day of the Dead skull was a conglomerate of pre-Hispanic icons and Christian symbols of death. I will advance Nutini's argument by discussing how the first version of the skull as we know it today, commencing with the calaveras of the turn-of-the-century broadside movement, incorporated both pre-Hispanic and-Catholic imagery, eventually associating with national identity. Roberto Berdicio and Stanley Appelbaum assert that these two traditions have "blended in the average stoic, but far from humorless view of death," a blend where the investigation begins.

Having introduced the notion of religious syncretism, it is essential to discuss Mexico's peculiar racial ideology of mestizaje and how this ideology cultivated national identity after the Mexican Revolution. Due to the violence and force that the Spanish conquest introduced into Mexican history, Guillermo Bonfil Batalla holds that Mexico is composed of two fundamentally opposed civilizational projects: the Indian "Mexico profundo" and Western "imaginary" Mexico, which cannot coexist "in harmony, to each other's benefit." In Bonfil Batalla's view, while mestizaje might imply racial mixing, it is not truly possible for the respective cultures affiliated with these races to merge. Yet, this argument is highly untenable when considering the Day of the Dead, which, quite intentionally, fuses cultures in a celebration that would not otherwise be possible. When analyzing the Day of the Dead celebrations in Lordes Portillo's La Ofrenda: The Days of the Dead in Oaxaca, released just after Bonfil Batalla's 1985-1987 analysis, it is obvious that these purportedly incompatible civilizational models overlap. When portraying the ofrendas, crucifixes lay atop the cempasúchil flowers, the Virgin Guadulupe is syncretized with both the Virgin Mary and Aztec godmother Tonantzin, and cross-shaped pan de muerto are sold in the seasonal markets. Bonfil Batalla's argument is also problematic when considering his proposed survival mechanism of religious appropriation, in which Indians have only practiced Catholicism as a means of survival, using the veneer of assimilation to perpetuate their indigenous culture. Yet, every element associated with the modern rendition of la calavera upholds the entrenchment of mestizaje in Mexican national identity at the turn of the twentieth century.

The first incarnation of la calavera as we know it today originates from the broadside movement of the late-nineteenth and early-twentieth centuries. One figure in particular, José Guadalupe Posada, left a legacy both on future artists, and collective national consciousness. While his art was not celebrated at the time, his work would ultimately connect ideas of nationhood to the la calavera figure. The broadside movement saw the proliferation of cheaply-printed urban popular art, which commented on political and social events, usually in a satirical manner. The mass proliferation of such art is largely attributed to the "penny press" the prensa de un centavo, which extended the audience of political discourse. When describing the broadside movement, the term "calavera" has two referents-the skull imagery itself, and the satirical epithets associated with them. These verses often portrayed political cynicism in a form of "peaceful protest," and symbolically situated "public figures on the level of the common

man." The most important implication of the broadside movement is how it extended to the population at large, picturing the "daily pleasures and chagrins of the common people from a proletarian or lower middle class P.O.V." The image of la calavera was not exclusive to an elite few, but the population at large.



Figure 1. José Guadalupe Posada, La Catrina (1910)

Furthermore, Posada's art in particular seems to connect the icon of the skull with the inevitability of death in a humorous manner. With the majority of his calaveras printed around the beginning of the Mexican Revolution, it is not surprising that his work both alludes to contemporary political developments, and grapples with the prospect of death. The death rate from 1910-1920 ranged from 46.6 to 48.3 per thousand per year demonstrating the horrific violence that ensued in this time.14 Perhaps a normalization of the omnipresence of death motivated Posada. In 1910, he printed what has today become his most celebrated calavera-La Catrina. By portraying a skeletal servant girl in European garb, Berdecio and Appelbaum suggest that Posada is championing death to the aristocratic ways of the Porfiriato. In addition to the visually mocking depictions of death, Diane Miliotes describes how Posada's La Calavera Bicicleta upholds the certitude of death through his words, "la muerte inexorable no respeta ni a los que veis aquí en bicicleta" [inexorable death does not respect even those seen here on a bicycle]. While Posada's work evolved into a popular form of resistance for a populace "needy of an outlet for public criticism," it concurrently extended to a mass audience, displaying a nonchalant, light-hearted approach to death. Nevertheless, its affiliation with national identity would not be realized until the decades after the Mexican Revolution, when the indigenismo movement nationalized la calavera.

Posada's work was wholeheartedly celebrated in the context of national identity only after his death. It is not until the 1920s when Posada's calaveras were "discovered" by French artist Jean-Charlot that the intersection of death, humor, and national identity came into being. This "discovery" transpired concomitant to artistic and literary movements in the 1920s– 1940s, which sought to elucidate Mexican history and rediscover the legacy of indigenous populations. Desperate to reconstruct a "culturally homogeneous society" grappling with the universality of death, mestizaje was increasingly promoted as a means to unify national identity. The art that consequently flourished after the collective national death of the Mexican revolution expressed a desire to uncover the indigenous aspects of this national identity. Many muralists, especially Diego Rivera and José Clemente Orozco believed that Posada's work aligned with the revolutionary cause, and marveled at his "forward-looking power to the oppressive Diaz regime," inspiring them to solidify the connection between the calavera imagery and national identity. All the while, the indigenismo movement, which recognized images "of the Indian" as "official symbols of nationalism" solidified in the decades following the Mexican Revolution. Therefore, Posada's 1910 depiction of La Catrina must be put into conversation with Diego Rivera's 1946-7 mural, Dream of a Sunday Afternoon in Alameda Central. Unlike Posada's Catrina, Rivera's subject wears a boa around her neck which can be interpreted as a reference to the Nahuatl creation myth, and Quetzalcoatl-"the 'Plumed Serpent', the great Mesoamerican creator god." Rivera's Catrina was additionally flanked by quintessential figures in Mexican history. Here, la calavera did not merely insinuate death, but was associated with Mexican national identity through its introduction into Mexico's history.



Figure 2. Diego Rivera, Dream of a Sunday Afternoon in Alameda Central (1946-7)

Now that the calavera has been attached to a satirical outlook on death, the populace at large, and national identity, its last attachment relevant to this analysis must be evaluated-profit. While Posada's work had monetary value at the beginning of the twentieth century, its raison d'être was not merely for the purpose of accumulating capital. Yet a framework shift would occur in Mexico starting in the 1970s that would permanently alter the purpose of la calavera. When conducting ethnographic fieldwork in Tzintzuntzan in 1974, Brandes experienced first-hand the government's efforts to promote domestic and international tourism for economic stimulation. In 1971, large-scale tourism was encouraged by the government in the implementation of "ferias," or fairs, that hosted commercial activities and recreational events. Not only has tourism inaugurated a lucrative craft industry, but because of the calavera's attachment to Mexican national identity, it has also made skull and skeleton trinkets available all year long. While the image I have detailed may seem exploitative to Mexican culture, it is important to note that this increased tourism is not necessarily resisted by members of the local community. In conducting interviews of locals throughout Mexico in 1988 and 1989, Carmichael and Sayer found that Mexican communities generally welcome these visitors and the capital they bring with them. At the ferias in Toluca, alfenique artist Consuelo Garcia Urrutia appreciates how the governmental efforts of the past couple decades have "enhanced Toluca's image," imploring "visitors to spend money in the town," and ultimately, on her own products. The governmental actions promoting tourism incited a ripple effect that has propagated throughout Mexico to the present.

Since the uptick in tourism from the 1970's, the calavera now relates to overarching trends of secularization, modernization, and globalization. When considering the consequences of globalization, neighboring countries have been affected the most, especially the United States and Mexico. The profitseeking venture beginning in the 1970s was only exacerbated by NAFTA, which increased international tourism, media coverage, and the flow of labor and capital across the Mexico-United States border. In addition to the effects of globalization, Nutini makes a critical distinction between secularization and modernization-while the infiltration of urban developments through modernization may keep a community's structure intact temporarily, its prolonged extension will result in secularization leading to deterioration and attrition. While the version of la calavera I have traced from the beginning of the twentieth century was never necessarily associated with specific practices, I argue that its modern "secularization" stems from the fact that it has become detached from the ritual celebration of the Day of the Dead itself. Instead, la calavera has become cultural capital embracing Mexican nationality in general, rather than any specific celebration, to maximize profitability. To conclude the economic component of my analysis, it is worth citing Nutini, who holds that secularization is complete once the "economic domain [is] on par with the social and religious domains." It would seem that from the 1970s onward, this economic domain has indeed triumphed over these other competing domains. Nevertheless, before considering the lasting impacts of the economically-motivated calavera, it is crucial to speculate about its counterpoint.

To a large extent, the image of la calavera that I have articulated is an urban development, and its mass distribution and association with national identity is challenged by rural stability and an alternative symbol of the Day of the Dead—la ofrenda. As Carmichael and Sayer hold, "in the countryside there are few if any skulls or skeletons." In Nutini's description of rural Tlaxcala, Christian symbols remain ubiquitous, as the Day of the Dead maintains a "sacred oriented ideology and worldview," which has "proved remarkably resistant to secularization."This additional symbol of the Day of the Dead must be explored as it has retained its sacred, religious association inherent to the traditional ritual practices of the Day of the Dead. Concerning ritual practices, Carmichael and Sayer have observed "to see the most traditional forms of celebration for the Day of the Dead one must leave Urban Mexico." Nutini reinforces this observation by noting how concern for widespread community celebration pales in comparison to the "effort, care, and symbolic importance, rural Tlaxcalans put, . . . to the composition of the ofrenda in the household." In addition, the concluding words of the narrator in La Ofrenda reinforce the absence of economic attachment to the ofrenda, "the offering means love, and love has no price." But if la calavera imagery is not inherent to rural spaces nor the tradition itself, what can be said about the authenticity of la calavera in urban spaces? When talking about tradition and authenticity, it is critical to consider the time, place, and function of a certain practice. To resolve the question of authenticity as it pertains to the calavera, one must not only consult the setting of urban Mexico, but leave the borders of the country itself.

In analyzing the celebration of the Day of the Dead in the United States, it becomes evident that a singular version of la calavera exists which is not necessarily relegated to the folk tradition of the Mexican Day of the Dead, but is situated in the larger sphere of Mexican popular culture and national identity. Increased Mexico-U.S. immigration persisted throughout the 1970s largely due to the end of the Bracero Program in 1964, and reforms to U.S. immigration law in 1965. According to Regina Marchi, this increasing presence would transfer the celebration of the Day of the Dead in the United States as a way to communicate Chicano identity beginning in the 1970s. What is distinct about the Day of the Dead celebrations in the U.S. is its objective to reach a wide audience of all different races, ethnicities, and nationalities. As a San Francisco community organizer in La Ofrenda notes, it is "important that there are people from all races here. That way, we are not alone." This general acceptance of incorporating diverse communities at large has had a profound effect on the conception of la calavera. Similar to how Posada's calavera extended to a national audience in the beginning of the twentieth century, the developments transpiring simultaneously in the United States and Mexico during the 1970s invited a wider audience to interact with Mexican national identity. In explicating the overlap of these national timelines, it is clear that the version of the calavera in the United States and the practice of the Day of the Dead itself were coming into being at the same time that la calavera in Mexico was undergoing a transformation. Therefore, I hold that the post-1970s calavera in Mexico and the only calavera to ever exist in the United States evolved together into a single entity shared by the international community. Because of the United States' desire to expand the scope of Day of the Dead traditions, and Mexico's willingness to welcome international tourism and capital, the modern calavera is a single, secularized entity, symbolic of Mexican national identity.

Part II: Bawon Samdi

The particular function of Bawon Samdi as an icon of Haiti is more complex to elucidate than its calavera counterpart as it has been subjected indiscriminately to oppressive cultural,

religious, and political forces. Nevertheless, it is pertinent to begin with a brief description of some of Bawon Samdi's modern characteristic features. Bawon Samdi, also known as Papa Gede-the head of the Gede family of loa, represents the crossroads between life and death. While he is afforded great power in the realm of the dead, Katherine Smith notes that Bawon Samdi has lust for life, and embodies it through "possession performance, healing, and grotesque humor." For practitioners of Haitian Vodou, he is often understood as a gentle, just manifestation of death who refuses to "dig a grave if it is not a person's time." Nevertheless, popular culture depictions of Bawon Samdi since the 1970s have relegated him to an evil figure, an extension of a preexisting Vodouphobic discourse. Thus, any modern understanding of Bawon Samdi must assess the role of Vodou in Haiti. While the modern expression of Vodou phobia traces back centuries, Bawon Samdi's association with fear has only come into fruition in the last quarter of the twentieth century.

Before explaining the incarnation of Bawon Samdi himself, it is essential to reconcile how Vodou functions for the people in Haiti. In Vodou, je me souviens: essai, Jean Fils-Aime' elucidates the ongoing scholarly discussion about whether Vodou should be considered a culture or a religion and ultimately argues that it should be a combination of both. Having considered this bipartite analysis, I hope to advance Fils-Aimé's argument by proposing a third element-survival. While Fils-Aimé alludes to Haitian resistance to the imposition of religion, namely in appropriating Catholic traditions, he does not detail the extent to which Vodou practitioners actually see their faith as a survival mechanism. According to Smith, the question of legitimate survival has been repeatedly questioned by Haiti's history of "slavery, revolutions, foreign occupations, military coups, insidious aid programs, and neoliberal structural adjustments." This conception of Vodou as survival will have several opportunities to be tested over the course of the twentieth and twenty-first centuries, articulating a function of Vodou peculiar to the triumphs and travesties of Haitian history.

By introducing the notion of survival, I intend to detail both survival of a cultural practice, and actual, physical survival. While the former unfolds via Vodou's interaction with Christian religions on Haitian soil, the latter must be considered alongside Haiti's violent, and repressive political history. Vodou was subjected to global disdain after the Haitian Revolution as it was seen as a "mauvais exemple" of the consequences of rebellious slaves. International disgust followed, restricting Haiti's access to global resources. Consequently, to reinvent the national image, Haiti adopted Catholicism with the Concordat in 1860. Despite Catholicism's newfound status as a national religion, Haitians have largely seemed to maintain their cultural practices. As Celucien Joseph holds, the Vodou faith is foundational to the Haitian spirit— "even though other faiths may superpose it, . . . they can never replace it." The apparent Catholic guise that Haitians have adopted is merely a veneer to perpetuate their true beliefs. This appropriation of Catholicism for the purpose of Vodou's survival is evident in Andrea Leland's (1989) Voodoo and the Church in Haiti. While there is obvious symbolism of a crucifix in the hounfour, or Vodou temple, the narrator asserts that instead of a Catholic cross, it actually represents the crossroads between life and death—just like Bawon Samdi. When listing the names of what appear to be paintings of Catholic saints, the narrator only mentions their syncretized Haitian loa equivalents. While I previously debunked Bonfil Batalla's argument in claiming that indigenous practices survived under the facade of Catholicism in Mexico where syncretism clearly existed, perhaps his conclusions are more apt to the Haitian context, where religion was perceived as a survival mechanism.

While Vodou might survive the imposition of Catholicism, it is also imperative to consider the increasing proliferation of Protestantism in the Haitian milieu. It becomes clear that Protestantism sought the complete eradication of Vodou. Unlike Catholicism, which offered pathways of syncretic appropriation, the Protestant church envisioned "la disparition de la dite culture" entirely. Considering this tension, Protestantism questions the aforementioned description of Vodou as survival. Yet, the two operate in completely different ways in Haiti. Contrary to Catholicism, the Protestant mission explicitly framed the Haitian religion in a Vodouphobic discourse whose "central premise was the rehabilitation and redemption of the Haitian people and their lost souls." While Catholic sentiment did exhibit a relative disdain towards Vodou practices, its central tenets did not consider them "the main obstacle to modernization and Christian evangelization of the Haitian people," responsible for their economic, physical, and political plights-decreasing their likelihood for survival. Protestantism could displace Vodou as the correct means of survival for Haitians. Becoming Protestant was the "best inoculation against Vodou spells" responsible for the curse of Haitian circumstance. Protestantism would increasingly encourage separation from the Vodou faith through the late twentieth and twenty-first century. While Bawon Samdi does not ultimately serve an authentic religious purpose for such Haitians, he is still bound to all manifestations of Haitian cultural identity due to his position at the crossroads.

The first references to Bawon Samdi occurred in early twentieth-century literature. Nevertheless, I will only briefly discuss the historical developments at the beginning of the twentieth century through the 1930s and 1940s, since Bawon Samdi had not yet assumed his role as a visual icon. For general reference, Bawon Samdi does not appear to have any equivalent in African or Afro-Atlantic religions and appears first in Antoine Innocent's 1906 ethnography Mimola ou l'histoire d'une cassette. Bawon Samdi was only mentioned three times in Innocent's text, and he was characterized by his "liberation, generosity, and benevolence." The apparent absence of Bawon Samdi before this point suggests that his modern incarnation is contextualized to a century's worth of Haitian history and developments. What happened between then and the 1940s that visually incarnated Bawon Samdi and what features did he develop? In the decades following his literary introduction,



Figure 3. Myrland Constant, Bawon Samedi (1990s-2000s) Modern portrayals of Bawon Samdi typically include a phallic figure, suggesting his explicit sexuality. Constant's ornate masterpiece, Bawon Samdi, is an ongoing project which strives to include Haitian national symbols in an intricately beaded Vodou flag.

Vodou confronted its first test of survival—the 1915-1934 American Occupation. While political and economic instability persisted through this period, survival through Vodou became a reliable means to forge continuity amidst flux. As Fils-Aimé notes, "en temps de crise, le Vodou est l'articulus stantis et cadentis de la nation" [in times of crisis, Vodou is the articulus stantis et cadentis of the nation]. When the veil was lifted off of the Haitian spirit in 1934, Bawon Samdi would emerge as a visual icon of Haitian culture.

The emergence of Bawon Samdi during the next two decades must be reconciled with a troubling contradiction-the criminalization of Vodou in 1935 and simultaneous Negritude movement by the intellectual elite. President Sténio Vincent's 1935 Anti-Superstition Laws prohibited "the performance of all acts, practices, etc. liable to foster superstitious beliefs harmful to the good name of the country," thus implicating all Vodou rituals., The conclusion of American Occupation coincided with a broader nationalistic movement forged by the intellectual elite to reinvigorate Haitian culture and resist foreign imposition. How do we account for this dynamic considering how the "imaginative and artistic energies of the Haitian people," and the "discovery of Haitian 'primitive art'" ensued throughout the 1940s? With such hostility amongst a people accustomed to Vodou as survival, Bawon Samdi acquired his post-Occupation characterization as an overtly sexual, flawed, witty trickster. Around the same time that la calavera came to represent national identity in Mexico, Bawon Samdi, as a benevolent, humorous, and sexual being, blossomed in the Haitian conception of cultural identity. Samdi's mischievous, illicit spirit rejected foreign imposition through its lewd sexuality and resisted the state by intentionally recreating Bawon Samdi in a self-deprecating national image. While this portrayal of Bawon Samdi persisted over the duration of the Belle Epoque from 1935-1957, a dictatorial conflation ensued changing the face of Bawon Samdi forever.

Bawon Samdi took a macabre turn beginning during Francois

Duvalier's 1957–1971 rule, as Papa Doc used Vodou as a means to control the people. Recognizing that Vodou was becoming increasingly associated with Haiti's cultural identity, Duvalier seized the opportunity to conflate this identity by impersonating Bawon Samdi—everything from "his black suits and glasses, to his nasal voice, mimicked" the Haitian gede. The function of Vodou as legitimate, physical survival becomes clear as Duvalier positioned himself as a living God, on "the axis between two planes of existence," whose loyal Tonton Macoutes could act on his abilities to control life and death. Therefore, practicing Vodou now became a question of legitimate survival. For the purposes of this project, it is critical to mark this period as a point of departure in which Bawon Samdi started to function differently for the Haitian people themselves compared to the international community.



Figure 4. François Duvalier as Bawon Samdi; Unidentified Ceremony

It was not until the descent of the Duvalier regime when the secrets of state-sponsored terror were widely acknowledged that Bawon Samdi increasingly developed into a more evil, cunning, vengeful character from an international perspective. Paul, Luce, and Blank assert that Papa Doc permanently tainted Bawon Samdi's reputation-the lwa who cared for the people had transformed into a "repressive god who randomly doled out life and death" which would ultimately diffuse across the world in various media depictions. While this vengeful trope may exist on the international stage, quite a different projection ensued for the Haitian people. According to Cosentino, the Duvalier years saw a "zombification" where Haitians' souls were indentured in their faith by state-sponsored violence. This "zombification" was the corporeal reality for Haitians by the end of the Duvalier years. The Haitian selfimage in the 1970s was characterized by a "body inarticulate and submissive" to intimidation and violence. The Haitians themselves knew they were zombies. In Frankétienne's (1975) Dezafi, the zombified body is portrayed as a metaphor for the oppression experienced by the Haitian people. The zombie narrator, relaying the collective experience of the Haitian people, encourages the reader to "remember, the dead never rise," lamenting that Haitians are "lifeless under the cursed weight of our woes." Yet, Bawon Samdi, who represented a crossroads between life and death, would undergo an immense transformation for the purpose of remedying Haitian zombification. While the voudouphobia incipient since colonial Saint Domingue never subsided, the international media found a physical referent in Bawon Samdi because of the Duvalier regime.

Paul, Luce, and Blank trace the history of visual imagery in film and sport throughout the late twentieth and early twentyfirst century, and indicate that Bawon Samdi has been inappropriately represented in the international media. They discuss the villainous rendition of Bawon Samdi in the 1973 James Bond film Live and Let Die, which was one of the first media representations to connect the African race with "malevolence where empowerment comes only through the destruction of white society." Not only was Bawon Samdi evil in and of himself, but he was now racialized-antithetical to the purity and goodness of white society and religion, and dangerous to them. This characterization of Bawon Samdi was further reinforced by Charles Thomas Wright's assumption of his ring persona, "Papa Shanga," who induced supernatural phenomena in the wrestling ring much to the chagrin of his opponents. While this image developed externally, using Vodou as a cultural and physical survival mechanism subsided in Haiti after the Duvalier regime. Vodou would now have the chance to represent life. The modern version of Bawon Samdi for the Haitians would consequently become more human and carnal. Given the opportunity to vacillate between life and death, and now human and god, the modern Bawon Samdi gave the previously zombified Haitian bodies the opportunity to rise from the dead-to come back to life.

While Bawon Samdi may have started as a spiritual figure, his modern association situates him at the crossroads of both life and death, spirit and human. The ease in which Bawon Samdi interacts with both the spirit world and human world is evident by one Vodou ceremony in particular-possession. Unlike la calavera which exists as an inanimate and static entity of its own, Bawon Samdi actually embodies humans in the act of ritual possession. He is said to mount a Vodou practitioner, who "devient alors son chwal (cheval)" and "ne s'appartient plus"-the ways in which the human behaves are decided by Bawon Samdi's will. In this ritual, Bawon Samdi is relegated to the corporeal realm, and the human is used as a vessel-the human and divine literally intersect. While possession was long-established as a Vodou ritual, I hold that before the 1970s, Bawon Samdi was still thought to maintain status as a god in his human occupancy. After Vodou had become associated with life with the fall of the Duvalier regime, Bawon Samdi possessing practitioners was now acceptable as it was accepted for Bawon Samdi to truly become human by associating to Haitian bodies.

Similar to how la calavera increasingly sought to include a broad, popular audience, Bawon Samdi's newfound malleable human component made him more available to the community at large. Bawon Samdi's human counterpart is especially evident in Luke Wassermann's Fet Gede in Little Haiti, which displays footage from the celebration of the Night of Bawon Samdi in a Haitian enclave in Miami.69 At various points throughout the ritual dances, the rele lwa, Bawon Samdi is depicted as both male and female. Not only are both male and female performers clad with Samdi's characteristic garb, but some of the visual imagery in the Jakmel temple portrays Bawon Samdi with breasts, particularly Didier Civil's Gede Triptych.70 Bawon Samdi thus opened himself up to the Haitian community as male and female alike were worthy of being possessed. In addition, the sexual innuendo infused in Bawon Samedi's dancing renders him even more fundamentally human by acting on his urges and desires, his body's physical demands.70 Thus by the twenty-first century, Bawon Samdi seemed to captivate all humans at the crossroads of the living and the dead.



Figure 5. Didier Civil, Gede Triptych (2006)

The liminality between life and death in Haiti is seen in Bawon Samdi's role as the guardian of the cemetery, a physical location both literally and figuratively operating as a crossroads between worlds. Despite the increasing prevalence of Catholicism and Protestantism in Haiti, which either directly or indirectly suppress the true expression of Vodou, Smith holds that all cemeteries in Haiti serve as a large social network—an hounfour or a Vodou temple.69 It is evident in Leah Gordon's (2007) interview with Haitian folk artist and Vodouist Jean Claude Saintilus, that the Haitian people themselves perceive the cemetery as a quintessential space for all Haitians-Bawon Samdi was not the guardian of the cemetery for a specific religion, but for Haiti. As Saintilus asserts, "I believe in the cemetery, it's where we will all end up, whoever you are."69 Ending up in the cemetery, the crossroads between life and death, was unavoidable. In accepting the cemetery as a crossroads, all Haitians implicitly accepted its embodied referent-the transitory human, god, dead, and living Bawon Samdi.

Nevertheless, the "whoever" described by Saintilus is not the same "whoever" that the Mexican calavera would extend to. Saintilius is only describing the "whoever" of Haiti: those who have the ability to embody the true Haitian experience, just like Bawon Samdi.

In discussing the conflation of bodies with iconic conceptions of identity, it is apt to return to Mexico, where the iconography of the cult of La Santa Muerte seems to lie at the crossroads of the Mexican calavera and Haitian Bawon Samdi. Al-

though the cult of La Santa Muerte originated in Mexico city, over the course of the twenty-first century it has developed into an increasingly transnational social and cultural group susceptible to a certain "social vulnerability," namely in the labor market, healthcare, and education. Additionally, La Santa Muerte has become insinuated in a discourse of crime and drug-related violence and symbolizes the patron saint of drug traffickers, criminals, and cops. The figure of La Santa Muerte itself is a female personification of death in skeletal form and "just as humans carry the skeleton inside, so too does she long for food, drinks, cigarettes, clothing, and attention." While La Santa Muerte may resemble the Mexican calavera as an inanimate skeleton, she does indeed maintain a corporeal association on a par with Bawon Samdi. La Santa Muerte manifests on the body in the form of a tattoo, which both signifies membership to the cult, and proves devotion of the body, "a blank canvas on which they display their tattoos" to the demanding saint. Moreover, La Santa Muerte is not widely celebrated like the Mexican calavera; rather its association provokes fear and suspicion of illicit and criminal behavior. La Santa Muerte thus seems to selectively extract characteristic elements of the internationally-feared Bawon Samdi, the Haitian corporeal Bawon Samdi, and the Mexican Calavera.

Having primarily considered two different icons-la calavera and Bawon Samdi-it is crucial to conclude this project by comparison. The majority of my investigation sought to uncover what changes these figures endured over the course of the twentieth century, yet after analyzing both figures in their respective contexts, perhaps the question I was really trying to answer was who these figures eventually belonged to. While la calavera and Bawon Samdi endured a comparable shift in becoming associated with either national or cultural identity around the 1930s, their point of departure aligned in the 1970s. The economic motivation behind the Mexican calavera, especially amplified through tourism, rendered it cultural capital accessible to the world at large. After the 1970s, Bawon Samdi would become a villainous character to the international community, while simultaneously insinuating himself into the corporeal experience of the Haitian people. Despite an intrinsic association with death at one point or another in both of these icons' histories, their present-day function could not be more associated with a strong desire to livespecifically to live through these icons.

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References

Bonfil Batalla, Guillermo. Me´xico Profundo: Reclaiming a Civilization. Translated by Philip A. Dennis. 1st ed. Austin: University of Texas Press, 1996.

- Brandes, Stanley H. Skulls to the Living, Bread to the Dead: The Day of the Dead in Mexico and Beyond. Malden, MA: Blackwell Publishing, 2006.
- Carmichael, Elizabeth., and Chloe⁻⁻ Sayer. The Skeleton at the Feast: The Day of the Dead in Mexico. Austin: University of Texas Press, 1992.
- Cosentino, Donald J. "Gede Rising." In In Extremis: Death and Life in Twenty-First-Century Haitian Art, ed. Donald J. Cosentino, 76-85. Los Angeles, CA: Fowler Museum at UCLA, 2012.
- Dash, J. Michael. Haiti and the United States: National Stereotypes and the Literary Imagination. New York: St. Martin's Press, 1988.
- Fils-Aime´, Jean. Vodou, je me souviens: essai. Montre´al: E´ditions Dabar, 2007.
- Frankétienne. Dezafi, 1975. In The Haiti Reader: History, Culture, and Politics, edited by Laurent Dubois, et al., 368-372. Duke University Press, 2020. ProQuest Ebook Central.
- Guadalupe Posada, Jose[´]. Posada's Popular Mexican Prints: 273 Cuts by Jose[´] Guadalupe Posada; Selected and Edited with an Introduction and Commentary, by Roberto Berdecio and Stanley Appelbaum. New York: Dover Publications, 1972.
- Joseph, Celucien L. "Redefining Cultural, National, and Religious Identity: The Christian–Vodouist Dialogue?" Theology Today 73, no. 3 (2016): 241–62.
- Leland, Andrea, director. Voodoo and the Church in Haiti. Andrea Leland, 1989. 43 min. https://www.kanopy.com/en/rochester/ video/172361.
- Marchi, Regina. "Hybridity and Authenticity in US Day of the Dead Celebrations." The Journal of American Folklore 126, no. 501 (2013): 272–301.
- "Migration Between the U.S. and Mexico." Pew Research Center, Washington, D. C. (April 13, 2012) https://www.pewresearch. org/hispanic/2012/04/23/ii-migration-between-the-u-s-andmexico/.
- Miliotes, Diane. José Guadalupe Posada and the Mexican Broadside. Chicago: Art Institute of Chicago, 2006.
- Nutini, Hugo. Todos Santos in Rural Tlaxcala: A Syncretic, Expressive, and Symbolic Analysis of the Cult of the Dead. Princeton, N.J: Princeton University Press, 1988.
- Pansters, Wil G. "La Santa Muerte: History, Devotion, and Societal Context." In La Santa Muerte: History, Devotion, & Society, ed. Wil. G. Pansters, 1-57. Albuquerque: University of New Mexico Press, 2019.
- Paul, John, Krystal Luce, and Sharla Blank. "Playing Vodou': A Visual Essay of Imitation and Meaning in Political and Popular Cultural Depictions of Baron Samedi." International Journal of Humanities and Social Science 3, no. 6 (2013): 287-301.
- Perdigón Castañeda, Judith Katia, and Bernardo Robles Aguirre. "Devotion That Goes Skin Deep: Tattoos of La Santa Muerte, In La Santa Muerte: History, Devotion, & Society, ed. Wil. G. Pansters, 158-182. Albuquerque: University of New Mexico Press, 2019.
- Portillo, Lordes, director. La Ofrenda: The Days of the Dead. Lordes Portillo, 1988. 50 min. https://www.kanopy.com/en/ rochester/video/361620.
- Saintilus, Jean Claude. Interviewed by Leah Gordon. "I'm Dead Already . . . I Just Haven't Been Buried Yet." In Extremis: Death and Life in Twenty-First-Century Haitian Art, ed. Donald J. Cosentino, 143-161. Los Angeles, CA: Fowler Museum at UCLA, 2012.
- Smith, Katherine. "Gede Rising: Haiti in the Age of Vagabondaj." PhD diss., University of California, Los Angeles, 2010. ProQuest

(AAT 3462850).

- Vincent, Sténio. Anti-superstition Laws, September, 1935. In The Haiti Reader: History, Culture, and Politics, edited by Laurent Dubois, et al., 265-6. Duke University Press, 2020. ProQuest Ebook Central.
- Wassermann, Luke, director. Fet Gede in Little Haiti—A Festival of Vodou Drumming and Dancing. Ekulnamm Productions, 2006. 2 hr 20 min. DVD.
- Matthes, D. J., Sink, H., Kolodkin, A. L., & Goodman, C. S. (1995). Semaphorin II can function as a selective inhibitor of specific synaptic arborizations. Cell, 81(4), 631–639. https://doi.org/ 10.1016/0092-8674(95)90084-5
- Nguyen, D. N., Liu, Y., Litsky, M. L., & Reinke, R. (1997). The sidekick gene, a member of the immunoglobulin superfamily, is required for pattern formation in the Drosophila eye. Development, 124(17), 3303–3312.
- Shimono, Y., Rikitake, Y., Mandai, K., Mori, M., & Takai, Y. (2012). Immunoglobulin Superfamily Receptors and Adherens Junctions. In T. Harris (Ed.), Adherens Junctions: From Molecular Mechanisms to Tissue Development and Disease (pp. 137–170). https://doi.org/10.1007/978-94-007-4186-7_7
- Sieber, M. H., & Spradling, A. C. (2015). Steroid Signaling Establishes a Female Metabolic State and Regulates SREBP to Control Oocyte Lipid Accumulation. Current Biology, 25(8), 993–1004. https://doi.org/10.1016/j.cub.2015.02.019
- Siebert, S., Farrell, J. A., Cazet, J. F., Abeykoon, Y., Primack, A. S., Schnitzler, C. E., & Juliano, C. E. (2019). Stem cell differentiation trajectories in Hydra resolved at single-cell resolution. Science, 365(6451). https://doi.org/10.1126/science. aav9314
- Siegenthaler, D., Enneking, E.-M., Moreno, E., & Pielage, J. (2015). L1CAM/Neuroglian controls the axon-axon interactions establishing layered and lobular mushroom body architecture. The Journal of Cell Biology, 208(7), 1003–1018. https://doi.org/ 10.1083/jcb.201407131
- Snow, P. M., Bieber, A. J., & Goodman, C. S. (1989). Fasciclin III: A novel homophilic adhesion molecule in Drosophila. Cell, 59(2), 313–323. https://doi.org/10.1016/0092-8674(89)90293-6
- Spindler, S. R., & Hartenstein, V. (2010). The Drosophila neural lineages: A model system to study brain development and circuitry. Development Genes and Evolution, 220(1–2), 1–10. https://doi.org/10.1007/s00427-010-0323-7
- Vogel, C. (2003). The immunoglobulin superfamily in Drosophila melanogaster and Caenorhabditis elegans and the evolution of complexity. Development, 130(25), 6317–6328. https://doi.org/ 10.1242/dev.00848
- Wu, X., Tanwar, P. S., & Raftery, L. A. (2008). Drosophila follicle cells: Morphogenesis in an eggshell. Seminars in Cell & Developmental Biology, 19(3), 271–282. https://doi.org/10.1016/ j.semcdb.2008.01.004

About the Author

In this particular research project, I was able to explore my own particular research interests while also investigating topics related to my senior thesis. For my thesis, I am tracing the trajectory of refugees from Saint-Domingue to New Orleans after the Haitian revolution—a project which must account for the spread of Vodou. I took an interest in the unique celebration of the Day of the Dead in my Spanish courses in high school, and had always wanted to find a way to learn more about it. I would encourage undergrads interested in research to take the opportunity to investigate something you are genuinely interested in.

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A Response to Alternative Decision-Making Capacity Standards

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Section I: Introduction

When patients are faced with difficult medical choices, it is imperative that they can make autonomous decisions. However, not all forms of capacity are equal. In "Three Kinds of Decision-Making Capacity for Refusing Medical Interventions," Navin et al. describe three distinct types of medical decision-making capacity: burdens-based decision-making capacity (DMC), goals-based DMC, and comparative DMC.

Burdens-based DMC describes a patient's ability to make a medical choice based on an important objection that the patient deems overriding. Similarly, goals-based DMC describes a patient's ability to exercise a choice guided by an overriding goal. With comparative DMC, patients must be able to compare the risks and benefits associated with all relevant treatment paths (Appelbaum 2007; Drane 1985; Grisso and Appelbaum 1998). Navin et al. note that comparative DMC is currently the necessary standard in "clinical ethics literature" (2021, 3).

The authors provide compelling arguments to suggest that burdens-based and goals-based refusals can serve as morally and medically authoritative alternatives to comparative refusals when patients lack this latter form of decision-making capacity.

The determination of overridingness is argumentatively critical in legitimizing burdens and goals-based refusals; in other words, only when a burden or goal is deemed to be overriding does it have the weight to justify a refusal when patients do not meet the standard threshold of comparative DMC.

Despite their extensive exploration of alternative manifestations of acceptable DMC, Navin et al. fail to consider that their arguments, in actuality, illustrate the necessity of comparative DMC: to ensure that patients' goals and objections to burdens are indeed overriding and therefore morally relevant, patients must be able to compare options completely. In light of this perspective, comparative DMC should be maintained as the necessary standard of capacity, contrary to the views of Navin et al.

Section II: On Burdens-Based Refusals

Although a patient may not be able to compare the costs and benefits associated with their treatment options, a patient may express an overriding objection that applies to all relevant interventions.

Indeed, Navin et al. describe three ways overridingness can be determined: first, patients may be able to communicate their

burden or goal as overriding any other interest; second, when patients are presently unable to communicate how their burdens or goals are overriding, there may be a record of previous communications illustrating the idea; third, a patients' life history may strongly suggest that certain burdens or goals are overriding.

If it can be established that this overriding burden exists, Navin et al. would seem to claim the patient may refuse treatment, even if the patient lacks the typically required comparative DMC.

Navin et al. extend their arguments to somewhat general burdens. They describe, for instance, a patient who objects to tubing without clear reasoning from the patient: "[Mrs. P's] statement that 'I don't want to be tied up here any longer; I don't want any more procedures or tubes in me' is a good indication that there may be overriding burdens associated with possible interventions that she rejects" (2021, 4, emphasis original).

Patients may indeed identify medically relevant burdens when faced with treatment options, but these burdens may be alleviated or countered if patients were able to understand their treatment options on a comparative level.

As an analogy, I may initially object to travel insurance plans, claiming, "The cost is simply an overwhelming burden given my financial circumstances. I do not want to pay for any extra frills. Instead, I want to spend the money on other things I deem necessary." Despite what seems to be a morally authoritative burdens-based refusal, I might change my mind if I truly understood the benefits of travel insurance compared to the harms of traveling without insurance. In other words, my initial refusal was potentially based on an incomplete understanding of travel insurance and a subsequent categorization of travel insurance as a mere frill. If I lacked comparative decision-making capacity, even compelling explanations as to why insurance may be the best plan of action might not meaningfully resonate with me. In this sense, informed refusal is perhaps not possible. Similarly, as a patient, I might say, "I don't want to be tied up here any longer; I don't want any more procedures or tubes in me" (2021, 4). However, I might concede that treatment is necessary, including treatment involving some derivative of tubing, if I truly understood the importance of such treatment relative to other available options.

The authors further describe an individual with an overriding objection to the burden of moving homes to illustrate their idea in non-clinical settings. According to Navin et al., we do not typically question a homeowner's decision to refuse to move, even when this individual lacks knowledge about alternative properties. Here the authors again fail to consider that, even with a general objection to some burden, people can and often do change their minds in response to large gains associated with a particular decision or the desire to avoid even larger burdens in the future. It is quite possible that someone would endure the burden of moving if a large benefit was at stake. Say, for example, the theoretical homeowner was offered a very attractive price for their property and found an affordable condominium to purchase. Comparing the financial gain with the burden of moving, the individual may, in fact, decide to move despite the initially overwhelming burden. Notice that the comparison of gains and burdens was indeed necessary to make this fully informed decision. Similarly, with treatment options, patients may or may not decide that the net benefit of a particular treatment path outweighs the burden to which they initially objected, yet this sort of evaluation requires comparative DMC. In an alternative scenario, say the homeowner was liable to face some significant burden - bankruptcy, for instance - if they did not sell their home for cheaper accommodations. It seems quite possible to me that the homeowner would endure the burden of moving to avoid this unfortunate outcome. Similarly, when patients with comparative DMC understand that their objection to burdensome treatment opens them up to risks of facing alternative future burdens (including death), the comparative assessment may lead them to make a choice to which they initially objected.

Section III: On Goals-Based Refusals

Navin et al. also describe cases where patients exercise goalsbased refusals while lacking comparative DMC. In these cases, a patient expresses some overriding goal that is inconsistent with the proposed treatment. For instance, certain patients "may want their disease to follow its natural progression. They may accept comfort measures, but they do not want to further prolong the course of their disease; they may have the goal of dying, or at least of no longer treating the disease" (2021, 5).

Further, the authors claim that "a patient can make a goalsbased refusal decision without understanding the details of their diagnosis and the nature and probable outcomes of possible treatments" (2021, 5). Similar to my above claims, it may be true that a patient deeply holds goal X. However, the goals of individuals change frequently when faced with difficult decisions and the comparative risks and benefits associated with each choice; therefore, without the ability to compare action plans, it is difficult to understand how a goal can legitimately be deemed to be overriding.

Further, the path towards an overriding goal may be complex and require a sophisticated type of capacity to achieve. For example, I may have a goal to be a productive student, prompting me to refuse treatment that would require some form of rest. However, it is possible that if I refused treatment, I would face consequences of the imagined condition, leading to less total productivity in the future. Therefore, despite my fervent goal of never missing the opportunity to study, I may accept the proposed treatment, but only if I comparatively understood the calculus of risks and benefits associated with treatment.

It is also possible that patients hold important yet mutually exclusive goals; in these cases, a patient will need to possess comparative DMC to identify which goal is indeed overriding.

The authors provide an example of an individual wishing to remain celibate to illustrate the role of overriding goals in nonmedical contexts; the authors note that this decision would appear to be authoritative, even though the individual does not know what could have otherwise been potential sexual partners. Navin et al. acknowledge the differential magnitude of consequences between choosing celibacy and choosing to refuse life-saving treatment. Nevertheless, Navin et al. do not appropriately consider that medical or legal decision-making capacity is perhaps importantly different from other types of decision-making capacity in casual settings. Of course, we cannot monitor each choice people make in their lives, and it would be an unrealistic and unjustified exercise of paternalism for government bodies to assess capacity when any individual makes any weighty choice. However, when patients are given medical information, it seems there is some professional duty for the healthcare provider to ensure that the patient, or proxy, understands and can compare the benefits and drawbacks of treatment paths. This obligation does not imply that providers ought to convince patients to act in particular ways; patients should be able to exercise autonomous choices, even when providers would not have made the same choice for themselves (assuming the choices made by patients do not unjustly harm others).

Section IV: Final Notes

Ultimately, I do not mean to suggest that goals or burdens are meaningless in medical settings. Of course, patients' wishes hold moral weight (Joosten et al. 2008; Pope 2013; Shay and Lafata 2015), even if these patients are only capacitated to exercise burdens or goals-based refusals. Further, scholars have noted the clarifying role expressed reasons, goals, or burdens may play in ambiguous advance directives (Tomlinson and Brody, 1988; Wicclair, 2010). However, it is one thing to use goals and burdens to clarify decisions presumably made with comparative DMC; it is quite another thing to use goals and burdens to clarify the components of comparative DMC. It is not enough for patients to meet the lower threshold of "understanding, appreciation, and reasoning required for these other kinds of DMC" (Navin et al. 2021, 7).

i o e t h i c s

D

References

- Appelbaum, P. S. 2007. Clinical practice. Assessment of patients' competence to consent to treatment. The New England Journal of Medicine 357 (18):1834–40. DOI: 10. 1056/NEJMcp074045.
- Drane, J. F. 1985. The many faces of competency. The Hastings Center Report 15 (2):17–21. DOI:10.2307/ 3560639.
- Grisso, T., and P. S. Appelbaum. 1998. Assessing Competence to Consent to Treatment: A Guide for Physicians and Other Health Professionals. New York: Oxford University Press.
- Joosten, E. A., L. DeFuentes-Merillas, G. H. De Weert, T. Sensky, C. P. F. Van Der Staak, and C. A. de Jong. 2008. Systematic review of the effects of shared decision-making on patient satisfaction, treatment adherence and health status. Psychotherapy and Psychosomatics 77 (4): 219–26. DOI:10.1159/000126073.
- Navin M.C., Brummett A.L., and Wasserman J.A. 2021. "Three Kinds of Decision-Making Capacity for Refusing Medical Interventions." The American Journal of Bioethics. DOI: 10.1080/15265161.2021.1941423.
- Pope, T. M. 2013. Making medical decisions for patients without surrogates. The New England Journal of Medicine 369 (21):1976–8. DOI:10.1056/NEJMp1308197
- Shay, L. A., and J. E. Lafata. 2015. Where is the evidence? A systematic review of shared decision making and patient outcomes. Medical Decision Making: An International Journal of the Society for Medical Decision Making 35 (1): 114–31. DOI:10.1177/0272989X14551638.
- Tomlinson T. and Brody H. 1988. "Ethics and communication in do-not-resuscitate orders." The New England Journal of Medicine 318 (1): 43-6. DOI:10.1056/NEJM198801073180109.
- Wicclair M. 2010. "Medical Futility: A Conceptual and Ethical Analysis." In Biomedical Ethics, ed. Mappes T.A., DeGrazia D., and Ballard J., 7th ed., 359-363. New York, NY: McGraw-Hill.

About the Author

Samuel Streicher is a pre-medical student majoring in bioethics and minoring in biology at the University of Rochester in Rochester, New York. Studying the natural sciences alongside the humanities, Samuel's work critically evaluates conventional approaches in health policy to uncover areas for future improvement. Delving further into his philosophical and bioethical studies, Samuel began exploring endof-life issues - including euthanasia, physician-assisted suicide, and refusal of life-saving treatment - by engaging with the Philosophy Department at the University of Rochester as well as the Department of Health Humanities and Bioethics at the University of Rochester Medical Center School of Medicine and Dentistry. For students interested in research, Samuel recommends pursuing a field that truly speaks to you - even if it is seemingly unconventional!

Improving Pronto Performance with Buffered Persistence

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Abstract

JUR

The rising number of general-purpose persistence frameworks has provided programmers with rich interfaces to interact with nonvolatile memory, reducing the effort required to develop new data structures. These frameworks, however, provide strict and immediate persistence at a high cost to latency and throughput, even when such a feature is unnecessary.

We introduce Buffered Pronto, a modified version of the Pronto persistence system that uses buffered durable linearizability, to show the possibility of modifying an existing persistence framework to use buffered persistence. Because of its buffered nature, Buffered Pronto requires significantly fewer resources for persistence at high throughput. It also uses the same annotations as Pronto with the addition of an optional sync feature to block until an operation persists.

his paper evaluates Buffered Pronto against the original Pronto and Montage, a general-purpose buffered durable linearizable persistence framework, and reports on our findings. We found that while Buffered Pronto cannot match the performance of the specialty designed buffered system Montage, its 1.26 to 6.77 times performance increase over the original Pronto makes it a viable drop-in replacement where data loss contained within a 1ms interval is acceptable.

Introduction

Modern nonvolatile memory (NVM) technology provides ample capacity for persistent storage with reasonable access latency. Many general-purpose persistent data structure frameworks have been developed over the years [7][8][9] to support the idea of durable linearizability [4]. The data structure can fully recover an up-to-date copy in the wake of a crash. However, these strict and durable linearizable persistent data structures are still significantly less performant than their volatile counterparts by several orders of magnitude. The buffered durable linearizability relaxed persistence model provides a viable alternative [4]. While it still guarantees failure atomicity, it improves performance by buffering operations on the persistent data structure, allowing for some degree of data loss. Such a model can be beneficial when there is a need to prioritize data structure performance and crash consistency, and users can tolerate losing small amounts of data.

Unfortunately, most existing persistent data structure frameworks do not support buffered durable linearizability. In order for a data structure to use this relaxed model, it must be written entirely from scratch using new systems, introducing a non -trivial amount of programmer effort. Converting an existing general-purpose persistent data structure framework to use a buffered persistence may be a viable alternative, provided there is significant performance gain over the original. This paper looks at modifying an existing strict durable linearizable persistence framework, Pronto, by Memaripour et al.[5] to use buffered persistence. We compare Buffered Pronto with Pronto-Full and Pronto-Sync variants described in the original paper and the Montage general-purpose buffered persistence framework developed by Wen et al. and discuss whether this conversion is feasible [9].

Motivation and Related Work

This section discusses the Pronto persistent data structure framework and the limitations that led to Buffered Pronto and other existing approaches in building persistent data structures.

Pronto

Pronto is a general-purpose persistent framework, developed by Memaripour et al., built on top of libpmem[5][3]. It uses a high-level semantic log called the Asynchronous Semantic Log (ASL) and a volatile copy of the data structure to maintain persistence. A snapshot mechanism periodically checkpoints the volatile data structure. On a crash, Pronto replays all committed ASL entries on top of the last snapshot to recover the persistent data structure to a consistent state. To convert an existing volatile data structure to a Pronto persistent data structure, the user simply annotates the data structure's operations with Pronto's op_begin() and op_commit() macros.

The original paper introduces two variants of Pronto called Pronto-Full and Pronto-Sync. While Pronto-Full writes to the log using dedicated persister threads, Pronto-Sync performs log operations inline on the worker threads that operate on the data structures. This difference allows Pronto-Sync to forgo the thread communication overhead by having the workers complete the ASL logging operation.

However, Pronto has several limitations: Pronto annotated data structures are significantly slower than the original volatile versions since all operations have to wait for persistence during an op_commit(). Pronto-Full requires processor SMT to be enabled. Each worker thread spawns a dedicated persister thread on the same physical core, effectively cutting the number of usable cores on a machine by half. These persister and worker threads also update data in the same per-ASL persistent metadata that resides on a single cache line in NVM, introducing significant false sharing problems. Meanwhile, Pronto-Sync writes to the ASL inline and cannot hide the latency introduced by flushing and fencing data to NVM. Because of these limitations, we decided to design a buffered variant of Pronto called Buffered Pronto that uses buffered durable linearizability, reducing the number of persister threads needed while also hiding some of the costs of flushing and fencing to NVM. We believe that improving throughput, latency, and resource usage with the tradeoff of losing the last sub-millisecond worth of operations on a system failure is reasonable for most users.

Related Work

Like Pronto, most existing work on general-purpose persistent data structure frameworks use strict durable linearizability. Mnemosyne [8] is one of the earliest systems that exposes persistent memory in a program's virtual address space to allow for building data structures on top of NVM. The static keyword allows the programmer to explicitly place a variable in NVM. Meanwhile, Mnemosyne's user library provides persistent primitives such as heap allocators and transaction logs. It also supports Durable Memory Transactions that allow for in-place updates.

The OneFile system provides a Persistent Transactional Memory interface for using NVM [7]. It uses a wait-free algorithm built on a multi-word Compare and Swap instruction. OneFile is immune to starvation because of this implementation and offers consistent latency regardless of thread contention and the persisted transaction size.

The closest existing work to our Buffered Pronto is Montage, a general-purpose framework for building buffered durable linearizable persistent data structures designed by Wen et al. [9]. Unlike Pronto, which keeps a copy of the complete data structure in volatile memory (DRAM), Montage keeps only the pointer bookkeeping data in DRAM; the rest is stored directly in NVM using the Ralloc allocator [1]. An epoch-based payload system persists the minimal amount of data required to reconstruct the data structure on a recovery. Individual operations are guaranteed to lie within an epoch boundary. Data in the last two epochs (e and e-1) are lost during recovery, while data from previous epochs persist.

Buffered Pronto Overview

Buffered Pronto is a modified version of the Pronto persistent data structure framework that uses buffered durable linearizability. Relaxing the persistence model allows for several optimizations that were not possible with the original Pronto. We also included an explicit thread_sync() operator to block until the data structure commits all operations up to a specific point.

Reduced Access to ASL Metadata in NVM

We designed Buffered Pronto to access the log metadata in NVM without worker threads. Accessing log metadata is a bottleneck in the original Pronto. All worker and persister threads needed to access some data in NVM that all resided in the same cache line. In our Buffered Pronto design, metadata frequently accessed by workers has a transient copy in DRAM that the workers operate on and is then updated periodically in NVM by the persister during persistent operations.

Persistent and Logical Tail

We made additional changes to the ASL tail, which indicates the offset where the last valid log entry ends in the log. We use the term persistent tail to denote this log tail as described in the original Pronto paper, indicating that there are no persisted operations beyond this offset. We introduced a new tail, called the logical tail, marking the end of buffered log entries. This logical tail is transient and is beyond or equal to the persistent tail. The data structure loses all operations logged between the persistent and transient tail on a crash. The logical tail is updated and read by worker threads only.

Simplified Single Persister

Our Buffered Pronto always uses a single persister instead of one per core to reduce resource usage. A simple persister design allows Buffered Pronto to avoid the persister from bottlenecking the worker threads. Because of this, the persister in Buffered Pronto does not write log entries on behalf of the workers. Instead, it waits for the workers to write the log entries, then flushes and fences the log. During each persister iteration, the persister finds the longest prefix of committed log entries starting from the current persistent tail. These changes are persisted and made visible to the recovery mechanism by updating the ASL metadata in NVM accordingly. We also designed the persister to suspend for a short period in case updates on the log are not particularly busy, further freeing up processor time.

Function to sync with Persistent Data Structure

We introduce a thread_sync() function to allow a thread to sync its last operation with the persistent data structure explicitly. Calling thread_sync() blocks the calling thread until every prior operation on the data structure is guaranteed to have been linearized and persisted. This mechanism allows users to ensure critical data has persisted before executing further operations on the data structure. However, it requires users to annotate their existing Pronto code to use. thread_sync() was the only modification made to the Pronto API in Buffered Pronto. Not using it does not affect Buffered Pronto's operation. The only downside is that there is no guarantee of how many operations will be visible on the recovered data structure during recovery.

Implementation Details

We describe the implementation details and challenges in this section. The majority of the changes in Buffered Pronto are in the persister. We decided to rebuild the persister from the ground up because of the significant differences in persister characteristics between the original Pronto-Full and Buffered Pronto. Several other components of Pronto were changed as well to make them compatible with and optimized for the new buffered persister.

Log Structure and Log Operations

We modify the log entry structure in the ASL to contain a log size field. This change allows Buffered Pronto's persister to traverse variable-length log entries without calculating its size during traversal. While this adds an 8 byte overhead to each log entry, it allows for significantly faster log traversal. We use this optimized traversal in Buffered Pronto's persister and recovery operations.

The persister checks for valid commit IDs at the persistent tail during Buffered Pronto execution; once it discovers a valid commit ID, it reads the next 8 bytes to discover the length of the log entry. The persister then uses this length to find the beginning of the following log entry. It repeats this process until it obtains the longest prefix of log entries at a given point in time. The persister then fences and flushes this prefix and updates the metadata's persistent tail. It then waits for a variable time before resuming operation.

Meanwhile, worker threads operate on the ASL directly, like in Pronto-Sync. Workers write the log entry with the commit ID omitted as soon as possible so that the system can write the data back to NVM whenever it is available. op_commit() fetches and writes the commit ID. The filled-in commit ID marks the log entry as visible to the persister and allows it to persist the data written by the worker explicitly.

Our current implementation of Buffered Pronto requires worker threads to not hang indefinitely between an op_begin() and op_commit() during a data structure modification operation. This requirement allows the persister to meet liveness requirements during program execution. We expect users to ensure that the data structure code they provide does not cause deadlock or livelock when Buffered Pronto executes the operation. Suppose the program cannot meet this requirement: in this case, delaying the call to op_begin() on the worker until right before the call to op_commit() can prevent issues with the persister, with lower performance due to unhidable logging overhead.

Recovery Changes

The recovery thread reads all log entries after applying the snapshot during recovery. It sorts the log entries (as they may be out of order) and then replays them in order on top of the snapshotted data structure image. Log entries with unreachable committed IDs are ignored, zeroed out, and treated as unpersisted entries.

We had to ensure that previously buffered-but-not-persisted operations beyond the persistent tail do not interfere with the persister's ability to detect new log entries. The persister may misread existing data written to the to-be-persisted log entries as valid commit IDs during operation post-recovery. Therefore, we decided to zero-set the entire empty log range after the recovery process. We recognize that this is not the most efficient solution, but it works well enough in Buffered Pronto, where we are primarily interested in general operation throughput and latency. Alternative solutions to this problem can be clearing only the commit ID using the size field or zero setting a maximum log range that the workers can operate on in a given time window. The optimal solution to this problem is open to future research.

Volatile Metadata

We have moved several variables frequently accessed by worker threads to a dedicated per-ASL metadata structure. This struct is volatile and contains the last commit ID, the logical tail, the number of active transactions, and other bookkeeping data. Initialization of the volatile metadata happens during ASL allocation and data structure recovery. The worker thread indexes the data structure's UUID into a hash table to find the volatile metadata for a specific ASL.

All variables in the volatile metadata fit in a single cache line to further improve performance. We took this design approach as we discovered that these variables are frequently accessed together during a single worker's operation. Giving specific variables a dedicated cache line reduces performance, as more misses and cache line writebacks would have to occur.

The persister also periodically reads some of the metadata copied from ASL persistent metadata (i.e., the last commit ID) and writes the changes back to NVM. This process is currently unnecessary as Buffered Pronto's recovery mechanism does not depend on these variables. However, we decided to implement this regardless, as it introduces negligible overhead and allows further improvements that depend on original Pronto's log metadata to be applicable in Buffered Pronto.

thread_sync() Implementation

Finding the exact log offset where all prior operations have persisted requires traversing the log and introduces latency to the workers. To improve thread_sync() performance, we use an overestimated approximation of a point in the log where all operations in the linearization are guaranteed to be written and persisted. We obtain this specific address via keeping track of the logical tail during each call to op_commit(). We will discuss the correctness of this approach in another section below.

Calling thread_sync() causes the thread to busy-wait until the offset in the ASL's persistent tail is larger or equal to the stored offset. Returning from thread_sync() indicates that all log entries have persisted before that point, and the worker thread can continue its operations. We decided to busy-wait instead of suspending because the persistent tail updates relatively quickly, making suspending introduce unnecessary latency. One potential future improvement would be to use Intel's new UMWAIT user wait instruction to hardware wait until the persistent tail updates, which is available on Intel's new Alder Lake processors [2].

Correctness

The modifications we made in Buffered Pronto require careful argument to ensure correctness. We have identified three cases where such a reevaluation is necessary and present our arguments in this section.

Failure Atomicity

We argue that all failures in Buffered Pronto can recover to a persistent state. We achieve this by having a single variable, the persistent tail, determine whether a log entry is visible to the recovery process. All log entries made visible by the persistent tail are guaranteed to have been entirely written and committed as the persister checked them during operation. Meanwhile, recovery ignores all remaining partially written log entries. A single persister updates the persistent tail through a single store instruction. Therefore, there are no possible race conditions or partial writes updating the variable.

If Buffered Pronto crashes before the persistent tail can be written or persisted, it uses the previous persistent tail. The previous tail still marks a valid range of persisted log entries. The only downside is that it may not be as up-to-date as the to-be-written persistent tail.

thread_sync() Correctness

We assert that the range from the log head to the logical tail directly after an op_commit() contains the log entries of all previous operations in the linearization. The commit ID determines the linearization order of the log entries written during op_commit(). Meanwhile, the logical tail indicates the end of the last partially-written log entry at any point in time. Since the commit ID only increases, calling op_commit() ensures that all prior commit IDs have been written somewhere in the log, in which the set of committed log entries is a subset of all log entries in the log. Thus the logical tail pointing to the end of all log entries can be used as an indicator that all prior commit IDs have persisted, regardless of their ordering in the physical log file.

Snapshot Recovery

Pronto's recovery process of replaying log entries on top of the last completed snapshot holds for Buffered Pronto, regardless of whether the snapshot is ahead of the persisted log. If the snapshot includes operations not visible in the persisted log entries, the recovery process can ignore the log and recover only using the snapshot to a consistent state. Original Pronto's snapshot mechanism ensures that no snapshot is taken during the middle of an operation, preventing inconsistent snapshots.

Evaluation

This section reports our findings for Buffered Pronto's performance on a bucket-based hash table and a simple CAS-based non-blocking concurrent queue [6]. We compare Buffered Pronto not only against Pronto-Full and Pronto-Sync but also Montage, an existing buffered durable linearizable persistent data structure framework. The data we gathered includes the throughput of operations, the latency of operations and thread_sync(), and the relationship between sync interval and latency for Buffered Pronto.

Test Environment

All tests were conducted on node2x20a.cs.rochester.edu, a server with the following configuration:

- CPU Configuration: 2 x Intel Xeon Gold 6230 (20 cores/40 threads each)
- Volatile Memory: 2 NUMA Nodes, with 187 GB memory and 20 cores/40 threads on each node, 18 GB of swap
- Non-Volatile Memory: 732 GB Intel Optane DC Persistent Memory configured in App-Direct mode
- Operating System: Fedora 30
- Linux Kernel Version: 5.3.7
- GCC Version: 9.3.1

We compile all microbenchmarks with -O3. The Pronto volatile memory allocator used for snapshotting is disabled to prevent bottlenecking at high thread counts. To ensure a fair comparison with Pronto-Full, which requires two threads per worker on the same physical core, we pinned all threads by the physical core on the first socket, then per core on the second socket. The microbenchmarks prefill all data structures with 50k entries, then perform a mix of insert, delete, and lookup operations at the ratio of 4:4:2. thread_sync() and its Montage equivalent sync() are called on average once per 1000 operations.

Code for the benchmarks and Buffered Pronto can be found at: https://gitlab.com/xythrez/pronto/.

Throughput and General Operation

While Montage outperforms all Pronto variants, Buffered Pronto still significantly outperforms Pronto-Full and Pronto-Sync for both the Hash Table and the Non-blocking Queue. On a single NUMA node, Buffered Pronto outperforms Pronto-Full and Pronto-Sync by x6.77 to x1.65 on the Hash Table and x4.70 to x1.26 on the Queue consistently. However, at higher thread counts where the threads reside on two different NUMA nodes, Buffered Pronto's performance drops as the cost of remote memory access takes over. Buffered Pronto keeps most of its bookkeeping data in volatile memory on the first NUMA node, where access time on a remote NUMA node can take up to x3.3 times as accessing it locally. The general performance degradation of Buffered Pronto with increasing thread count is related to not only the persister having to perform more work, but also contention on the volatile metadata, which needs to be atomically updated by each worker thread to fetch valid commit IDs and log ranges for new entries.

It should be worth noting that while Montage outperforms Buffered Pronto, it is possible to use data structures interchangeably between Buffered Pronto and the two original variants of Pronto, requiring no modification. Porting an existing data structure to Buffered Pronto from the original Pronto requires little programmer effort. The user only needs to insert thread_sync() in program locations where strict persistence is preferred.

Sync Latency

By comparing the sync latency between Montage and Buffered Pronto, we discover that Buffered Pronto's thread_ sync() is significantly faster than Montage's sync() in most cases. Buffered Pronto's thread_sync() involves waiting for the persistent tail to catch up with a past logical tail. The fastmoving persistent tail could have caught up with the logical tail before the call to thread_sync(), making the return happen almost immediately. However, this does subject Buffered Pronto to variable sync time. More worker threads operating on the data structure means that the persister must perform more work to catch up with the logical tail, increasing latency.

Meanwhile, sync() in Montage requires updating the epoch to ensure all persistent payload changes are made visible. Other than a few outliers, this operation generally takes a constant amount of time, allowing Montage to have a consistent sync time between 400s to 500s. Pronto's approach leads to a more variable sync time, ranging anywhere between 50s to 600s depending on the number of workers.

To determine how the number of operations affects sync performance for Buffered Pronto, we measured the latency of thread_sync() with different call frequencies and different numbers of worker threads. We discovered that while increasing the number of operations between syncs generally leads to high latency, extremely frequent syncs also result in higher sync latency at low thread counts. This higher latency is due to the persister suspending while waiting for additional work, leading to the worker threads waiting for this suspension period to end. Overall, having the sync frequency be between 100 to 1000 operations leads to the best response time. Calling sync with threads split across NUMA nodes results in significantly higher latency.

Interestingly, we discovered that the sync latency eventually reaches a cap where it does not increase any further. 40 worker threads with thread_sync() per 10k operations perform about the same as 40 worker threads with thread_sync() per 100k operations. The sync latency never exceeds 650s. This information ensures that regardless of the number of explicit calls to sync, Buffered Pronto can always contain data loss on a crash under one millisecond, which should be acceptable to most users.

Operation Latency

While operation latency of Buffered Pronto can be considerably lower than Pronto-Full and Pronto-Sync, requiring only 16 of the time in some instances, all variants of Pronto underperform Montage by a significant margin. This behavior is because Pronto writes all operation parameters to NVM and DRAM. On a data structure write operation, Pronto updates the volatile data structure in DRAM, then writes the changes to the ASL. Pronto's design is particularly problematic in Pronto-Full, where it copies the data to another shared buffer between the worker and persister before being written to the log. Pronto writes each operation parameter to memory multiple times during execution. The effect of this behavior worsens when some of the writes happen across NUMA nodes, which occurs over 20 worker threads in the benchmark. Meanwhile, Montage only keeps a single copy of the arguments in the persistent payload residing in NVM. Montage's design allows significantly fewer writes for the same operation than Pronto, leading to lower overall latency and higher operation throughput.

Conclusion

We have introduced Buffered Pronto, a variant of the Pronto persistent data structure framework that uses buffered durable linearizability instead of strict durable linearizability. By annotating existing Pronto programs with thread_sync(), it is possible to convert the program to use buffered persistence, improving performance at the cost of sub-millisecond data loss. While Buffered Pronto is not as fast as specially designed buffered durable linearizable frameworks such as Montage, it requires significantly less programmer effort to convert an existing Pronto data structure to Buffered Pronto. Remaining performance issues are limited by persister operation speed, and also the quantity of writes to both NVM and DRAM.

Acknowledgements

We want to thank Dr. Michael Scott for his contribution to buffered durable linearizability and guidance on implementing a buffered version of the Pronto system. We would also like to thank Wentao Cai and Haosen Wen for their assistance in fixing issues with the original Pronto code and for introducing us to the Montage API.

References

1. Wentao Cai, Haosen Wen, H. Alan Beadle, Chris Kjellqvist, Mohammad Hedayati, and Michael L. Scott. 2020. Understanding and Optimizing Persistent Memory Allocation. In Proceedings of the 2020 ACM SIGPLAN International Symposium on Memory Management (London, UK) (ISMM 2020). Association for Computing Machinery, New York, NY, USA, 60–73. https://doi.org/10.1145/3381898.3397212

2. Intel Corporation. 2020. Intel® Architecture Instruction Set Extensions and Future Features Programming Reference. Vol. 319433-038.

3. Intel Corporation. 2020. libpmem. Retrieved Dec 11, 2021 from https://pmem.io/pmdk/libpmem

4. Joseph Izraelevitz, Hammurabi Mendes, and Michael L. Scott. 2016. Linearizability of Persistent Memory Objects Under a Full-System-Crash Failure Model. In Distributed Computing, Cyril Gavoille and David Ilcinkas (Eds.). Springer Berlin Heidelberg, Berlin, Heidelberg, 313–327.

5. Amirsaman Memaripour, Joseph Izraelevitz, and Steven Swanson. 2020. Pronto: Easy and Fast Persistence for Volatile Data Structures. In Proceedings of the Twenty-Fifth International Conference on Architectural Support for Programming Languages and Operating Systems (Lausanne, Switzerland) (ASPLOS '20). Association for Computing Machinery, New Y o r k, N Y, U S A, 789 - 806. https://doi.org/

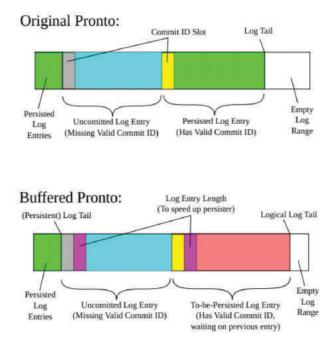
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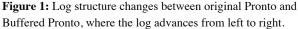
6. Pedro Ramalhete, Andreia Correia, Pascal Felber, and Nachshon Cohen. 2019. OneFile: A Wait-Free Persistent Transactional Memory. In 2019 49th Annual IEEE/IFIP International Conference on Dependable Systems and Networks (DSN). 151–163. https://doi.org/10.1109/DSN.2019.00028

7. Haris Volos, Andres Jaan Tack, and Michael M. Swift. 2011. Mnemosyne: Lightweight Persistent Memory. In Proceedings of the Sixteenth International Conference on Architectural Support for Programming Languages and Operating Systems (Newport Beach, California, USA) (ASPLOS XVI). Association for Computing Machinery, New York, NY, USA, 91–104. https://doi.org/10.1145/1950365.1950379

8. Haosen Wen, Wentao Cai, Mingzhe Du, Louis Jenkins, Benjamin Valpey, and Michael L. Scott. 2021. A Fast, General System for Buffered Persistent Data Structures. Association for Computing Machinery, New York, NY, USA. https:// doi.org/10.1145/3472456.3472458

Additional Figures





lgorithm 1 Buffered Pronto Persister Pseudocode loop	
$asl \leftarrow get_next_asl()$	
$ptr \leftarrow asl.tail.ptr$	
ptr \leftarrow asi.tan_ptr	
// Repeat if there is a valid commit ID stored at ptr	
while valid_commit_id(*ptr) do	
// Add the log entry size to ptr and move to the next entry	
$ptr \leftarrow ptr + *(ptr + sizeof(commit_id))$	
end while	
// Persist the log range	
$pmem_persist(asl.tail_ptr, ptr - asl.tail_ptr)$	
// Update persistent tail	
asl.tail_ptr \leftarrow ptr	
// Persist the metadata	
$pmem_persist(\&asl.tail_ptr, sizeof(asl.tail_ptr))$	
// Suspend if there not enough work	
if enough_time_to_sleep() then	
sleep()	
end if	
end loop	

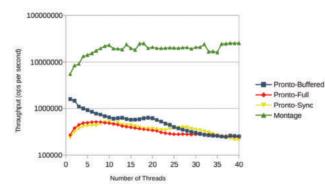
 Algorithm 2 thread_sync() Pseudocode

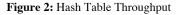
 asl ← get_current_asl()

 // Per thread last logical tail, stored during last call to op_commit()

 $last \gets get_last_logical_tail()$

while asl.persistent_tail < last do
 busy_wait()
 lfence()
end while</pre>





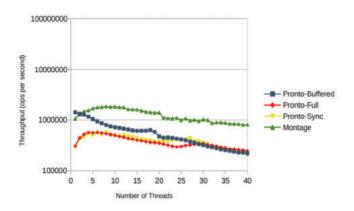


Figure 3: Non-blocking Queue Throughput

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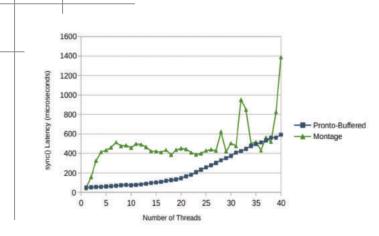


Figure 4: Non-blocking Queue sync() Latency Comparison

About the Author

I got into research during winter of the 2020 pandemic. I had some spare time and was interested in the work Prof. Scott was doing so I decided to reach out to him. Seeing how new technologies like nonvolatile memory can change traditional applications is something that quickly grabbed my interest, so I jumped into this project right away. For others trying to get into undergraduate research, I would say send that initial email as soon as possible. It doesn't matter if you don't know the professor that well, as long as the first step is done, everything else comes naturally. Introduce yourself, talk about your research interests, and good luck!

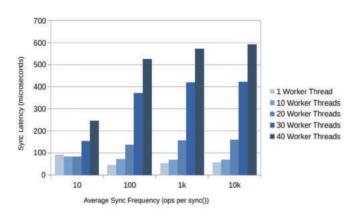


Figure 5: Pronto Non-blocking Queue sync() Latency as a Function

of Frequency

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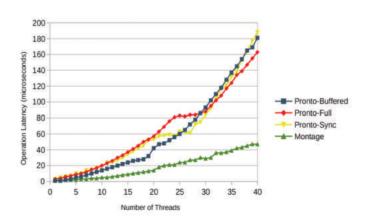


Figure 6: Non-blocking Queue Operation Latency Comparison



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Featured in This Issue

In The Main: Exploring The Interdisciplinary Anthropological Work Of Zora Neale Hurston Sasha Murray '22 (p.13)

Harlem renaissance writer Zora Neale Hurston, popular for her 1937 novel, "Their Eyes Were Watching God", was also captivated by dance. Fed by her anthropological studies of folk dance and songs in the US and Bahamas, Hurston put on a folk show on January 10th,1932 that aimed to accurately represent black dance on stage. This study contributes to the existing conversations surrounding Hurston's impact on dance by investigating the seldom talked about connection between literature and dance in her work. The study culminated in a creative dance project, translating the findings of this research to the concert stage.

Drinking Water as a Source of PFAS Exposure in Pregnant Women: Well versus Municipal Water Sources in Monroe County and

the City of Rochester Evan Bushinsky '22 (p.22) An ever-growing body of research points to the serious adverse health effects that PFAS presents to humans, especially pregnant women, their fetuses, and infants. A large and consistent source of PFAS exposure is drinking water. Using data from the UPSIDE (2021) study, it was found that in Monroe County and the City of Rochester, the estimated percent difference in PFAS exposure was lower in private well water sources than in the municipal water supply. It was also found that the sources of Rochester and Monroe County's municipal water, respectively, significantly differ in concentrations of PFAS.

Usefulness of the Reactive Strength Index (RSI) in Assessing Burnout in Division III College Athletes Domenica Verdi '22, Iva Savic '23, Hannah Keiper '23 (p.37) The aim of the study was to determine if reactive strength index (RSI) scores could be used to assess burnout (SMBM) in NCAA Division III student-athletes. The sample of participants consisted of 100 male and female students. The main statistical test determined that there was not a significant statistical relationship between SMBM scores and RSI scores. However, other statistical methods suggested that the study might have not been able to accurately determine the causal relationship. The study concluded that more research is needed to determine the statistical relationship between RSI scores and SMBM scores.

Mauritian Creole (Kreol) Kinship Terms of Reference Jillian Breithaupt '22 (p.44) The goal of this project is to examine what happens in a multiethnic, multilingual community when one language comes in contact with a number of "heritage languages". In the case of Mauritius and Kreol, kinship terms serve as salient markers of ethnic identity. Therefore, this project focuses on Kreol kinship terms of reference as an avenue to the broader questions of cross-language contact. In this paper, I will outline the methods of data collection, discuss each kinship system and relevant terms, and present my findings.

Achieving Abolition: Punishment, Public Sentiment, and Harm Prevention in Pakistan Mahnoor Raza '24 (p.53)

In the aftermath of gender-based violence in Pakistan, there is often a call for perpetrators to be executed. There is a disconnect between Western abolitionist frameworks, which might denounce this response, and on-ground social, cultural, and emotional realities in Pakistan. Currently, the death penalty is the only option victims and other affected individuals have in the absence of homegrown abolitionist imaginations. This paper suggests models that can create genuine spaces for healing, prevention, and accountability in non-punitive ways within the Pakistani context in the short term, and eventually engender abolitionist solutions in the long-term.

Investigating the Role of TRMT1L in tRNA Modification Chesna Apere '23 (p.58) Transfer RNAs (tRNAs) are one of the most highly modified RNAs. Lack of particular modifications can lead to many disease phenotypes caused by improper folding or more frequent degradation. Previous research suggests that tRNA methyltransferase-1-like (TRMT1L) plays a role in m2,2G modification of tRNA tyrosine-isoacceptors. We hypothesize that re-expression of TRMT1L into TRMT1L knockout cells will rescue the modification of tRNA-Tyr at position 27. To test this hypothesis, lentiviral constructs encoding TRMT1L were created to produce lentivirus in 293T cells. We plan on using this TRMT1L expression system to further understand TRMT1L and its role in tRNA modification.

Evolution and Evil-ution: Revealing the Assertion of National and Cultural Identities Through the Twentieth-Century Transformation of la Calavera in Mexico and Bawon Samdi in Haiti Megan Emery '23 (p.63) La calavera in Mexico and Bawon Samdi in Haiti share a century-long cultural, economic, and political history. This comparative project shows how their fundamental association shifted according to local, national, and international needs. I intend to articulate how both figures underwent two fundamental contextual transformations—first in the 1920s-1940s and subsequently in the 1970s-1980s. While la calavera has made the interaction with Mexican national identity wholly accessible, and while both adequately fulfill the cultural needs of their respective communities, only those within the Haitian community can fully appreciate Bawon Samdi as a crossroads figure between life and death. crossroads figure between life and death.

A Response to Alternative Decision-Making Capacity Standards Samuel Streicher '23 (p.71) In "Three Kinds of Decision-Making Capacity for Refusing Medical Interventions," Navin et al. describe the viability of multiple decision-making capacity (DMC) frameworks: burdens-based DMC, goals-based DMC, and comparative DMC. Although comparative DMC is typically held as the capacity standard in the literature of clinical ethics, Navin et al. argue that burdens-based and goals-based refusals can serve as morally and medically authoritative alternatives. Ironically, however, Navin et al.'s arguments can be reanalyzed to illustrate the necessity of comparative DMC: to ensure that patients' goals and objections are indeed overriding, patients must be able to compare options completely.

Improving Pronto Performance with Buffered Persistence Yiyao Yu '22 (p.74)

Persistence frameworks built with nonvolatile memory enable programs to retain data in the event of system failure, allowing fast data recovery at the cost of operating performance. We optimized an existing system called Pronto with buffered persistence to improve performance. Our evaluations showed that while our Buffered Pronto may not retain modified data within the last 1ms before a crash, its significant performance increase may prove beneficial for applications where performance matters more than full data recovery.

