Venture: The University of Mississippi Undergraduate Research Journal

Volume 6 Article 1

10-2-2024

Complete issue

Journal Editors

THE UNIVERSITY of MISSISSIPPI

UNDERGRADUATE RESEARCH JOURNAL

VOLUME NO. 6

2023-2024

VENTURE

University of Mississippi Undergraduate Research Journal

VOLUME NO. 6

FALL 2024

MISSION

Venture: The University of Mississippi Undergraduate Research Journal is a yearly double-blind, peer-reviewed publication of research written by students from across academic disciplines. We provide students the opportunity to experience the review and publication process first-hand. “Venture” embodies what we think drives new and dynamic scholarship: the courage to take risks, explore the unknown, and chart a new path. Our publication shares student scholarship that pushes limits and redraws boundaries for what research can do and where writing can take us.

ABOUT

Our journal takes its name from two previously separate publications at the University of Mississippi. In 2009, the Department of Writing and Rhetoric launched Venture, a magazine showcasing freshman student writing, especially creative works of prose, poetry, and art. The original Venture ran until 2013.

In 2015, Whitney Greer, a UM Writing Center consultant, created the U*niversity of Mississippi Undergraduate Research Journal (UMURJ)* to provide an outlet for undergraduate researchers to publish their work. The Associated Student Body recognized the importance of this endeavor and fully sponsored the journal for its first two years of publication. The journal continued until 2019. Sadly, during the COVID-19 pandemic and temporary shift to online and remote learning that followed, previous editors graduated and publishing the journal became very difficult.

In Fall 2021, a group of students and faculty decided to revive these efforts to celebrate excellence in undergraduate student scholarship by combining Venture and the UMURJ. Today, we are a registered student organization housed in the UM Writing Centers, with generous support from the Office of Research and Sponsored Programs as well as the Department of Writing and Rhetoric.

REVIEWERS

Reagan Allen

Christian Boudreaux

Grace Brian

Ellie Crane

Thomas Dyminski

Avery Flynn

Cayleigh Garrard

Sujung Ham

Sreya Maddali

Olivia Mardis

Grace Mattingly

Claire Reynolds

EDITORIAL BOARD

Lyle Tobin: Editor-in-Chief

Christian Boudreaux: Natural Sciences

Avery Flynn: Social Sciences

Grace Mattingly: Arts and Humanities

Graphic Design: Alexa Tamburlin

Faculty Advisor: Claire Mischker

Cover design: Lyle Tobin, Claire Mischker, & Alexa Tamburlin

ACKNOWLEDGEMENTS

Special thanks to the staff of the Graduate Writing Center for assistance with proofreading and copy-editing, including Jacob DeBrock, Caroline Brandon Kirkland, Elizabeth Pringle, Micah Russell, Ira Sen, and Regina Young.
We are especially grateful to Stephen Monroe and Jason Hale for their continuing time, expertise, and support.

If you would like more information about Venture or would like to be a reviewer, please email the editorial team at venture@go.olemiss.edu or visit our website. https://rhetoric.olemiss.edu/venture

CONTENTS

SUBMISSIONS

Pre-Service Teachers' Attitudes About Gender, Education, and Mathematical Aptitude: A Quantitative Study

Paige Barnett

ABSTRACT

Females and mathematics are often not grouped together due to the gender bias surrounding the two. Perhaps attending an all-girls high school has instilled a higher level of confidence in my own mathematical abilities than many women exhibit. The focus of my research is to examine preservice elementary teachers for their confidence levels in mathematics, as well as whether they hold a form of unconscious gender bias. I completed this research by developing a six-question survey instrument. The survey was administered in the spring and fall of 2023 to Math 245 and Math 246 classes, both of which are required math courses for elementary education students. These classes cover methods for teaching elementary level mathematics. Because education is a major populated predominantly by females, the highest percentage of participants in the survey identified as a woman. The survey results indicated a higher level of math confidence than predicted, which provided hope that not all females are being pushed out of math. While these participants had a higher confidence level, they still held a level of gender bias. When picking a working partner for a graded problem, the majority of participants chose partners based upon math confidence scores. However, when the problem is ungraded, the highest chosen characteristic in a working partner is gender. This research has helped to highlight the importance of both mathematical confidence and gender when selecting a working partner in math class. It has also indicated that gender bias is present in many differing forms, which is a key point in understanding how to combat it in the classroom.

Introduction

From the start, I knew I wanted my capstone project to be focused on the realm of mathematics and gender. Both topics have fascinated me throughout both my high school and collegiate years. Because of my experiences, I feel privileged to have received encouragement from previous teachers in regards to my math ability. However, not every woman is as lucky to have a constant support system promoting confidence in science, technology, engineering, and math (STEM) related fields. This lack of confidence is a main focus of my research and study. Throughout this research, I hope to gain an understanding of how gender plays a role in math confidence levels.

Attending an all-girls private high school was perhaps the most pivotal part of my education. It was there that I learned it is perfectly normal to make mistakes, and, hopefully, we can learn from our mistakes and grow as human beings. Being in an environment of all women allowed for true growth from these errors instead of potential embarrassment that may come from having males in the classroom. My teachers encouraged every student to work their hardest in all aspects of their education, not just in one field. This created a culture where females felt comfortable pursuing their interests in STEM-related fields, and we were supported instead of discouraged. Without this positive reinforcement, I can confidently say I would not have the courage to be a secondary math education major. My high school teachers instilled a level of confidence in me that I am equally as worthy and knowledgeable as my male classmates. Had I attended a coeducational school, I cannot say I would have chosen the same degree path.

Many females begin to be pushed out of STEM-related fields in their secondary education. Up until that point, they may have been encouraged to try their best in these courses. However, once they entered middle school, that encouragement may have begun to decrease. There are two factors that play a role in women's decreased interest and participation in STEM-related fields. First, many teachers, even females, have an unconscious bias that favors male students. Since this is unconscious, many teachers will not recognize they are encouraging boys more, as well as giving them more opportunities. The second factor is negative peer response. Many students still believe males should excel in math and science courses, and females should outperform in literature and history classes. With this belief, many women receive pushback from peers if their academic choices are in STEM subjects, which instills discouragement instead of confidence level for STEM subjects. While these are not the only factors that can cause a decreased confidence level in mathematics for female students, I believe they are two of the most prominent and disruptive causes.

When I begin my teaching endeavors, I will be teaching in a coeducational classroom. Instead of condoning the traditional approach of separating males and female students into separate fields of study, I will fight for equal opportunities for both genders. Thus, it is one of my highest priorities to boost the math confidence levels of all of my students, especially the females. I want to be an advocate for those students who believe they cannot pursue a career in a STEM field because it is a traditionally male-dominated field.

The classroom environment I intend to foster will be conducive to learning and growing from mistakes rather than being embarrassed by them. By emphasizing the importance of mistakes, I believe students will begin to feel more comfortable in the classroom and about math in general. Building a safe environment is the first step to boosting confidence. Once the environment is safe, then students should be more willing to learn and grow their skills. An inclusive and welcoming classroom is vital to the success of students on their learning journeys. This inclusivity encompasses the use of encouragement instead of discouragement. In my future classroom, my hope is that no student will be discouraged during their learning process. Discouragement can cause a negative impact on their confidence levels and lead to a dislike of the subject. To further build up courage and competence, students should receive encouragement throughout their learning. By incorporating a safe, welcoming environment and minimizing

the use of discouragement, students' math confidence levels should prosper.

I plan on using this research to better inform my teaching methods as well as those of my peers. I want to be able to take the results and learn how to better serve my students. I hope the results will act as a guide when creating more effective lessons and intentional interactions to boost confidence levels among learners. Information gathered will help to decipher whether coed groups or same-gender groups are more beneficial to students when completing group work. The research also serves as a reminder to survey students about their likes and dislikes. If a female student is not comfortable working with male students and will not be as successful in that setting, it is imperative to place them with another female. Without asking students about their comfort levels, we, as educators, are not promoting the safest environment for them. Thus, I hope to discover how confidence levels in math can affect a student's choice of partners when relating to group work.

To gather information about gender and confidence levels relating to mathematics, I developed an instrument to survey college students. The focus of the research is to identify whether preservice elementary teachers hold a form of gender bias, specifically in mathematics. If a teacher holds an unconscious bias, then it is likely the students in the class will be affected. Teachers with gender bias often tend to favor the male students and sometimes do not give equal attention to the female students. The data gathered will indicate whether there is any form of gender bias present among preservice teachers relating to mathematics. The survey was administered to all of the Spring 2023 and Fall 2023 courses of Math 245 and 246,each of which is a required course for all elementary education majors. The focus of Math 245 is to introduce students to sets, the real number system, and its subsystems. The students learn methods of how to teach these topics to their future students. Math 246 covers the best teaching methods for introducing geometry and mastering measuring and the metric system. The students also spend time reviewing the best teaching practices for probability and statistics.

The survey presents a series of six multiple choice questions. The first question asks participants to identify their gender. The students are given six different options: Woman; Man; Transgender Woman; Transgender Man; Nonbinary; Prefer not to respond. The next question gives a baseline of what the students believe their math confidence level is. They have the option to choose a level from zero to five, with zero indicating no confidence and five indicating extremely confident. The third question asks students what type of high school they attended; the only two answer choices are public or private.

The fourth question states: "You are in a room with a group of peers you do not know. If you are asked to solve a math problem that *will be graded* and you can select a working partner from the people in the room, which gender are you most likely to select for your partner?" The students are given the same answer choices as the first question: Woman; Man; Transgender Woman; Transgender Man; Nonbinary; Prefer not to respond.

In the fifth question participants are given the same prompt as before, but with slightly different wording: "You are in a room with a group of peers you do not know. You are asked to solve a math problem that *will be graded* and you can select a working partner from the people in the room. If you know their math confidence score, which characteristic will most impact your decision when selecting a partner?" There are five answer choices for this question: Gender; Sexual Orientation; Math Confidence Score; Public vs. Private School Attendance; Other.

The final question is almost the same as the fifth question. However, the math problem is no longer being graded. The question states: "You are in a room with a group of peers you do not know. You are asked to solve a math problem that *will not be graded*. You can select a working partner from the people in the room to help you solve the problem. If you know their math confidence score, which characteristic will most impact your decision when selecting your partner?" The answer choices for this question are: Gender; Sexual Orientation; Math Confidence Score; Public vs. Private School Attendance; Other.

For each of the survey questions, I have developed predictions of potential results. For

the first question, I predicted that the majority of survey takers will be female, with the other categories making up the minority. The hope for the second question was to receive a data representation similar to a traditional bell curve. With this hypothesis, I predicted that about 25% of survey takers will have a confidence level between 0 and 1. I anticipated around 55% will have a level of either a 2 or a 3. Finally, I predicted close to 20% of students will feel extremely confident with a 4 or a 5. While private schooling is becoming more popular, I still believed the public schools would be more dominant among survey respondents. Thus, I predicted only 30% of students will have attended a private school, and the majority of students will have attended a public high school.

The fourth question asks students to pick the gender of their preferred classroom working partner. I anticipated a majority choosing a man, with woman being the second picked category. For the fifth question, I foresaw the math confidence score being the top answer. Because of this prediction, I thought gender would be the second most dominant answer with the math confidence score receiving a majority of the selections.

For the final question, I anticipated a swift change from the results of the fifth question. I believed there would be a switch from math confidence being the most important to gender. Thus, I predicted around half of the survey population would choose gender as the most important, and math confidence score would then move to second highest chosen.

Through attending an all-girls high school, I was constantly supported and challenged to further my knowledge in mathematics. However, this is often not the case for many female students. By the time they reach high school, they have often already been discouraged and pushed away from pursuing a degree in mathematics. My hope as a teacher is to provide a positive learning environment for all of my students. This research will help guide me to develop practices that allow students to feel comfortable and also supported in their learning processes. Every student should have the opportunity to pursue a degree in a STEM field, not just males. The purpose of this research is not only to get an accurate baseline of data from students, but also to guide future research questions. I hope to develop more effective teaching methods to promote the confidence levels of my students, as well as take steps to decrease gender bias in mathematics. This study will serve as a reminder to all preservice teachers to be aware of their unconscious bias and how it can impact a student's future opportunities.

Literature Review

Confidence Levels in Mathematics

A key part in my research is preservice teachers' confidence levels in mathematics. However, before they were elementary education majors, they once were middle and high schoolers. At a young age, students begin to develop confidence in school subjects. Some students may excel more in a language arts class than the students who do well in a math course. This plays a vital role in the development of confidence. These levels can change over time depending on many factors, including environment, teacher, and school. Thus, the purpose of this literature review is to investigate the effects of confidence levels in mathematics on K-12 students. The findings from these studies will help to inform the research and interpret the results.

Before delving into the research and results from these various studies, the definition of self confidence, specifically relating to math, must be explored. First, it is important to understand that confidence can be measured and conceptualized in various ways for research (Sheldrake et al., 2022). Self-confidence is an individual's belief that an outcome will occur as expected. The confidence is developed through past and current experiences and events (Schuh et al., 2023). These past influencing experiences can be categorized into four types: mastery experiences, social persuasions, vicarious experiences, and physiological states (Sheldrake et al., 2022). When relating to mathematics, self-confidence is the concept of how well the student

thinks they will perform on certain mathematical tasks and skills (Schuh et al., 2023). Confidence can often be tied to a particular task. If a student is able to complete a challenging word problem, their confidence will increase when going into the next problem set. This can also be the case for struggling students as their confidence levels are likely to decrease (Schuh et al., 2023). Research has proposed that if a student has a higher confidence level, they will be more inclined and motivated to surpass their normal performance; while lower confidence may be an inhibitor (Sheldrake et. al., 2022). Therefore, based upon previous research, self confidence can play a large role in a student's future endeavors and successes.

Attitudes towards math can greatly affect a student's confidence. Often, if a student has a negative attitude towards math, they will have a lowered confidence level. Attitude is a multidimensional construct that includes engagement, likes, confidence, anxiety, and beliefs (Christensen & Knezek, 2022). Each one of these factors plays a different role in developing a student's attitude. Previous research has shown that students with high math anxiety often enjoy math less and will have a lower confidence level. From this, it is believed that confidence can be a predictor of how a student will perform in their math courses (Christensen & Knezek, 2022). Students begin to develop their attitudes towards math in their early years of school.

Confidence, however, is not always beneficial to students. Overconfidence and underconfidence can be detrimental to a student if they let it dictate their study habits and participation in class. If a student is overconfident, this may lead them to believe they do not need to study for a test. The student would then do poorly on their test due to the overconfidence. Students who have underconfidence typically have a lower satisfaction with their math performance, perhaps indicating the two have an impact on each other. These levels of over- or under- confidence are more often seen in lower-performing students rather than the higher performing students (Sheldrake et. al., 2022). While some research appears to indicate that there is no correlation between student confidence levels and performance/success in mathematics, overconfidence and underconfidence have shown this hypothesis to be incorrect.

Each of the articles provided a slightly different approach to investigating confidence levels in mathematics. However, the results can all be linked together to form an interesting outcome. In Christensen and Knezek's (2022) research, they found there were significant differences throughout each grade level in math enjoyment, confidence, and attitudes towards school. The data also pointed out a trend that the previously stated dispositions became lower as grade levels advanced, specifically in the period between 5th and 8th grade (Christensen & Knezek, 2022). The confidence levels in 5th grade did predict a student's success in the class. However, when the students got to 9th grade, their confidence levels positively predicted performance. The findings from this study affirm to some extent that over- and underconfidence on mathematics tasks may link with their generalized math beliefs and performance (Sheldrake et. al., 2022).

Gender and Mathematics

When it comes to mathematics and other STEM-related fields, there tends to be a smaller ratio of women to men (Muzzatti & Agnoli, 2007). Even though the number of females in these fields is continuously increasing, many people in society still believe untrue stereotypes (Kane & Mertz, 2012). The widespread nature of these beliefs may cause women to consider other lines of work due to the lack of support from their peers. Stereotype threats have also been a tool used to prevent many women from pursuing mathematics degrees. Women have come a long way from representing less than a tenth of the field, but it is unclear whether they have reached the point to close the gender gap (Muzzatti & Agnoli, 2007). The goal of this literature review is to examine if there is still a gender gap in mathematics, specifically in school aged children and young adults.

There tend to be many stereotypes surrounding gender and mathematics. It is commonly

thought that math and science are male domains where females are unable to succeed. Many parents and teachers believe their female children/students are inferior compared to their male peers when it comes to mathematical ability. This mindset is passed onto their children, who may believe boys and girls have equal mathematical abilities, but male adults are better than females in math. In one study, fathers believed their sons had on average a 12- point higher IQ level than their daughters (Lindberg et al., 2010). The mothers also believed their sons' IQs to be higher, but only by six points (Lindberg et al., 2010). Another common stereotype is that women tend to choose fields that focus more on nurturing instead of quantitative skills (Kane & Mertz, 2012). While many people are fighting against these stereotypes with time and money to increase the female representation in mathematics-intensive fields, these efforts can quickly be thwarted. This has caused some to believe it is a waste of time and resources to include more women in the field, since they will often choose a more nurturing career instead (Kane & Mertz, 2012). These stereotypes are shaping certain fields to push women out because they are seen as "incapable" instead of creating a welcoming environment where everyone is invited to become a scholar of mathematics.

Another large factor contributing to the lack of women in mathematics is the theory of stereotype threat. The theory consists of the idea that a situational decrement in a person's performance may occur due to the awareness that their own ingroup is rated as less skillful in the domain they are going to be tested in (Muzzatti & Agnoli, 2007). This threat model has been found to have effects in younger (ages 5-7) and older girls (ages 11-13) but not necessarily in intermediate age girls (ages 8-10) (Muzzatti & Agnoli, 2007). When it comes to high school females, the discrepancy between performance and recognition is quite detectable. These girls will achieve better grades than their male peers in the classroom. However, in Mathematics Olympiads, the girls who compete do not perform as well as the boys. This can be attributed to the underrepresentation of women at these types of events (Muzzatti & Agnoli, 2007). College females are also affected by stereotype threat. Studies have shown that college women underperform compared to men in the threat condition. Whenever the threat condition was removed, the women performed at an equal level as the men (Lindberg et al., 2010). Creating a stereotype threat for females will indeed impair their mathematical performance.

Other research explores whether there is a true gap between male and female performance in mathematics. The studies found that in elementary and middle school there was little to no difference in mathematical ability between the two gend ers (Kane & Mertz, 2012; Lindberg et al.,2010). It was only in high school and college where there was a small difference between males and females. In these situations, the males were *favored* to have higher ability, especially in relation to complex problem solving. This difference can be attributed to the parents' and teachers' estimates of their children. If both parties believe the boys to have a higher ability than the girls, this can affect the students greatly causing a difference in ability level. However, the findings indicate there is no longer a gender difference in mathematics performance (Lindberg et al., 2010).

Teacher Perceptions and Mathematics

Teachers play an important role in students' success in school. Their perceptions can have both positive and negative effects on students' perceptions of themselves. Oftentimes teachers carry unconscious biases and perceptions that can be harmful to students when implemented into the classroom and workday lessons. These biases can have a detrimental effect on students and their future success due to a lack of support. Teachers tend to overestimate the mathematical ability of the male students in the classroom and underestimate the abilities of female students. This places the males on a pedestal, giving them more support for success. Overestimation of a student's abilities can lead to a positive impact on their own beliefs and ability level. One focus of this literature review is to investigate how teachers' perceptions and biases impact both female and male students.

The way a teacher interacts with their classroom can have a large impact on their students. Thus, it is important to recognize and understand the different biases and perceptions many teachers hold. It is more common for implicit stereotypes about gender differences in mathematics to be present in a classroom than explicit ones. Gender bias is so socially ingrained into society it can be a difficult subject to grasp (Riegle Crumb & Humphries, 2012). Teachers and parents are the top socializing agents in the construction of these gender norms (Robinson-Cimpian, 2014). Due to these norms, elementary teachers tend to rate the mathematics skills of the girls lower than the boys, even though the girls will behave and perform in a similar manner to their male counterparts. Teachers often give boys more specific and positive feedback due to higher expectations. This is in part due to the belief that boys have a natural gift for excelling in math. In this article, the teachers attributed female student failure to lack of abilities and male student failure to lack of effort (Robinson-Cimpian, 2014). It was found that the only time teachers tend to rate their female students higher than the boys is in the spring of kindergarten (Robinson-Cimpian, 2014). After this period, the male students are perceived to have higher abilities. By understanding the biases teachers hold, one can further examine how teachers' biases impact their students.

A teacher's perception of a student is sometimes associated with a negative impact. However, there are many studies indicating that the perception a teacher holds of a student can have a positive impact on their ability level. The student-perceived teacher ability-beliefs can be used as a prediction of the student's academic achievement (Gniewosz & Watt, 2017). If a teacher or parent holds a more optimistic view about a student's ability, then the student feels supported and is likely to reach a level of competency. Thus, the overestimation of skill level by a teacher can promote students' intrinsic motivation. This perceived teacher overestimation of ability does not have any predictive power of intrinsic values until the student reaches eighth grade. After that point, the more the teacher overestimated the skill level, the more the student's utility and intrinsic values increased. This was specifically detected during the period between eighth and tenth grade. A study revealed that students are motivated by believing their teachers think highly of them and their ability levels (Gniewosz & Watt, 2017). Even if the student did not feel strong in their math abilities, the positive perception from the teacher helped to boost their confidence and increase their competency level. However, the use of overestimation by the teacher should be used moderately and not to an excessive amount. If a student is receiving too much pressure from the teacher, it can lead to a negative effect on their skill level. Thus, teachers must learn how to use their perceptions about a student's ability to positively create change in the learner.

Capstone

The survey instrument was created with the purpose of identifying any gender bias present in preservice elementary teachers in relation to mathematics. Since the instrument was implemented in two different semesters, the data will be broken down into two groups. The first group will include only the Spring 2023 classes of Math 245 and 246. The second group will examine both the results from the Spring 2023 and Fall 2023 classes to include a wider review and analysis of the data. The results from each question will be further analyzed for both groups. From the research and results, I will develop action steps and recommendations to further prevent gender bias in the mathematics classroom. The data will also be used to inform how to implement these steps into my future classroom.

Question 1

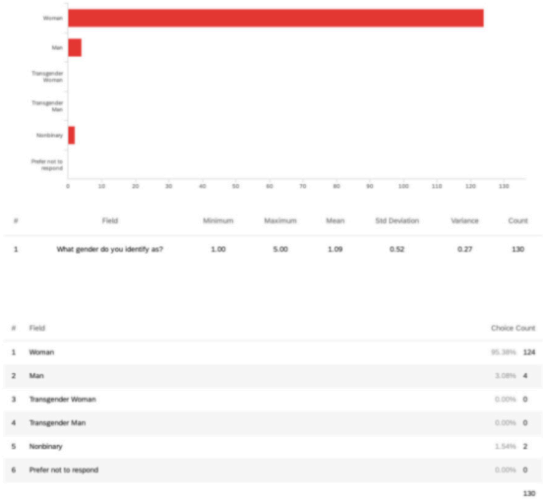
The first survey question asked participants to identify their gender. As mentioned in the introduction, I predicted the survey takers would be majority female, with a small percentage of male, transgender woman, transgender man, and nonbinary participants. The results from the first round of the surveying indicated there were 70 participants. Of these 70, 66 indicated they identify as a female, which is around 94%. Only two people identified as male, which equaled 3%. Nonbinary identifiers also equaled 3%. No one identified as a transgender woman or man. Once the survey was administered again, there were 130 participants. Of those survey takers, 124 identified as female, equaling close to 95.5%. There were four males, and the number of non-binary participants stayed the same. The other categories remained unidentified. These results indicated the majority of elementary education students taking these two required courses were females, which coincides with the fact there are more female teachers than males. With there being a high population of women taking the survey, this could potentially skew the data. A common belief associated with gender bias is that males are better at math than females. However, it is possible that because a majority of the survey takers were women there will not be as strong of a bias towards students of their own gender.

Table 1 (Spring): Data results from the spring 2023 administration of Question 1.

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	What gender do you identify as?	1.00	5.00	1.14	0.68	0.47	70

#	Field	Choice Count
1	Woman	66
2	Man	2
3	Transgender Woman	0
4	Transgener Man	0
5	Nonbinary	2
6	Prefer not to respond	0
		70

Table 2 (Spring and Fall): Data results from the spring and fall 2023 administration of Question 1.



#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	What gender do you identify as?	1.00	5.00	1.09	0.52	0.27	130

#	Field	Choice Count
1	Woman	124
2	Man	4
3	Transgender Woman	0
4	Transgender Man	0
5	Nonbinary	2
6	Prefer not to respond	0
		130

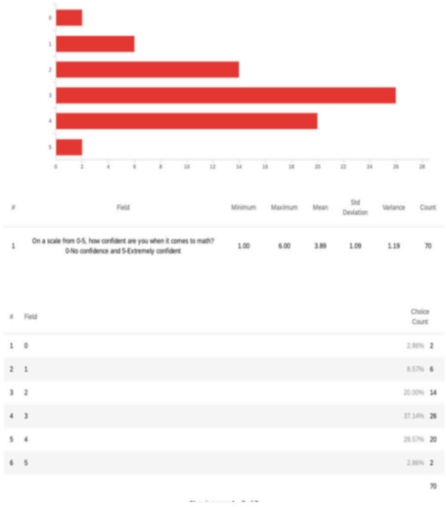
Question 2

The second question explored student confidence levels. The participants were asked to self-identify their confidence levels when it comes to math. In my previous predictions, there were three groups. The first group included levels 0 and 1, which was predicted to be 25% of the population. The second group was the largest group, predicted with 55% in the 2 or 3 levels. The final group was levels 4 and 5, which received a smaller prediction of 20% of participants. The actual data results proved to be quite different. At both the 0 and 5 levels, there were only two students, which totaled 3% of participants for each level. Level 1 received about 8.5% of survey takers, with six students. The confidence level of 2 ended up being 20% of the population, with 14 students. The third level received the highest number of students, resulting in about 37%. Level 4 was the second highest identified category with 28.5% of participants. After the completion of the second round of surveying, the data changed ever so slightly and became more centralized to the middle. Levels 0, 3, and 5 saw decreases, while levels 1, 2, and

4 had slight increases. Level 0 received five participants, and level 5 saw the second least with seven participants. Levels 1 and 2 received around a 1% decrease to 7% and 18.5%, respectively. The largest decrease was in the 4th level, which went down 4.5% to allow for level 3 to increase by that same number. Level 3 ended up with 41.5% of survey participants, and level 4 received 24%.

In the second round of surveying, the data changed slightly towards the middle confidence levels. Level 0 increased from 3% to 4%, while level 1 decreased down to 7%. The levels where the most change occurred are 2, 3, and 4. Both levels 2 and 4 saw decreases.
A possible explanation for the results being higher than predicted is the date of instrument administration. The survey was not implemented until the middle of the semester. The students had a longer time to increase their confidence in their math skills, which potentially led to a higher level of confidence reported. The next round of surveying took place at the beginning of the semester to see if there were any differences. The data from the next round did not indicate there was much of a difference in confidence level in relation to the time in the semester it was administered.

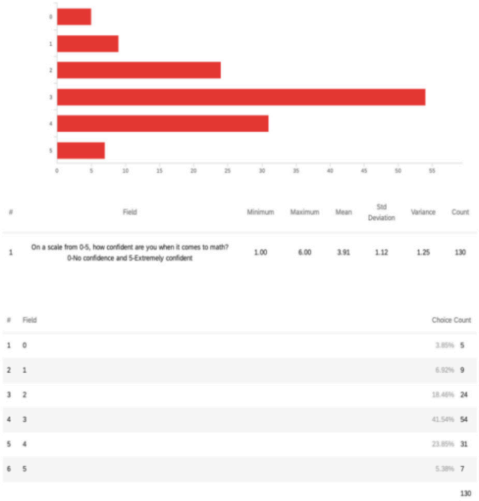
Table 3 (Spring): Data results from the spring 2023 administration of Question 2.



#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	On a scale from 0-5, how confident are you when it comes to math? 0-No confidence and 5-Extremely confident	1.00	6.00	3.89	1.09	1.19	70

#	Field	Choice Count
1	0	2
2	1	6
3	2	14
4	3	26
5	4	20
6	5	2
		70

Table 4 (Spring and Fall): Data results from the spring and fall 2023 administration of Question 2.



#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	On a scale from 0-5, how confident are you when it comes to math? 0-No confidence and 5-Extremely confident	1.00	6.00	3.91	1.12	1.25	130

#	Field	Choice Count
1	0	5
2	1	9
3	2	24
4	3	54
5	4	31
6	5	7
		130

Question 3

The third question asked participants to identify which type of high school they attended. Students were given the choice of either public or private high school. I predicted a majority of the participants to have attended a public high school, averaging about ⅔ of the target population. This left around ⅓ of survey takers to have attended a private high school. The results from the instrument show a similar response. Around 73% of the participants attended a public high school, and 27% attended a private high school. These results remained the same after the second administration of the survey. This question was examined because both high school settings can provide a different amount of support to students. Oftentimes, students in a private high school may receive more support and individualized attention compared to their public-school peers. This extra support can foster a higher confidence in mathematical abilities. Many students in a public-school setting will receive that level of attention and support; however, it is not as common. Thus, we use these results to examine whether the public-school students or the private school students will have a higher math confidence level. After further investigation, the public-school students had a slightly higher average confidence level of 2.904. The private school students average was 2.842.

Table 6 (Spring): Data results from the spring 2023 administration of Question 3.

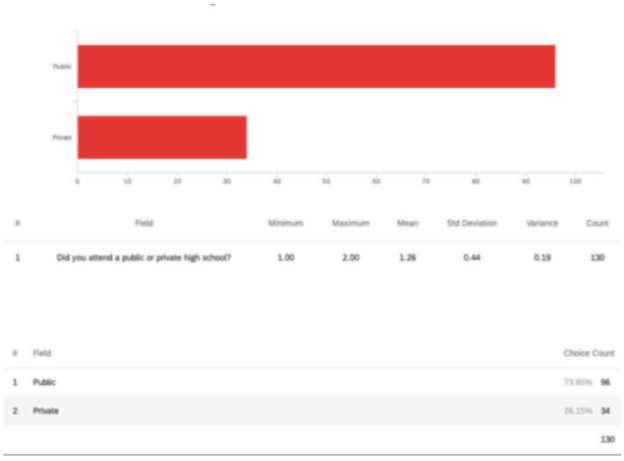


Table 7 (Spring and Fall): Data results from the spring and fall 2023 administration of Question 3.

Question 4

The fourth question had perhaps the most surprising results. As previously stated, I predicted around half of the participants would most likely choose a male as a working partner. The next highest chosen gender was predicted to be a female. In actuality, the highest picked gender for a working partner was a female. There were over 59 students who chose this gender, equivalent to about 84%. The next highest picked gender was a male, which received about 14% of participant votes. The transgender woman, transgender man, and non binary all received zero votes. Only one participant chose not to respond, equating to about 2%. The second round of surveying produced similar results. The female working partner received 85% of participant votes, the male working partner stayed at 14%, and those who preferred not to respond decreased down to 0.78%.

This response was shocking due to the widely accepted gender norms for males and

females. One would expect the male working partner option to be the most widely chosen due to the common belief that men are inherently better at math. There are two possible explanations for this type of response. The first would be due to the fact there were only four students who did not identify as a woman. With a class full of females, the students may have felt more comfortable and confident in choosing a female for a working partner. The other possibility is that the population sampled does not have as strong of a gender bias relating to mathematics. If their bias is not as strong, then they are more accepting of all genders in math compared to those who believe math is a male-dominated field. The continuation of this survey in the Fall 2023 semester indicated that gender bias is present, so the second explanation is not as feasible. The results, however, are likely to have been influenced by the high population of females in this major.

Table 8 (Spring) : Data results from the spring 2023 administration of Question 4.

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	You are in a room with a group of peers you do not know. If you are asked to solve a math problem that will be graded and you can select a partner from the people in the room, which gender are you most likely to select for your partner?	1.00	6.00	1.18	0.55	0.30	129

#	Field	Choice Count
1	Woman	85.27% 110
2	Man	13.95% 18
3	Transgender Woman	0.00% 0
4	Transgender Man	0.00% 0
5	Nonbinary	0.00% 0
6	Prefer not to respond	0.78% 1
		129

Table 9 (Spring and Fall): Data results from the spring and fall 2023 administration of Question 4.

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	You are in a room with a group of peers you do not know. If you are asked to solve a math problem that will be graded and you can select a partner from the people in the room, which gender are you most likely to select for your partner?	1.00	6.00	1.21	0.67	0.45	70

#	Field	Choice Count
1	Woman	84.29% 59
2	Man	14.29% 10
3	Transgender Woman	0.00% 0
4	Transgender Man	0.00% 0
5	Nonbinary	0.00% 0
6	Prefer not to respond	1.43% 1
		70

Question 5

The purpose of the fifth question was to investigate which characteristic most impacts the decision when selecting a working partner for a graded question. The math confidence score is a known variable, but is also used as a characteristic. The prediction was the math confidence score would be the highest chosen characteristic. The second highest was gender, and the other three categories: Sexual orientation, Public vs. Private education, and others were predicted to make up the smallest percentage. The results proved to be quite similar to the predictions. The math confidence score was the highest chosen characteristic with 61 participants or an 87% selection rate. Gender was the second rated characteristic with 11.5% of students choosing it. One student selected other and indicated they would pick based upon math grades. The sexual orientation and public vs. private education characteristics were not chosen in the first survey administration. After the second round of surveying, the data remained consistent. Eighty-seven percent of survey respondents chose math confidence score, 11% chose gender, 1.5% specified other, and .78% chose sexual orientation.

This indicates the students are the most confident with a working partner who has a high level of math confidence. If the partner does not feel confident in their abilities to complete the problem, the student is not likely to select them due to it being graded. Students also found gender to be an important characteristic as well. The gender of the working partner might help

the students to feel more comfortable expressing their ideas and opinions. Thus, this question indicates students need to either feel comfortable to solve a graded problem or have someone else who is confident in their abilities.

Table 10 (Spring): Data results from the Spring 2023 administration of Question 5.

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	You are in a room with a group of peers you do not know. If you are asked to solve a math problem that will be graded and you can select a partner from the people in the room. If you know their math confidence score, which characteristic will most impact your decision when selecting for your partner? - Selected Choice	1.00	5.00	3.67	0.97	0.93	70

#	Field	Choice Count
1	Gender	8
2	Sexual Orientation	0
3	Public vs. Private Education	0
4	Math Confidence Score	61
5	Other	1
		70

Table 11 (Spring and Fall): Data results from the Spring and Fall 2023 administration of Question 5.

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	You are in a room with a group of peers you do not know. If you are asked to solve a math problem that will be graded and you can select a partner from the people in the room. If you know their math confidence score, which characteristic will most impact your decision when selecting for your partner? - Selected Choice	1.00	5.00	3.67	0.96	0.92	129

#	Field	Choice Count
1	Gender	14
2	Sexual Orientation	1
3	Public vs. Private Education	0
4	Math Confidence Score	112
5	Other	2
		129

Question 3

The final question of the survey examined who a student will choose for a working partner when the math problem is ungraded. When the last question was posed as a graded problem most participants chose a working partner based upon confidence score. The prediction for the ungraded problem was most participants would switch from math confidence score to gender as the most important characteristic. I predicted a majority would select gender and the next highest selection would be math confidence score. The actual results for the first round of survey administration proved to be very similar to the predictions. Gender was the highest selected characteristic with a 51.5% selection rate. The second highest characteristic was math confidence score and it was chosen 39% of the time. Public vs. private education and the other categories were both chosen by 3 people each, totaling to a combined 8%. Only 1 participant chose sexual orientation as the most important characteristic. When the survey was readministered, the results remained similar to the results from the first administration. The gender decreased to 47.5%, and the math confidence score saw a slight increase to 45%. The other three categories make up the remaining 7.5% of respondents.

From this big switch between math confidence score and gender we can see how prevalent gender is in mathematics. When students know they are going to be graded they tend to choose a working partner that will help them succeed. However, when the stakes are taken away the participants choose working partners of the gender they feel most comfortable with. The results of this question paired with the results from question four are a large indicator of the level of gender bias in mathematics the group holds. A majority of the students in question four chose to work with a female partner. This shows there is still a higher level of gender bias in this group. The participants believe they can achieve an ungraded problem when choosing a working partner based upon gender. However, they do not feel as confident in choosing the same characteristic when the problem is graded. While the students chose female working partners in the fourth question, they still hold a form of gender bias which can be easily seen by

the switch in responses for this question.

Table 12 (Spring): Data results from the Spring 2023 administration of Question 6.

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	You are in a room with a group of peers you do not know. You are asked to solve a math problem that will not be graded. You can select a partner from the people in the room to help you solve the problem. If you know their math confidence score, which characteristic will most impact your decision when selecting for your partner? - Selected Choice	1.00	5.00	2.43	1.52	2.30	70

#	Field	Choice Count
1	Gender	35
2	Sexual Orientation	1
3	Public vs. Private Education	3
4	Math Confidence Score	27
5	Other	3
		70

Table 12(Spring and Fall): Data results from the Spring and Fall 2023 administration of Question 6.

#	Field	Minimum	Maximum	Mean	Std Deviation	Variance	Count
1	You are in a room with a group of peers you do not know. You are asked to solve a math problem that will not be graded. You can select a partner from the people in the room to help you solve the problem. If you know their math confidence score, which characteristic will most impact your decision when selecting for your partner? - Selected Choice	1.00	5.00	2.55	1.51	2.28	129

#	Field	Choice Count
1	Gender	61
2	Sexual Orientation	2
3	Public vs. Private Education	4
4	Math Confidence Score	58
5	Other	4
		129

Recommendations

The research gathered from this case study has led me to develop three action steps and recommendations I plan to implement in my future classroom. The first of these ideas is to provide positive role models for all students. It is important for students to see someone who looks like them as a role model. If there are only white male mathematicians being displayed in the classroom, the students who do not identify as such will feel discouraged from becoming a mathematician. There needs to be an equal representation of role models both widely diverse in race and gender. All students deserve to have a role model who they can identify with.

The second recommendation is to incorporate more encouragement in the classroom. In math the boys tend to get more praise than the rest of their classmates. This excess of encouragement often leads to the females feeling like they do not belong or cannot succeed in math. Thus, it is important that all students receive praise and encouragement throughout the entirety of the learning process. When students are all getting an equal amount of encouragement then they all feel they are worthy of succeeding in mathematics. I want to ensure the students are getting the amount of support they need to build their confidence and mathematical skills. The final action step I have developed is to use learning materials that are equitable for all students. This means examining the curriculum and other resources in order to provide examples of both boys and girls. The materials will need to portray all genders as being skilled in mathematics, not one being better than another. This also goes to the use of examples that reach all genders of the students. Only having examples about trucks and sports can be discouraging for students who are not interested in these topics. Thus, the examples need to have a wide variety of interests to get the students excited about math. By implementing these three steps into my classroom, I believe I can significantly reduce the amount of gender bias present. While it will take more steps than just these three, I am committed to trying many different recommendations to achieve a classroom with little to no gender bias.

After inspection, the survey instrument yielded slightly different results than predicted.

There was a greater number of female participants than expected, which potentially could have caused most students to choose a female partner in the fourth question. The confidence levels of the students were significantly higher than the predicted average of 2. The instrument also revealed a level of gender bias the participants as a whole hold. When a question is graded, students feel more confident in a partner with a higher math confidence level.

However, when the question is no longer graded the students choose a partner based upon gender. From these results, I have developed action steps to help prevent gender bias in my own classroom. Through more encouragement and fair learning materials and role models for all genders, I believe I can reduce the amount of gender bias students are exposed to in the math classroom. In combination with the action steps, this instrument can be used in the future to assess the success of the action steps on eliminating gender bias in mathematics.

Conclusion

Through this research I have examined different aspects of gender, math confidence levels, and bias in the mathematics classroom. There are two questions that help narrow the field of research:first, how does gender play a role in the math confidence levels of students? The second research question examines whether preservice elementary teachers hold some form of gender bias in relation to mathematics. The results showed that for pre-service elementary teachers, they value mathematical confidence, and that gender plays a large role when working with partners in a math classroom. There still appears to be some form of gender bias held by preservice teachers, even though a majority of them are females. The data also showed that gender is not the only factor in the development of math confidence scores. Where a student attended school and the amount of encouragement and support they received also made an impact on the level. This research is important because gender bias is constantly pushing females out of STEM fields and into areas they are less passionate about. Everyone deserves to have the opportunity to pursue a career in whatever field they so choose, not what is traditionally male- or female-dominated.

There are a few main points that have impacted the way I have thought about and approached gender bias in mathematics. The first of these being confidence levels begin to drop in the fifth grade for female students. By the time they reach ninth grade, confidence levels can be used as a predictor of student performance. Gender bias is introduced at a young age not only in schools but also in individual households. Previous research indicated fathers believe their sons to have an IQ 12 points higher than their daughters and mothers believe their sons to have an IQ 6 points higher. Not only can parent beliefs and perceptions affect their children, teachers have a large impact on performance. A teacher's perception of a student's mathematical ability is one of the biggest predictors of future grades and performance. The last two key points came from the capstone portion of the research. The most surprising results indicated the participants felt more comfortable with a female partner than a male partner. This was surprising due to the fact the predictions were in favor of the males due to the traditional form of gender bias present. The participants showed their gender bias in the later questions. When choosing a partner for a graded question, math confidence level was the most important characteristic. However, when the question was ungraded, they trusted their female partners more. All of these key points have shown that gender bias can be present in many different aspects, not just the typical avenues. Thus, it is important to be hyper-aware of where gender bias may be present in the classroom.

The results uncovered throughout the project are relevant and useful for all teachers, not just math or elementary teachers. It was discovered that gender bias is present in many different ways. The bias can be seen and modeled by parents, teachers, other students, and also in the way students are partnered up. The research also helped to remind teachers the impor-

tance of surveying students about their likes and dislikes so that students feel comfortable in the classroom, especially in relation to group work. The capstone project revealed that students prefer to be partnered up with another student of the same gender. However, the occasional coed groups can be beneficial to students. While my research has provided new insight on the topic, there is always further research to be done. An idea for future research would be to examine the most effective methods to combat gender bias in the classroom. This research would be a much more extensive process due to the nature of it. However, it would provide educators with valuable insight on how to eliminate gender bias in the classroom. Another possible avenue for more research would be to examine if students tend to endure more gender bias in a public or private school setting. The goal of not only this research but also future research is to pinpoint where gender bias is occurring and develop steps and methods to eventually eliminate it from schools.

My high school experience has been the catalyst for this entire research process. The support I received from all my teachers and peers at Nerinx has led me to take an interest in gender equality. Many students are not lucky enough to receive constant encouragement from their teachers to pursue an education in a STEM field. Thus, I took an interest in discovering where gender bias is present in the math classroom and how to combat it. I believe this capstone encompasses my mission to create a positive learning environment where all students are able to reach their full potential. However, until everyone examines the biases they hold, the cycle of gender bias in mathematics will continue. Our teachers must receive specialized training to be more inclusive of all students and provide them with a safe learning environment. The math field needs to be rebranded as an inclusive space for anyone wanting to further their mathematical knowledge. Because the math itself is not biased, it is our duty to work to eliminate all the bias we have allowed to build up around the teaching and learning of mathematics.

References

Christensen, R., & Knezek, G. (2020). Indicators of middle school students' mathematics enjoyment and confidence. *School Science and Mathematics,* 120(8), 491–503. https://doi.org/10.1111/ssm.12439

Gniewosz, B., & Watt, H. M. G. (2017). Adolescent-perceived parent and teacher overestimation of mathematics ability: Developmental implications for students' mathematics task values. *Developmental Psychology, 53*(7), 1371–1383. https://doi.org/10.1037/dev0000332

Kane, J. M., & Mertz, J. E. (2012). Debunking myths about gender and mathematics performance. *Notices of the American Mathematical Society, 59*(1), 10. https://doi.org/10.1090/noti790

Leder, G. C., & Forgasz, H. J. (2018). Measuring who counts: Gender and mathematics assessment. *ZDM Mathematics Education, 50*(4), 687–697. https://doi.org/10.1007/s11858-018-0939-z

Lindberg, S. M., Hyde, J. S., Petersen, J. L., & Linn, M. C. (2010). New trends in gender and mathematics performance: A meta-analysis. *Psychological Bulletin, 136*(6), 1123–1135. https://doi.org/10.1037/a0021276

Muzzatti, B., & Agnoli, F. (2007). Gender and mathematics: Attitudes and stereotype threat susceptibility in Italian children. *Developmental Psychology, 43*(3), 747–759. https://doi.org/10.1037/0012-1649.43.3.747

Tiedemann, J. (2000). Parents' gender stereotypes and teachers' beliefs as predictors of children's concept of their mathematical ability in elementary school. *Journal of Educational Psychology, 92*(1), 144–151. https://doi.org/10.1037/0022-0663.92.1.144

Reigle-Crumb, C. & Humphries, M. (2012). Exploring bias in math teachers' perceptions of students' ability by gender and race/ethnicity. *Gender and Society, 26*(2), 290-322. https://doi.org/10.1177/0891243211434614

Robinson-Cimpian, J. P., Lubienski, S. T., Ganley, C. M., & Copur-Gencturk, Y. (2014). Teachers' perceptions of students' mathematics proficiency may exacerbate early gender gaps in achievement. *Developmental Psychology, 50*(4), 1262–1281. https://doi.org/10.1037/a0035073

Sheldrake, R., Mujtaba, T., & Reiss, M. (2022). Implications of under-confidence and over-confidence in mathematics at secondary school. *International Journal of Educational Research, 116*, 102085. https://doi.org/10.1016/j.ijer.2022.102085

Schuh, K., Meiners, A., Ferguson, C., Hageman, K., George, S., Cox, M., Zou, Y. & Lin, C.J. (2023). Junior high school students' self-confidence during transition to above-grade-level mathematics courses. *The Journal of Educational Research, 116*(2), 61-76. https://doi.org/10.1080/00220671.2023.2186338

Urine-Derived Stem Cells Detect Renal Mitochondrial Toxicity in HIV Patients Receiving Antiretroviral Therapy

Daniella Beiner[1,2], Yu Pengfei[2], Hainan Zhu[2], Carol C. Bosholm[2] and Yuanyuan Zhang[2]

1 University of Mississippi, Oxford, MS 38655
2 Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC 27157

ABSTRACT

Antiretroviral therapy (ART) has significantly improved the clinical outcomes of patients infected with human immunodeficiency virus (HIV). However, long-term use of certain antiretroviral drugs has been associated with renal mitochondrial toxicity (MtT), leading to potentially severe renal dysfunction. To date, there is no available test for renal MtT. Urine-derived stem cells (USCs) have emerged as a valuable non-invasive tool for studying renal pathology. In this study, we examine the effects of antiretroviral drugs on mitochondrial function in USCs from HIV patients as a first step towards establishing USCs as a tool for renal mitochondrial toxicity testing. USCs were isolated from urine samples of adult HIV patients taking ART drugs and compared to an age-matched (30 years old) healthy donor as a control. No changes in cell morphology and cell proliferation were found at passage 3; however, mitochondrial DNA levels were significantly decreased, while superoxide dismutase-2 (SOD2), cytochrome P450 2E1 (CYP2E1), caspase-3, and kidney injury molecule-1 (KIM-1) levels were significantly increased in HIV patient USCs compared to control. Thus, USCs from HIV patients exhibit decreased mitochondrial function, excessive oxidative stress conditions, increased apoptosis and acute kidney injury markers, all indicating renal mitochondrial dysfunction. These findings highlight the potential of urine-derived stem cells as a promising platform for non-invasive monitoring of renal mitochondrial toxicity in HIV patients undergoing antiretroviral therapy.

Introduction

Nearly 28.7 million people receive antiretroviral therapy (ART) worldwide (UNAIDS, n.d.). ART has significantly reduced HIV-related morbidity and mortality; however, as the life expectancy of HIV+ individuals has increased, the long-term safety of ART has gained increasing attention. Long-term use of antiretroviral drugs often induces mitochondrial toxicity (MtT), leading to injury of various organs, particularly the kidney. Currently, there is no available assessment to test for renal MtT (Ding, 2022). Urine-derived stem cells (USCs), mitochondria-rich progenitor cells in the kidney, may provide indication of renal conditions and hold promise for detecting early signs of mitochondrial toxicity. They may also act as a model to better understand the role of mitochondrial dysfunction in HIV patients undergoing antiretroviral therapy. USCs represent an advantageous cell source due to their simple, non-invasive, low cost collection, abundant supply, and easy isolation without the use of tissue digestive enzymes (Bento, 2020). In addition, the use of autologous USCs from individual patients opens the door to potential precision medicine applications.

Our objective is to determine the effects of antiretroviral drugs on mitochondrial function in HIV patients' urine-derived stem cells as a first step to establish USCs as a biomarker for ART-induced mitochondrial toxicity. We examine mitochondrial conditions by assessing cell morphology and proliferation, as well as mitochondrial DNA, superoxide dismutase-2 (SOD2), cytochrome P450 2E1 (CYP2E1), caspase-3 and kidney injury molecule-1 (KIM-1) levels. SOD2 is a mitochondrial protein that catalyzes the dismutation of $O2\bullet-$ (superoxide radical anion), produced by the electron transport chain, to $H2O2$ with production of oxygen, thus playing a major role in mitochondrial ROS (reactive oxygen species) detoxification (Infantino, 2022). CYP2E1 is an enzyme that plays a role in drug metabolism and ROS production (Linhart, 2014), and caspase-3 is a protease essential for typical hallmark processes of apoptosis such as apoptotic chromatin condensation and DNA fragmentation (Porter, 1999). Thus, elevated levels of these molecules are an indication of increased oxidative stress conditions and apoptosis in the kidney. KIM-1 is a transmembrane glycoprotein that is normally only minimally expressed in renal tissue but is upregulated in response to nephrotoxic acute kidney injury (Magshaw, 2009) and therefore used as a biomarker for nephrotoxicity.

Materials & Methods

Urine samples were collected from adult HIV patients on prolonged ART and an age-matched (30 years old) healthy donor as control. USC samples were then isolated using standardized protocols including centrifugation of urine samples to sediment cells, antibacterial wash of pellets to avoid contamination and seeding cells onto normal culture plates without special substrates (Manaph, 2018). Cell samples were then characterized at passage 3 based on cell morphology using a light microscope and cell proliferation using a Countess3 automated cell counter.

USCs from HIV patients undergoing ART (HIV-USCs) and healthy USCs (control-USCs) were then further cultured to passage 5, when mitochondrial mass and superoxide dismutase 2 (SOD2) levels were analyzed by fluorescence assays. DAPI, a DNA stain that exhibits blue fluorescence upon binding to AT rich regions of dsDNA and is excited by violet (405nm) laser (Thermo Fisher, n.d.), was used to detect nuclear counterstain and determine the number of nuclei in order to assess gross cell count. MitoTracker Green is a mitochondrial-selective fluorescent dye that accumulates in the mitochondrial matrix, allowing for visualization of mitochondria in live cells (Chazotte, 2011). MitoTracker was used concurrently with DAPI staining to assess mitochondrial mass while accounting for the differences in cell count per image. We also analyzed gene expression by quantitative real-time polymerase chain reaction (qRT-PCR) and protein

expression by Western blot. Statistical significance of results was assessed using a two-tailed test.

Results

There were no observable differences in cell morphology and mitochondrial mass between HIV-USCs and control-USCs (Figure 1).

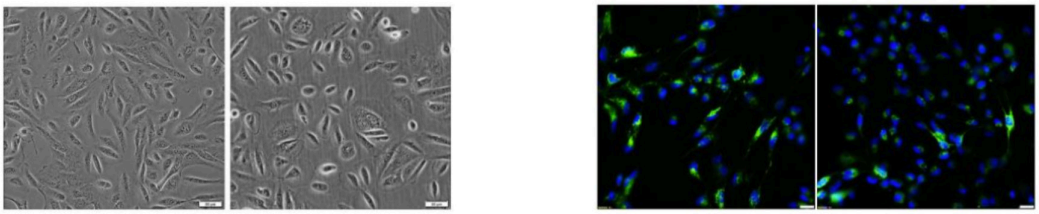


Figure 1: Cell morphology and mitochondrial mass of control-USCs (left) and HIV-USCs (right). A) USCs at p1, day 2, 20X magnification. B). Mito-Tracker mitochondrial staining (green) and DAPI nuclei staining (blue).

Superoxide dismutase-2 (SOD2) levels were significantly increased in HIV-USCs **(Figure 2).**

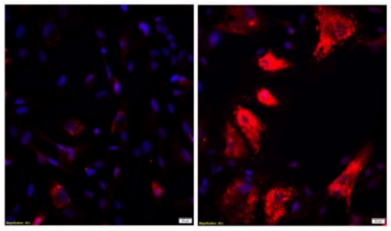


Figure 2: SOD2 expression in HIV-USCs (right) vs. control-USCs (left). SOD2 (red) and DAPI nuclei staining (blue).

Mitochondrial DNA levels were significantly decreased in HIV-USCs compared to control-USCs ($p<0.01$) (Figure 3).

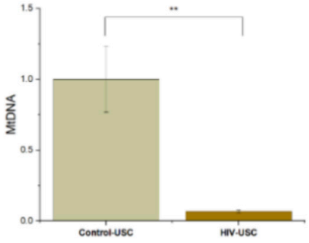
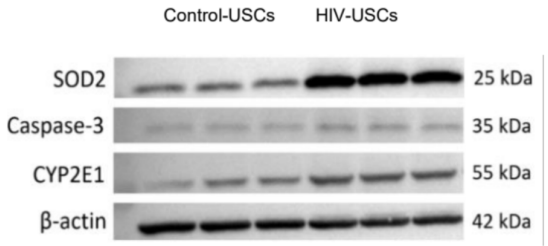


Figure 3: mtDNA levels in HIV-USCs vs. control-USCs assessed by RT-PCR.

HIV-USCs exhibited significantly higher cytochrome P450 2E1 (CYP2E1) levels ($p<0.01$) and elevated caspase-3 levels ($p<0.05$) compared to control-USCs **(Figure 4).**



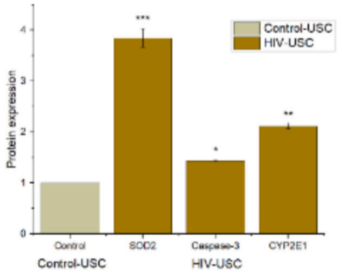


Figure 4: SOD2, caspase 3 and CYP2E1 expression of USCs assessed by western blot. Note: control-USC gene expression is normalized.

KIM1 expression was significantly higher in HIV-USCs compared to control-USCs ($p<0.05$) (Figure 5).

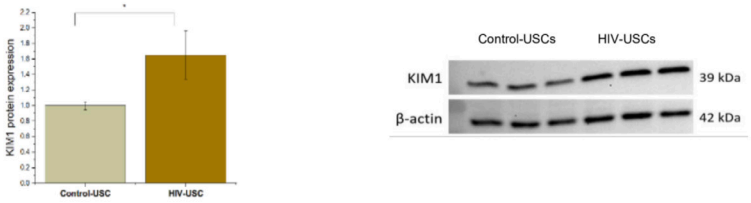


Figure 5: Kidney injury molecule-1 (KIM1) expression of USCs assessed by western blot.

Conclusion

Urine-derived stem cells from HIV patients exhibited decreased mitochondrial DNA levels, indicating reduced mitochondrial function. The elevated levels of SOD2, CYP2E1, and caspase-3 demonstrate excessive oxidative stress conditions and apoptosis, while the increase in KIM1 levels reveal signs of nephrotoxicity. Collectively, these results signal substantial renal mitochondrial dysfunction. This research demonstrates the potential of USCs as a platform for non-invasive monitoring of renal mitochondrial toxicity in HIV patients undergoing antiretroviral therapy. Early detection of mitochondrial dysfunction may provide valuable insights into the pathogenesis of antiretroviral drug-induced kidney injury, allowing for timely intervention and improved patient management. In addition, USCs may serve as a valuable in-vitro model to investigate the underlying mechanisms of renal mitochondrial toxicity and aid in the development of safer antiretroviral regimens. While these initial findings are promising, larger studies with diverse patient populations and longer follow-up periods are warranted to validate the utility of USCs as a reliable biomarker for detecting renal mitochondrial toxicity in HIV patients receiving ART. Nevertheless, this research opens new possibilities for personalized medicine approaches and could lead to the development of novel strategies to mitigate renal side effects associated with antiretroviral therapy, thereby improving the overall quality of life of HIV-infected individuals.

Acknowledgements

This research was conducted as part of a summer research position at the Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, NC, and was supported by the NIH NIDDK Undergraduate Summer Research Award #R25DK121572, NIH NIAID R21 AI152832 and R03 AI165170 (to PI: Yuanyuan Zhang) and the Rena Shulsky David Fund.

References

Bento, G., Shafigullina, A. K., Rizvanov, A. A., Sardão, V. A., Macedo, M. P., & Oliveira, P. J. (2020). Urine-derived stem cells: Applications in regenerative and predictive medicine. *Cells, 9*(3), 573. https://doi.org/10.3390/cells9030573

Chazotte, B. (2011). Labeling mitochondria with MitoTracker dyes. *Cold Spring Harbor Protocols, 2011*(8), 990–992. https://doi.org/10.1101/pdb.prot5648

Ding, H., Jambunathan, K., Jiang, G., Margolis, D. M., Leng, I., Ihnat, M., Ma, J. X., & Zhang, Y. (2022). 3D spheroids of human primary urine-derived stem cells in the assessment of drug induced mitochondrial toxicity. *Pharmaceutics, 14*(5), 1042. https://doi.org/10.3390/pharmaceutics14051042

Infantino, V., Todisco, S., & Convertini, P. (2022). Mitochondrial physiology: An overview. In M.R. de Oliveira (ed.), *Mitochondrial intoxication*, 1-27. Academic Press.

Linhart, K., Bartsch, H, & Seitz, H.K. (2014). The role of reactive oxygen species (ROS) and cytochrome P-450 2E1 in the generation of carcinogenic etheno-DNA adducts. *Redox Biology, 3*, 56-62.

Magshaw, S.M, & Bellomo, R. (2014). Kidney function tests and urinalysis in acute renal failure. In Ronco, C., Bellomom, R, & Kellum, J.A. (eds), *Critical care nephrology* (2nd Edition), 251-259. W.B Saunders.

Pavathuparambil Abdul Manaph, N., Al-Hawwas, M., Bobrovskaya, L., Coates, P. T., & Zhou, X. (2018). Urine-derived cells for human cell therapy. *Stem Cell Research & Therapy, 9*(1), 189. https://doi.org/10.1186/s13287-018-0932-z

Porter A. G., Jänicke R. U. (1999). Emerging roles of caspase-3 in apoptosis. *Cell Death and Differentiation, 6*(2), 99–104. https://doi.org/10.1038/sj.cdd.4400476

Thermo Fisher Scientific. (n.d.). *DAPI (4', 6-diamidino-2-phenylindole)*. Thermo Fisher Scientific – US. Retrieved Apr 12 2024, from https://www.thermofisher.com/nl/en/home/life-science/cell-analysis/fluorophores/dapi-stain.html

UNAIDS. (n.d). *Global HIV & AIDS Statistics – Fact Sheet.* Global HIV statistics. Retrieved April 12 2024, from https://www.unaids.org/en/resources/fact-sheet

Building a Better Security Force Assistance Brigade

William Bittner

ABSTRACT

This paper examines the effectiveness of United States Army Security Force Assistance Brigades (SFABs) in training and advising partner militaries while preserving conventional force readiness. A shortage of volunteers, the manpower demands of embedded security, and lack of a Congressional funding authority have made SFABs and the conventional force less ready for operations. These challenges will be analyzed through the lens of institutional theory to identify systemic threats to the viability of SFABs. This paper posits that an advisor career field should be created in response to an institutional aversion to non-combat roles. Further, the Army should eliminate or shrink the embedded forces providing security for SFABs, reducing their impact on conventional force readiness. Finally, a clear SFAB funding authority should be created to allow faster and more flexible spending. SFABs could be the Army's long-sought tool to develop partner militaries, but only with intentional policy change.

Building a Better SFAB

In 2014, fighters of the Islamic State invaded Iraq, sweeping as far as the outskirts of the capital city of Baghdad (Malsin, n.d.). Four American-trained Iraqi divisions collapsed in the face of the much smaller jihadist force, leading the United States to intervene again in the country despite having spent the last eleven years building an Iraqi military that, it was hoped, could stand on its own (Levy & Yusuf, 2021). Stung by this costly failure, the United States Army created the first Security Force Assistance Brigades (SFABs) in the hope of developing an expert corps of Security Force Assistance (SFA) professionals.

These units, specialized in training and advising partner militaries, have shown potential in deployments around the world, but they face continuing challenges. The first SFAB sent to Afghanistan offered $5,000 bonuses to volunteers but was still being staffed right up to deployment (SIGAR, 2019). Only one in three soldiers remained in this SFAB after completing their tours (CRS, 2020). Further, embedding security teams in the first two SFABs sent to Afghanistan left an entire brigade combat team, the Army's basic deployable force for conventional warfare, understrength (Anderson, 2022). Additionally, as there is no Congressional authority designed to fund SFABs, their activities and responsiveness are severely restricted by reliance on funds with requirements incompatible with effective SFA. For example, a 2020 reinterpretation of funding rules led to 60% of SFAB activities in Africa being canceled for the 2021 fiscal year (Probst, 2022).

The purpose of this paper is to identify challenges facing the Army's six SFABs – manpower shortages, negative impact on conventional force readiness, and inflexible funding – as well as offer policy alternatives to address them. This will be done through the lens of institutional theory, emphasizing the role of the Army's structure and culture in understanding policy challenges. This paper will first provide an overview of recent SFA challenges and the role of SFABs in addressing them. Second, it will analyze the effectiveness of SFABs. Third, it will examine policy alternatives to improve SFAB effectiveness. Finally, this paper will provide policy recommendations to ensure that SFABs can better support America's strategic objectives.

Background of American Security Force Assistance Policy

The United States military defines "Security Force Assistance" (SFA) as "unified action to generate, employ, and sustain local, host nation or regional security forces in support of a legitimate authority" by "organizing, training, equipping, and advising" partners (CRS, 2023, para. 1). SFA spans a range of activities, from teaching foreign soldiers how to use American weapons to standing up entirely new militaries in failed states. In Iraq and Afghanistan, where the U.S. built new militaries largely from scratch, the U.S. Army used regular soldiers and reservists with little pedagogical knowledge to train partner nation conventional forces while Army Special Forces, experts in SFA, trained elite units (CRS, 2023). The result was unmotivated and underprepared partner militaries, with the rare exception of units trained by Special Forces (Matisek & Commons, 2021). By 2015, the U.S. had trained 350,000 Afghan and 625,000 Iraqi security force members (Anderson, 2022), none of whom could compete with the Taliban or the Islamic State in conventional battle.

The pitiful results were in large part due to poor SFA practices. Advisors were pulled from conventional units, received limited training, and were withdrawn before they could master the environment or develop relationships with host nation counterparts (Matisek, 2018). These deployments impacted American readiness by leaving brigade combat teams (BCTs) without their senior officers and non-commissioned officers (whose experience made them ideal choices to serve as advisors), a serious liability as the United States pivoted towards preparing for great power conflict (CRS, 2023).

To professionalize SFA and preserve BCT readiness, the Army developed the Security Force Assistance Brigades (SFAB) (SIGAR, 2019). These 816-person teams are composed of experienced volunteers who undergo fifty-four days of cultural and advising training (Anderson, 2022) and serve multi-year terms (CRS, 2023). Five of the six SFABs are aligned with a geographic Combatant Command, allowing advisors to develop understanding of and relationships with partner security forces by serving in the same region for several years (Anderson, 2022). The inaugural deployment of the SFABs came in 2018, when 1st SFAB began training and advising the Afghan National Army (GAO, 2018). Since then, SFABs have deployed to areas of national security interest across the globe, including Ukraine prior to Russia's 2022 invasion (Eversden, 2022).

Current State of SFAB: New Opportunities and Persistent Challenges

Security Force Assistance Brigades (SFABs) have engaged with fifty-four countries as of 2022 (McEnany, 2022), with promising results. Afghan National Army (ANA) units partnered with 1st and 2nd SFAB saw a "marked increase in morale and enthusiasm" (Pelham, 2022, p. 45). In 2018, there was a two- to three-fold increase in the number of ANA-led offensive operations in areas where 1st SFAB was deployed (SIGAR, 2019). Such progress was due in large part to the greater training and enthusiasm of soldiers serving in SFABs compared to previous advisors (Joyce et al., 2021).

SFABs, however, have faced several challenges rooted in the structure and culture of the Army. First, recruiting and retaining advising talent is difficult because SFABs are not viewed as career advancing assignments (Matisek & Reno, 2019). The Army's emphasis on conventional combat means that advancement is easiest for those with leadership experience in units such as brigade combat teams (BCTs) (Metz, 2023). As such, many officers and non-commissioned officers (NCOs) "feel that being an advisor hurts their career [and] diminishes prospects for promotion" and may not volunteer even if their skills and interests align with service in an SFAB (Payne & Osburg, 2013, p. 16). This is evidenced by the experiences of both 1st and 2nd SFAB, which barely met their manpower requirements before deploying to Afghanistan despite offering $5,000 bonuses to volunteers (SIGAR, 2019). After finishing its first deployment, only thirty percent of 1st SFAB agreed to a second tour with the brigade (CRS, 2020). Late entry and high turnover limits training and cohesion-building, which reduces effectiveness.

Second, providing SFABs with embedded security negatively impacts conventional force readiness. A culture of risk aversion within the Army – minimizing casualties is both a commander's responsibility and vital to their career – has led combat units to be pulled from their BCTs and reassigned to protect SFABs. In Afghanistan, each SFAB was protected by an infantry battalion from the 32nd Infantry Brigade Combat Team, leaving that BCT severely understrength. This occurs to a lesser extent even in more permissive theaters such as Africa, and, as regional and global instability spreads, demands on the conventional force to protect SFABs will continue to grow (Anderson, 2022).

Third, SFABs lack a dedicated funding authority. Outside conflict zones with designated funding (such as Afghanistan prior to 2021), SFABs must draw on money from Title 10, Sections 321 and 333 to fund their activities. Section 321 provides funds for training with foreign partners to develop *American* capabilities, which SFABs can only employ on the grounds that SFA develops both American and partner skills. However, requests must be approved by Congress and cannot be easily repurposed to meet changing needs. Section 333 provides more flexible funds for partner capacity-building, but each request to Congress takes two to three months before funds are dispersed. Additionally, since fiscal year 2021, the Department of Defense no longer allows unused Section 333 funds to roll over from year to year. As a result, the Southern European Task Force–Africa was forced to cut sixty percent of its SFAB activities in

fiscal year 2021. Unable to rely on the consistent and timely funding needed to plan and carry out advising activities, SFABs are regularly pushed into "a cyclical, self-induced, functional separation from their partners" (Probst, 2022, p. 84).

Policy Alternatives

Create an Advisor Career Path

Creating an Advisor Functional Area (a career path for officers with niche skills) and a Military Occupational Specialty (MOS) for non-commissioned officers (NCOs) would allow for professional advancement within the advising field, providing time to master the craft and build relationships with partners without harming one's career (Tishman, 2020). Within this community, promotion boards would judge advisors based on their experience and success advising partner nations rather than the traditional command and education opportunities they may have missed out on. New professional development programs would be provided to advisors, and tours in the SFABs would no longer be limited to three-year broadening assignments. Such three-year assignments could still be offered as a way for soldiers to try out advising before making a commitment. Even experienced advisors could be allowed to return to their original career field with the skills they have gained training and mentoring foreign partners, ensuring that SFABs and the conventional force complement rather than compete for motivated, knowledgeable soldiers.

Employ SFABs With Less Embedded Security

An excessive security presence within SFABs reduces conventional force readiness. Accepting a higher degree of risk by deploying SFABs without embedded security teams in all but the least permissive environments would reduce manpower demand, as well as allow more organic interaction with partners. Expanding combat and survival training for SFAB soldiers would partially mitigate the risks of reduced security (Anderson, 2022). It should be noted, however, that making advisors responsible for their own security could increase workload, and additional combat and survival training could slow the production of new advisors.

Create a Dedicated SFAB Funding Authority

Lack of a dedicated funding authority has forced SFABs to seek funding from Congressional authorities not tailored to their needs, reducing mission flexibility, predictability, and speed. Congress could instead create a funding authority specifically for SFAB exercises, training programs, and other engagements with foreign partners, with rapid dispersal of funds and flexible guidelines that allow SFAB activities to be modified to meet changing partner needs. Requiring quarterly reports to Congress could allow accountability to be maintained (Probst, 2022).

Recommendations and Conclusions

If Security Force Assistance Brigades (SFABs) are to become the flexible and professional advising forces they were designed to be, Congress and the Department of Defense must make service in SFABs more desirable, reduce embedded security, and provide a funding source tailored to the needs of SFABs. The Department of Defense should create an Advisor Functional Area and Military Occupational Specialty (MOS), and SFAB doctrine and training should be modified to allow SFABs to be security self sufficient. Additionally, Congress should create a single funding authority for SFABs to spend training and advising host nation partners. The merits of these policies are demonstrated through the evaluative criteria of effectiveness, efficiency, and political feasibility, as defined by Michael Kraft and Scott Furlong (2021).

Effectiveness

Creating an Advisor Functional Area and MOS would make SFABs more effective by encouraging talented officers and non-commissioned officers to become advisors and remain long enough to become experts. In addition to knowledge of the art of advising, long-term advisors can form stronger relationships with partners and better understand their languages and cultures. In the words of Staff Sergeant Richard Kennedy, an SFAB Medical Advisor who deployed repeatedly to Mongolia, "[My] previous time in Mongolia helped me to grasp the cultural and army differences better, allowing me to communicate, instruct, and create relationships more effectively" (Leasure, 2023, para. 8). By promoting expertise and U.S.-host nation relationships, creating an advisor career path would make SFABs more effective at building and supporting partner forces.

Employing SFABs with smaller or no embedded security teams would increase conventional force readiness by freeing up soldiers for service with their brigade combat teams. In addition, a greater degree of shared risk would allow closer bonds with partner security forces. This was observed in the Vietnam War, when American advisors often worked in small groups with South Vietnamese forces, sharing risks and hardships. These advisors could then earn the respect of their partners and better understand their responsibilities, which were assets then leveraged to build effective units in a generally ineffective South Vietnamese military (Anderson, 2022). Thus, besides its benefits to conventional force readiness, reducing embedded security and sharing partner risk would make for more effective advising.

Creating a dedicated SFAB funding authority will improve SFAB effectiveness by increasing the speed and flexibility of funding, both of which are vital to engaging consistently with partners. When the State Partnership Program, a National Guard SFA effort that initially lacked a funding authority as well, first received a line-item appropriation of $1 million, it was able to launch the innovative GUARDEX and Minuteman Fellowship programs to train foreign soldiers in the US and create exchanges for non-military officials, respectively (Howard, 2004). A dedicated source of funding that similarly allows the comparatively better–trained and more focused SFABs to pursue adaptive and innovative engagements with partners could enable even greater successes.

Efficiency

Making SFABs more effective increases the efficiency of American defense policy, as it allows the United States to work through partners to prevent or prevail in armed conflicts without direct US involvement (JCS, 2017). In Ukraine, for example, American training has helped Ukrainian troops seriously damage a peer competitor of the United States (McEnany, 2022). Creating an Advisor Functional Area and Military Occupational Specialty to make advising more effective would thus also contribute to efficiency.

Employing SFABs with smaller or no embedded security teams would allow for more efficient use of manpower. By shifting soldiers from security duty, the Army could increase its conventional force strength without increasing its end strength. Such a change could free up as many as two Brigade Combat Teams, assuming the ratio of one battalion per SFAB seen in Afghanistan (Anderson, 2022). This would save the Army the tremendous expenses of training and maintaining additional soldiers, especially in an unfavorable recruiting environment, while maintaining a meaningful global presence. Reducing embedded security would allow for more efficient use of limited personnel and reduced personnel costs.

Creating a dedicated SFAB funding authority would further increase efficiency. A single source of funding with uniform reporting requirements could simplify oversight, preventing waste (CRS, 2011). Additionally, manpower could be saved by removing the administrative chore of identifying, requesting, and tracking funds from funding authorities not designed with the unique challenges of advising in mind. A new funding authority would thus promote efficient use of both money and manpower.

Political Feasibility

Creating an Advisor Functional Area and MOS that give advisors more opportunities for advancement may be difficult due to the Army's emphasis on conventional operations and the resulting pushback both in the internal politics of the Department of Defense and the politics of its congressional allies. In the words of a Special Forces chief warrant officer, "The Army mindset is dead set on maneuver warfare and tank warfare, and it is not certain [that the Army] can make a genuine switch to…advising" (Payne & Osburg, 2013, p. 16). However, public and political leaders may favor investing in relatively low-cost improvements to SFA rather than the costly conventional capabilities needed to win wars should SFA fail. As such, creating an advisor career path would likely be politically feasible.

Employing SFABs with less embedded security may be politically challenging due to the Army's institutional culture of risk aversion. Any policy which raises the risk of casualties threatens the careers of military leaders and may be unpalatable to a casualty-averse American public. Special Forces teams, however, have long carried out SFA missions with no embedded security (Anderson, 2022). Thus, providing additional combat and survival training to SFAB advisors, like what Special Forces receive, could make reduced embedded security politically feasible.

A dedicated SFAB funding authority will likely not be politically controversial. It would not necessarily be more expensive, as funds currently used for SFABs could simply be moved from other authorities. The fact that a dedicated authority would allow for increased Congressional oversight may likewise make it acceptable to Congress, and the promise of greater governmental efficiency is generally popular with conservative and liberal voters alike.

Conclusions

The United States military has struggled for decades to effectively organize, train, equip, and advise partner nation security forces. Even the Security Force Assistance Brigade created in response to security force assistance failures in Iraq and Afghanistan has many weaknesses. The view of service in SFABs as a "career killer" means qualified volunteers are in short supply. The need for embedded security draws manpower away from the conventional force, reducing readiness. Without a dedicated funding authority, the speed and flexibility of SFAB spending is limited. These problems can be addressed by creating an Advisor Functional Area and Military Occupational Specialty, replacing embedded security with increased combat and survival training, and creating a dedicated SFAB funding authority. All policy alternatives, however, will struggle to overcome the US Army's institutional aversions to risk and non-conventional warfare. Further research should be directed towards the effectiveness of SFABs in training and advising partner forces in recent permissive theaters such as Africa and South America, as SFABs remain understudied outside of the most challenging conflicts in the Middle East.

SFABs have great potential to provide better security force assistance, a task which will remain relevant for the foreseeable future. In the words of former Chairman of the Joint Chiefs of Staff Mark Milley, "We are training, advising, and assisting indigenous armies all over the world, and I expect that will increase and not decrease" (Kauffman, 2018). Only with intentional policy change can the SFAB take its much-needed place as an effective tool of American defense policy.

References

Anderson, M. (2022, July 24). Alone and unafraid: Advisors and risk across the spectrum of conflict. *Journal of Military and Strategic Studies, 21*(4), 1-34.

Congressional Research Service. (2020, June 11). *Army Security Force Assistance Brigades (SFABs)*. https://www.everycrsreport.com/files/2020-06-11_IF10675_3d3c8ff7ee4a3fca305d69fd2823a4ec419bc2d2.pdf

Congressional Research Service. (2023, August 21). *Army Security Force Assistance Brigades (SFABs)*. https://crsreports.congress.gov/product/pdf/IF/IF10675

Congressional Research Service. (2011, August 15). *The National Guard state partnership program: Background, issues, and options for Congress.* https://apps.dtic.mil/sti/pdfs/AD1172021.pdf

Eisenstadt, M., & Pollock, J. (2020). Training better Arab armies. *Parameters, 50*(3), 95-111. https://doi.org/10.55540/0031-1723.2677

Eversden, A. (2022, September 26). *Army's military adviser brigades see increased demand in Indo-Pacific, Europe.* Breaking Defense. https://breakingdefense.com/2022/09/armys-military-adviser-brigades-see-increased-dem and-in-indo-pacific-europe/

Government Accountability Office. (2018, December 19). *U.S. Advising of Afghan National Army has expanded since 2015, and the U.S. Army has deployed a new advising unit.* https://www.gao.gov/products/gao-19-251r

Howard, P. (2004). The growing role of states in U.S. foreign policy: The case of the state partnership program. *International Studies Perspectives, 5*(2), 179-196. https://doi.org/10.1111/j.1528-3577.2004.00168.x

Joint publication 3-20: Security cooperation. (2017, May 23). Joint Chiefs of Staff. https://irp.fas.org/doddir/dod/jp3_20.pdf

Joyce, R. (2021, November 23). *The future of US Security Force Assistance. Modern War Institute.* https://mwi.westpoint.edu/the-future-of-us-security-force-assistance/

Kauffman, B. (2018). Preparing SFABs for the complexity of human interaction. *Military Review, 98*(4), 88-95. https://www.armyupress.army.mil/Journals/Military-Review/English-Edition Archives/July-August-2018/Kauffman-SFA/

Leasure, W. (2023, April 10). *US Army strengthens partnership in Mongolia*. U.S. Army Pacific. https://www.usarpac.army.mil/Our-Story/Our-News/Article-Display/Article/3357637/us army-advisors-strengthen-partnership-in-mongolia/

Levy, I., & Yusuf, A. (2022, March 13). The "Lightning" Brigade: Security Force Assistance and the fight against al-Shabaab. *Studies in Conflict and Terrorism,* 1-26. https://doi.org/10.1080/1057610X.2022.2045967

Malsin, J. (n.d.). The next war for Iraq: A revealing look inside the country's fight with ISIS—and itself. *Time*. Retrieved October 19, 2023, from https://time.com/isis-mosul/

Matisek, J. (2018, August 1). The crisis of American military assistance: strategic dithering a Fabergé Egg armies. *Defense & Security Analysis, 34*(3), 267-290. https://doi-org.umiss.idm.oclc.org/10.1080/14751798.2018.1500757

Matisek, J., and Commons, A. (2021). Thinking outside of the sandbox: Succeeding at security force assistance beyond the Middle East. *Military Review,* 33- 42. https://www.armyupress.army.mil/Portals/7/military-review/Archives/English/MA 21/Matisek-SFAB-Beyond-1.pdf

Matisek, J., & Reno, W. (2019). Getting American Security Force Assistance right. *Joint Force Quarterly,* 65-73. https://www.researchgate.net/publication/326534224_Getting_American_Security_Force _Assistance_Right_Political_Context_Matters_Published_2019_Joint_Force_Quarterly

McEnany, C. (2022, October 3). *The U.S. Army's Security Force Assistance triad: Security Force Assistance Brigades, special forces and the state partnership program.* Association of the United States Army. https://www.ausa.org/publications/us-armys-security-force-assistance-triad-security-force assistance-brigades-special

Metz, R. (2023, January 1). The cult of the persuasive: Why U.S. security assistance fails. *International Security, 47*(3), 95-135. https://doi.org/10.1162/isec_a_00453

Payne, L., & Osburg, J. (2013). *Leveraging observations of Security Force Assistance in Afghanistan for global operations.* RAND. https://www.rand.org/pubs/research_reports/RR416.html

Pelham, J. (2022, November 18). Security Force Assistance Brigades and US Indo-Pacific command multi-domain competition. *Parameters, 52*(4), 43-56. https://doi.org/10.55540/0031-1723.3186

Probst, S. (2022). The lesson of the Security Force Assistance Brigade in Africa: Find the authority to compete and win. *Military Review, 102*(2), 81-89. https://www.armyupress.army.mil/Portals/7/PDF-UA-docs/Propst-UA.pdf

Special Inspector General for Afghanistan Reconstruction (SIGAR). (2019). *Divided responsibility: Lessons from U.S. security sector assistance efforts in Afghanistan.* https://www.sigar.mil/pdf/lessonslearned/SIGAR-19-39-LL.pdf

Tishman, J. (2020, June 29). *Who gets a brown beret? Rethinking assignments to the Security Force Assistance Brigades.* Modern War Institute. https://mwi.usma.edu/gets-brown-beret-rethinking-assignments-security-force-assistance brigades/

Bothrocophias microphthalmus, one of the specimens processed in this study

WINNER

Outstanding Research Writing (Continuing Student)

In-Situ, High-Throughput DNA Sequencing and Methodology Refinement to Survey Biodiversity and Identify Candidate New Species to Science in a Largely Unprotected Biological Corridor in Ecuador

Christian Boudreaux

ABSTRACT

The world is undergoing a biodiversity crisis in which hundreds of species have gone extinct within the last few centuries, rivaling previous mass extinction events that have only occurred five times in the history of the planet. Anthropogenic stressors such as the climate crisis, over exploitation of natural resources, deforestation, and pollution have created and fostered a mass extinction event that is taking place in every corner of the planet. Endemic species, those that are hyper-specialized to specific environmental conditions are most at risk of going extinct. Endemism, therefore, is used as a tool for conservation and as an indicator of which areas of land should be prioritized to protect biodiversity and endemic species. The Llanganates-Sangay Ecological Corridor, in which this study took place is home to high rates of biodiversity and endemism, acting as a buffer zone for organisms to pass through between the protected areas. This corridor, which remains largely unprotected, is critical for the health of the ecosystems that exist in the region and the organisms that inhabit them. Through the use of high throughput sequencing techniques in the field, the project detailed here aimed to identify species new to science and catalog the biodiversity that exists within the region of study. In addition, methodology for sequencing in the field was revised and optimized for multiple gene regions across and within six taxa groups (frogs, snakes, tarantulas, pseudoscorpions, fungi, and velvet worms). Sixty-four new sequences were deposited in the National Institutes of Health BLAST, with a number of those representing putative novel species. The sequence data detailed here will provide the basis for further scientific inquiry and publications to describe the candidate new species. These findings will facilitate further research in the region as well as promote conservation of the corridor through the broadening of biodiversity and endemism metrics.

Introduction

An endemic species is one that lives in a specific area and cannot be found in any other part of the world. There are varying degrees of endemism at different spatial scales that could be as large as a hemisphere or as small as a particular mountain, forest, or cave. Hyperendemic species, such as those in the latter examples, have evolved to occupy particular niches. Due to their requirement of specific conditions to survive, these species are widely considered to be the most vulnerable to stressors, as small changes to the environment, such as that which is seen under global warming and the worsening climate crisis, can lead to their extinction (Manes et al., 2021; Jones et al., 2021; Price & Jenkins, 2022). The extinction of particular species as a result of climate alteration has become a topic that has garnered increasingly more attention from scientists and the international community. Conservation organizations typically prioritize areas that serve as habitat to the most at risk groups of individuals of a species as a means of preserving biodiversity.

Prominent wildlife conservation agencies such as the World Wildlife Fund and the International Union for Conservation of Nature (IUCN) note their commitment to protecting and discovering threatened species (WWF, 2023; IUCN ,2023). Given that endemic taxa are among the most endangered, endemicity often plays an important role in the protection of biodiversity and the assessment and prioritization of the conservation value of land (Waltert et al., 2011). As humans continue to expand throughout the globe, the resulting anthropogenic effects of our consumption has caused over 900 species to go extinct since 1500 CE. As a result, we are in the midst of a mass extinction on scale with those that have occurred five other times in the history of the Earth (Rull, 2022). Overexploitation of resources is a leading factor in this problem, with deforestation, such as that which is currently taking place within the Llanganates-Sangay Ecological Corridor. Without proper legal protection of this crucial biodiversity hotspot, the endemic species that are found there will become just another statistic in this extinction. These stressors, such as deforestation, lead to high rates of species extinction, specifically in the tropics, a region which contains 75-90% of species on Earth (van Uhm, 2016) . As a way to combat this crisis and these heightened rates of extinction, it is necessary that biodiversity and endemic species be cataloged before silently going extinct. A more comprehensive knowledge of these metrics will promote conservation in the region and make a case for the preservation of the habitat that is home to a number of species that are not found in any other part of the world. It is through further investigation into these metrics and the prioritization of conservation of natural areas that we can best combat the climate crisis and the mass extinction that currently plague our planet.

Despite only making up 0.2% of the Earth's landmass (Initiative 20x20, 2023), Ecuador is one of the 17 megadiverse countries in the world, denoting it as particularly rich in living organisms and crucially important for biodiversity conservation (Yang et al., 2020). Among this biodiversity, Ecuador is particularly abundant in reptiles, amphibians, and endemic vascular plants (Kleemann et al., 2022). The country is home to an incredibly diverse range of habitats, 26 in total, that are found within four broader ecosystems: the Andes mountains, the cloud forest, the tropical Amazon rainforest, and the islands of the Galapagos (EF, 2023). Each of these habitats vary greatly and are defined according to their altitude and precipitation. Within these ecosystems are three of the 10 biodiversity hotspots in the world, the northwestern humid forests, the northeastern Amazonian jungle and the edges of the Andes mountain range (Convention on Biological Diversity, 2023). Due to this high degree of biodiversity and wide range of habitat types filled with niches to exploit, Ecuador is also home to one of the world's highest rates of endemism (Borchsenius, 1997; Liria, 2022; Büttner 2023; Convention on Biological Diversity, 2023). It is, therefore, a priority to identify endemic species that are yet undescribed to science as a powerful tool for promoting conservation of natural areas in the country. The

endemism that is explored in this study is specifically within the region of study, but may extend beyond depending on the habitat distribution of the species in question.

The Sumak Kawsay reserve, where this study took place, is located in the Pastaza Province of Ecuador in the upper Amazon and foothills of the Andes at an altitude of 1,475 meters and average precipitation of 5,500 millimeters a year. The protected area within the reserve is located just below the edge of the Llanganates National Park and right above Sangay National Park, within what is known as the Llanganates-Sangay Ecological Corridor (LSEC). This corridor exists between the upper cloud forest and lower Amazonian regions, allowing for a unique mix of highland and lowland ecosystems as well as flora and fauna to exist within the reserve. Additionally, the reserve is located in the upper watershed of the Rio Anzu, which provides additional water and nutrients to the area and allows for another degree of habitat diversification, leading to increased rates of endemism (Sterner, 2023).

Samples for this study were also collected at nearby Waska Wild Reserve, which is 95 hectares of protected forest located southeast of Sumak Kawsay at the base of the Eastern side of the Andes mountain range at an altitude of 1,239 meters. This protected area is found on the transitional boundary between the foothill and low montane evergreen forests. The reserve is unique due to the system of subterranean caverns and aquifers that run throughout the property. These caves and the limestone formations found throughout the reserve are home to a number of unique and undescribed species, including the two species of pseudoscorpions that are explored in this study. The topography of the area, housing both above-and-below–ground elements, lends itself to the development of microclimates that allows biodiversity and endemism to flourish. The site also experiences minorly varied environmental conditions than Sumak Kawsay, being at a lower elevation and, therefore receiving less precipitation, about 4,500 millimeters a year (Bentley et al., 2023).

While each reserve houses its own endemic flora and fauna, there is also a great deal of overlap in the organisms that exist between the two areas. Both reserves are characterized by a humid climate and frequent precipitation. Fog forms in the valleys of the Anzu River and De Los Monos River frequently, traveling into both reserves, creating a system of horizontal precipitation. Due to their location in the upper Amazon, they provide habitat for lowland species that are found in the highest part of their distribution, mountain species in the lowest part of their distribution, and species that are unique to the ecological transition zone that exists within the reserve. This unique blend of ecosystems and organisms makes the two reserves particularly conducive to genetic study, as the investigation could lead to discovery of new species and the growth of the biodiversity metrics within these protected areas (Bentley et al., 2023). Due to the reserves' placement between the two Ecuadorian National Parks, much of the land around these conserved areas remains unprotected, leaving species utilizing the LSEC vulnerable to anthropogenic stressors (Fig. 1). The buffer zone that this creates poses a threat to the biodiversity that exists in these areas. A specific emphasis needs to be placed on the protection of this crucial biological highway, a process that will be aided through scientific endeavors aimed at describing the biodiversity and ecological importance of the region.

This study utilized high-throughput, in-situ genetic sequencing techniques and bioinformatics to produce reference sequences for several taxa groups that are traditionally overlooked in classical conservation strategies. This was done in an effort to catalog biodiversity and identify candidate novel species (either new to science or previously unrecorded in the area). This was accomplished through DNA barcoding, a method that utilizes variable gene regions of DNA within an individual that are bookended by more conserved regions to track evolution across taxa.

In order to generate a broad baseline of data that can be used to compare the taxa sequenced in this study at different evolutionary scales, this study focused on the COI, 12S, 16S, ND1, RAG-1, and 18S/28S gene regions. DNA barcoding studies, such as this one, are crucial

in the construction of databases which can inform future, more in-depth research. In an effort to solve for this database availability and broaden barcoding information across taxonomic groups for better comparison, several gene regions have been identified as the most beneficial areas to replicate and sequence. The use of these areas have thus been standardized between a wide range of organisms and specific primers have been developed to isolate and replicate them. The first of these markers used in this study is the mitochondrial cytochrome oxidase subunit I (COI), which is a short mitochondrial marker of about 650 base pairs (bp) (Yang et al., 2019). It is themost commonly utilized barcoding gene in the identification of animal specimens (Rubbmark et al., 2018). COI is particularly effective at identifying even recently split species. This is due to multiple factors, including the mitochondrial region's rapid evolution, the mitochondrial genome's maternal inheritance pattern, and that it is widely utilized in barcoding studies, meaning that the reference database for the gene region is extensive (Krehenwinkel et al., 2019). Through replication and identification by means of COI primers a curated database of COI sequences known as the Barcode of Life Database (BOLD) has been created. This database has contributed to the popularity of COI due to its robust size. COI, however, does have its drawbacks, as its small gene region of amplification coupled with the rapid mutation rates in the region, the very aspects that make it so useful for novel species identification, can lead to non-specific binding of primers and difficulty replicating the region (Elbrecht et al., 2016). This limited availability of informative sites on the COI region results in decreased phylogenetic accuracy and difficulty analyzing deeper evolutionary relationships and divergent lineages (Krehenwinkel et al., 2019).

The 12S and 16S primer sets utilized in this experiment each replicate other sections of the mitochondrial genome. Specifically, they amplify a mitochondrial gene coding for ribosomal RNA that is utilized by the mitochondria for the translation of messenger RNA into mitochondrial proteins (Chan et al., 2022). Due to the haploid nature of mitochondria and its elevated rates of replication, the mitochondrial genome evolves four times faster than the average nuclear gene, making this method very useful for tracking genetic divergence within closely related taxa or even species (Merheb et al., 2019). Large differences exist between the mitochondrial genomes across species of animals, but there are certain sections within the 12S and 16S gene regions that are well conserved between taxa (Yang et al., 2014). Primers can, therefore, be applied to this region with relatively high rates of accuracy in binding and replicating the less conserved, more rapidly evolving regions important in species identification discovery.

The mitochondrially encoded NADH dehydrogenase 1 gene (MT-ND1), or ND1 for short, contains the necessary instruction for building the NADH dehydrogenase. This protein acts as an electron acceptor and is utilized in the first step of the electron transport chain step of oxidative phosphorylation as a part of the process of cellular respiration (Yusnita et al., 2010). Mitochondrial DNA undergoes rapid evolution, therefore making it ideal for the examination of population genetics and the identification of recently diverged taxa (Wu et al., 2014). ND1 diverges from this trend, as most taxa have a slower evolving, more conserved ND1 region, allowing it to be particularly useful in determining more distant evolutionary relationships in the construction of phylogenies (Morgan & Blair, 1997; Zang et al., 2023). Zang et al. (2023) found that, alongside ND2, ND1 was the most useful in constructing phylogenies, as it had the greatest number of parsimony-informative sites, meaning that the information found within ND1 often provided a simple, parsimonious, explanation as to the evolutionary history of the taxon in question.

The recombination-activating gene 1 (RAG-1) is primarily found in lymphoid organs and is an essential part of the adaptive immune system in vertebrates that is responsible for differentiating between mature and immature B and T cells. Due to its integral relationship with the adaptive immune system, which is specific to jawed vertebrates (Bartl et al., 2003) RAG-1 is particularly useful in determining the phylogenetic relationships among and within vertebrate

lineages (Chiari et al., 2009). The RAG-1 gene region contains several sections of DNA, some of which are highly conserved throughout evolution and others that change more readily (Villa et al., 2019). This makes the gene excellent for use in creation of phylogenies and examining evolutionary relation, both on a recent timescale of species divergence as well as with more distant vertebrate taxa relation.

With the recent advent of long-read sequencing technologies, it is now possible to sequence long, contiguous lengths of DNA. The application of this technology to DNA barcoding can provide more information for phylogeny creation and differentiation between taxa. The primers utilized in this study replicate specific sections of the ribosomal genome, about 8 kb in total, by targeting short sequences, the identity of which depends on the primers used and the organism of focus (Hadziavdic et al., 2014; Krehenwinkel et al., 2019; Milan et al., 2020). This type of DNA barcoding is more typically utilized in fungi, plants, and invertebrates (Murienne et al., 2014; Cheng et al., 2016; Banos et al., 2018). One of the main differences between shorter mitochondrial amplicons, such as those mentioned above, and long rDNA amplicons, is the difference in evolutionary rates between the regions. COI tends to evolve rapidly, making it suitable for studying recent evolutionary events and identifying closely related species, as explained above, while long rDNA amplicons are more conserved and evolve more slowly, making them ideal for studying deeper evolutionary relationships andidentifying distantly related species (Hillis & Dixon 1991). In this study, for the taxa that underwent replication for the nuclear ribosomal 18S and 28S primers, the entire region between the 18S and 28S genes of the ribosomal genome was replicated. 18S is the gene region responsible for protein synthesis of the 40S ribosomal subunit and 28S houses the information necessary to create the large ribosomal subunit (Lodish & Darnell, 1995; Hałakuc et al., 2022). This variation in length depended on the ITS region of the organism, a section of DNA that is highly variable as compared to the 18S and 28S regions which are very conserved. These conserved regions do not change rapidly over evolutionary time and it is, therefore, easier to discern distant relations between taxa. In contrast, the variable ITS region allows for differentiation of more closely related taxa and species.

The sequencing technique utilized in this study, nanopore technology, was first conceptualized in 1989 and later described in a publication in 1996 (Oxford Nanopore Technologies, 2023). The technology has been further developed since that time, but follows the same basic concept that was originally proposed. Nanopore sequencing works by passing strands of DNA or RNA through a protein channel, known as a nanopore that is embedded in an membrane that is electrically resistant. As the molecule passes through this protein and the bases are read, a current is released that is specific to the particular nucleotide that has passed through the protein. This raw data in the form of electrical current can be read and translated back into a nucleotide sequence, effectively sequencing the molecule in real time (Jurkowski, 2020). Depending on the model of sequencer being utilized, anywhere from 126 to 128,400 nanopores can be available for sequencing, with a single flow cell containing between 126 and 2675 nanopores. Nanopore has made DNA sequencing more widely accessible and affordable and has a variety of applications, from the study of infectious diseases and cancers, to epigenomics and DNA sequencing in the field (Wang et al., 2021).

The National Institutes of Health Basic Local Alignment Search Tool (BLAST) is a sequence database that is widely used for comparing genetic sequences. The algorithm behind BLAST works to align a query (the sequence being used for comparison) that is input into the system with the bank of sequences that are available online. BLAST identifies comparable sequences up to a certain threshold of overlap and presents them for review. The sequence that is input is compared with a sequence that is already in the database through query cover, which is the percentage of alignment that occurs between the two sequences for the gene region in question, and percent identity, which is the percentage of identical base pairs between

the two sequences at the aligned positions, are given for each consensus. The database serves as a powerful tool for determining taxa, species, and individual relatedness and can have a function in the identification of species, establishing phylogenetic relationships between and within taxa, and DNA mapping and comparison (Nirjhon, 2023). Although the amount of genetic divergence necessary to claim that one species is different from another varies by taxa and by the gene region that is being studied, the generally accepted value for the COI, the 18S/28S ITS, and 16S gene regions is a sequence divergence of about 3% or more (Beye et al., 2017; Fungal Diversity Survey, 2022; Lawson, 2023). This genetic divergence is the grounds on which to promote future study of the species in question and, through more sequencing, phylogeny creation, mapping of the species distribution, and other metrics, the species can be formally declared as new to science. This 3% variance is observed in fungi and in frogs as an unwritten rule rather than a formal expectation (Fungal Diversity Survey, 2022; Lawson, 2023). While this agreed upon value does exist in many cases, there is no discrete rule for how much sequence divergence is needed to declare a species as having split from another (Xu, 2020). For example, in Fungi, within certain taxonomic groups, a specimen can have a 99% sequence similarity to another and the two may be from closely related, but separate, species (Fungal Diversity Survey, 2022).

The purpose of this study was to extract and sequence DNA from a variety of organisms found within the Sumak Kawsay and Waska Wild Reserves in an effort to better understand and catalog the biodiversity of the region as well as identify potential novel species within it. Additionally, in the process, methodology used in DNA processing of six taxa was validated. Specifically, all genetic work with invertebrates and fungi was novel for the Sumak Kawsay laboratory. Therefore, this study first reports the methodology determined to be most effective for the sampled taxa. This information was used to extract and sequence DNA from a variety of organisms found within the Sumak Kawsay and Waska Wild Reserves in an effort to better understand and catalog the biodiversity of the region as well as identify potential novel species within it. The project consisted of taking samples, DNA extraction, optimization of primer sets and annealing temperatures per taxa, the refinement of DNA sequencing procedures, and the comparison of individuals to their most closely related genetic relative represented in a genetic database. The findings of this study will not only promote future study into these taxa through the optimized procedures that have been herein described, but they will provide the grounds for an array of publications to describe the candidate undescribed species that are first reported here. For the sake of this study, a 3% or higher sequence divergence was used to determine a species that has either not been sequenced in the database or is entirely new to science. The study possesses additional conservation value, as it will promote further scientific inquiry, visitation to, and protection of the region in which it took place.

Methods

Specimen Collection

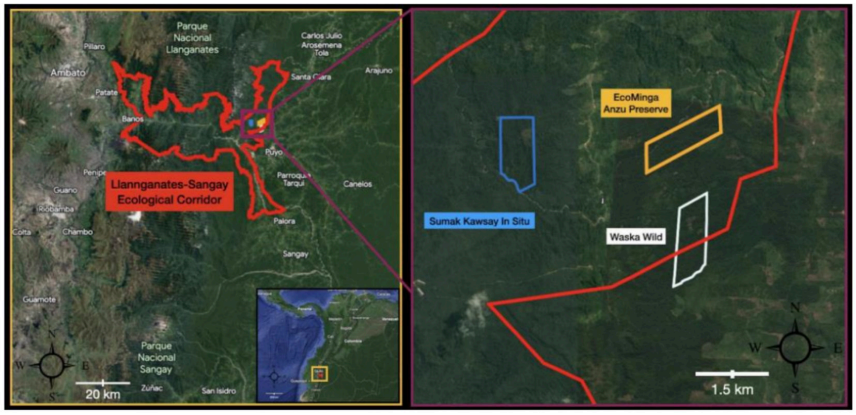


Figure 1: Study area map. Google Earth map depicting the Llanganates-Sangay Ecological corridor, outlined in red, the EcoMinga Anzu Preserve, outlined in yellow, and the Sumak Kawsay (blue) and Waska Wild preserves (white) from which the specimens utilized in this study were gathered.

All specimens utilized in this study were collected within the Sumak Kawsay (1,475 meters) or Waska Wild reserves. Collection of samples occurred over the course of several independent expeditions and was performed by various individuals involved in a variety of projects. The researchers involved in collecting samples included Zane Libke, Alex Bentley, Christian Boudreaux, Andy Better, Alan Rockefeller, Jordan Jacobs, Amanda Schabdach, Rosa Batallas, Roberto Leon, Pedro Peñaherrera, Ariel Gutiérrez, and David Diaz. Samples were taken from pseudoscorpions, tarantulas, velvet worms, fungi, frogs, and snakes. The sample type varied by taxa and is detailed below. For immediate use in DNA extraction, samples, other than fungi, were placed into a 1.5 mL microcentrifuge tube and preserved in at least 200 µL TL Buffer (more buffer was used for larger samples) at a temperature of -20° C. Specimens that were not to be utilized immediately in DNA extraction were preserved in at least 500 µL of 70% alcohol (more buffer was used for larger samples) and frozen at -20° C for later use. Fungi samples were housed in plastic bags containing desiccant beads and in darkness to ensure that the samples remained dry and preserved. The sample collection of the tarantulas and snakes required the use of tweezers and scissors. Prior to use in collection, these utensils were placed into 10 mL tubes of 10% chlorine, distilled water, and 70% ethanol, respectively, for 10 seconds each. Utensils were then sterilized in an open flame to remove any contaminants such as ambient DNA molecules.

Due to the small size of the pseudoscorpions utilized in this study, entire specimens were collected from cave habitat in the Waska Wild Reserve. Tarantula specimens were obtained from both reserves and, once encountered, specimens were taken back to the lab and the most distal portion of one of their legs, the tarsus, was removed and preserved using sterilized scissors and tweezers. Velvet worm specimens were found within the Sumak Kawsay reserve and tissue samples were taken in the form of leg segments, again utilizing sterile scissors and tweezers. Fungi specimens were discovered within both the Sumak Kawsay and Waska Wild reserves, where they were placed in plastic bags and kept at room temperature until use in DNA extraction. Snakes found within the Sumak Kawsay reserve were captured and placed in holding containers until blood, ventral scale, and occasionally feces and tail samples were collected. Blood was extracted using a sterile needle inserted below the cloaca and into the caudal vein.

Ventral scale and tail samples were removed using sterilized scissors and tweezers and placed into tubes for preservation.

Special care was taken during sample collection to ensure specimen safety. Most sampling was non-lethal and individuals were released following collection. Sampling that required extraction of blood or clipping of a body part was followed by sterilization of the site with 70% alcohol. A number of frog specimens were deemed necessary to be sacrificed and sent to a herpetological museum in Quito for documentation of novel species. This was done with the injection of novocaine to the posterior end of the individual so as to initially induce sleeping and not cause pain.

DNA Extraction

DNA extraction was performed according to the Omega BioTek E.Z.N.A. tissue extraction kit (Norcross, GA, U.S.). Preceding the placement of specimens into test tubes, tweezers and scissors were sterilized according to the procedure detailed above in Specimen Collection. This process was repeated between each sample. Specimens were taken out from their preservation states and a small portion (about one millimeter in length) was cut from the original sample using the sterilized scissors and tweezers. This subsample was then placed in a 1.5 mL microcentrifuge tube with 200 µL of TL buffer inside and the larger sample was returned to its preservation state. In order to more easily lyse samples and break down the structure of the sample, such as the chitinous exoskeletons of the invertebrates (tarantula and pseudoscorpion) or the septa of the fungi cell walls, additional mechanical digestion by means of mashing with a pipette tip pestle or using scissors to cut the material into smaller pieces was utilized. When specimens were thoroughly mechanically digested, 25 µL of Proteinase K was added to each tube and they were incubated at 55° C overnight or until they were finished lysing. The incubation time varied by sample and a sample was deemed ready for DNA extraction qualitatively, when most of the starting sample material had degraded and a cloudy solution was formed in the tube.

DNA Binding, Washing, and Elution

Once lysed, samples were centrifuged at 14,000 rpm to separate the supernatant DNA region from the pellet of biological material such as proteins, cell walls, cell and nuclear membranes, etc. The supernatant of each sample was then transferred to a new, sterile, 1.5 mL microcentrifuge tube, where 220 µL of BL Buffer was added. Samples were shaken by hand to mix the contents and were incubated at 70° C for 10 minutes. Upon removal, 220 µL of 100% ethanol was added. A HiBind® DNA Mini Column was then placed into a 2 mL Collection Tube and the sample was transferred into the Mini Column. Samples were centrifuged at 14,000 rpm for one minute. The material that had accumulated in the Collection Tube was discarded, but the Collection Tube was reused. 500 µL of HBC Buffer diluted with 100% Isopropanol was then added into the Mini Column and samples were centrifuged again at 14,000 rpm for 30 seconds. The filtrate along with the Collection Tube was discarded. The Mini Column was inserted into a new Collection Tube and 700 µL of DNA Wash Buffer diluted with 100% ethanol was added. Samples were centrifuged at 14,000 rpm for another 30 seconds. Filtrate was discarded but the Collection Tube was reused. The previous step was repeated for a second wash with the DNA Wash Buffer. The Mini Column was then centrifuged at 14,000 rpm for two minutes in an empty Collection Tube reused from the previous step in order to dry the column and remove any excess ethanol. The Mini Column was then transferred into a 1.5 mL microcentrifuge tube. 200 µL of Elution Buffer, heated to 70° C was added and samples were left to sit at room temperature for two minutes. Samples were then centrifuged at 14,000 rpm for one minute. The previous step was repeated for a second wash with the Elution Buffer and completed samples were stored in a freezer at -20° C.

Fluorometer

To ensure that extraction had occurred successfully and there was sufficient DNA that

could be replicated in a given sample, a Promega Quantus™ Fluorometer (Madison, WI, U.S.) was utilized. To assess their DNA content in nanograms per microliter, 2 µL of each sample was taken and mixed with QuantiFluor® ONE dsDNA Dye (Madison, WI, U.S.) in a 0.5 mL PCR tube and shaken to homogenize the solution, followed by centrifuging at 4,000 rpm to spin down any liquid collected on the sides of the tube. The tubes were then covered for five minutes so as to not be exposed to the light and, after that time frame, placed in the fluorometer to be read. DNA concentration measurements were recorded for later reference and to inform future actions.

PCR

Polymerase Chain Reaction (PCR) was performed in 0.2mL PCR tubes. Amplification was conducted in a 12.5 µL volume reaction, consisting of 6.25 µL of DreamTaq™ Hot Start PCR master mix (Waltham, MA, U.S.), 1 µL DNA extract, forward primer, and reverse primer, and 3.25 µL of nuclease free water. The primers, thermocycling procedures, and optimum annealing temperature for each taxa follow what is outlined in Table 1. For each PCR sample type, two additional control tubes were run, one with a control created during the DNA extraction process consisting of 200 µL TL Buffer and 25 µL Proteinase K, and another containing only the PCR Master Mix of DreamTaq™ Hot Start PCR master mix, forward primer, reverse primer, and nuclease- free water.

Gradients

PCR gradients were performed to validate and optimize annealing temperature and primer combinations for each taxa studied. The contents of the PCRs performed were the same as those detailed above and cycling protocol follows what is outlined in Table1, excepting the cycled annealing step, which varied across the gradient of samples. Each gradient consisted of eight PCR tubes, all containing the same sample type, that were run at the annealing temperatures 51.6°, 52.9°, 55°, 57.6°, 60.6°, 63.2°, 65.2°, and 66.6° C, respectively. For each set of gradients run, two additional control (the same as explained previously) gradients of eight PCR tubes each were run.

Gel Electrophoresis

PCR amplification success and DNA fragment length were verified using gel electrophoresis. Upon review of the results of the gel, if the sample demonstrated a band that was consistent with that which was expected from the selected primers (approximate amplicon lengths can be viewed in Table 1), the sample proceeded to indexing. For gradients, the entire gradient of samples was reviewed to ascertain at which temperature or temperatures the annealing process was successful, indicated by the samples with a visible band at the correct fragment size location. The success of gradient PCRs was also evaluated on the specificity of binding and only deemed successful if a singular band was found at the expected amplicon location. This information was recorded and, if no band was present, used to inform future primer and annealing temperature optimization tests to ensure proper annealing and amplification. An example gel containing samples from both gradient and normal PCRs can be found below.

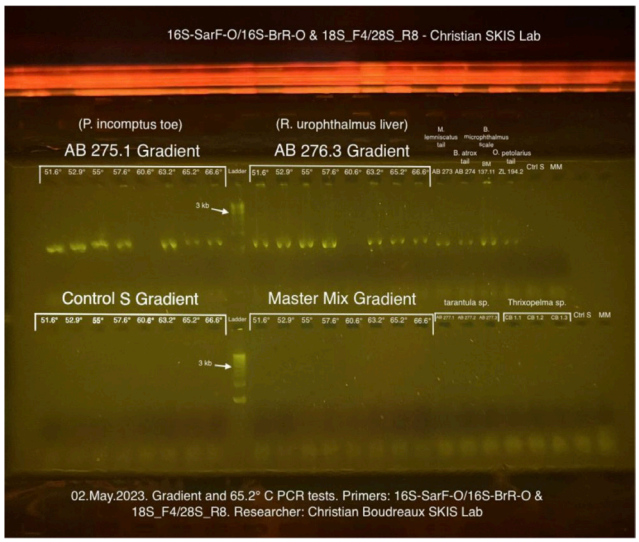


Figure 2: Validation of PCR results using a gel. A gel depicting the visualization of PCR samples run on a gradient (ex. AB 275.1) and under optimized cycling conditions for that taxa (AB 273). Bright green bands indicate replicated amplicons and size can be compared with the ladder that is included in each section of the gel, with 3,000 base pairs marked by the white arrows. PCR runs that failed to replicate (ex. AB 277.1) show no band for the corresponding well. Text above the gel details the primers used for the samples and text below indicates the date that the gel was run as well as cycling conditions.

Indexing

Upon successful amplification of the desired DNA region, samples underwent a second round of PCR, where they were tagged with a unique nucleotide sequence, giving them a distinct "code" that can be recognized by a flow cell once the samples are sequenced. In order to accomplish this, 9 µL each of 10 distinct forward and 10 distinct reverse primers were each placed into a 96 well plate according to what is detailed in Figure 3 (totalling 96 out of 100 possible unique combinations).

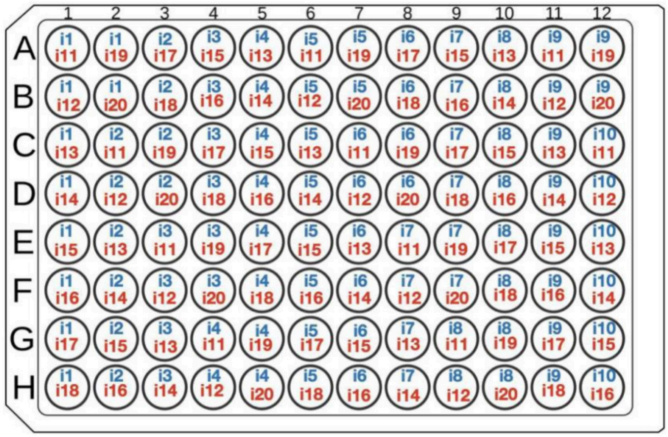


Figure 3: Indexing PCR primer scheme. Indexing scheme used to populate a 96 well plate with 96 unique primer combinations to be used indexing of samples. Forward indices are indicated in blue and reverse indices are indicated in red. Each index contains a sequence of nucleotides that is unique from that of the indices indicated by different numbers.

Indexing PCR

The indexing PCR was carried out in a different 96 well plate in a 12.3 µL volume reaction, consisting of of 6.25 µL of DreamTaq™ Hot Start PCR master mix (Waltham, MA, U.S.), 3.25 µL of nuclease-free water, 0.8 µL of sample, and 2 µL of the unique indexing primer set. Prior to pipetting into wells, each sample was assigned a specific set of primers that corresponded with a well code from the original indexing primer plate (ex. A1 for sample RJL001). The procedure that was followed consisted of an initial denaturation step of 95° C for 180 seconds, followed by 13 amplification cycles of (95° C for 15 seconds, 55° C for 15 seconds, 72° C for XX seconds), and a final extension step of 72° C for 180 seconds (Pomerantz et al., 2022). The long amplicons (18S/28S) were run for an extension of 180 seconds, while the short amplicons (16S/16S/COI) were run for 80 seconds.

Indexing Gel Electrophoresis

To validate the success of indexing, a gel was run with a subset of the indexed samples. One sample was chosen for each taxa included in the indexing run. The original PCR sample was placed alongside the indexed sample within the gel to ensure that the indexed amplicon was slightly longer than the original and, therefore, slightly above it. Procedure for loading and running the gel followed that which is described above.

Library Prep

Library prep and all procedures involved in completing it were performed according to the Ligation sequencing kit (SQK-LSK114) (Nanopore, 2023). Long (18S/28S) and short (Rag-1, ND1, 16S, 16S, COI) amplicons were separated into different sequencing runs and, therefore, underwent slightly altered library prep procedures. Depending on the desired amplicon length for each library, a proportional amount of Mag-Bind® Total Pure NGS (Norcross, GA, U.S.) magnetic beads, homogenized prior to use, were mixed with the sample (.6x for long amplicons and .65 for short amplicons).

For use in loading the flow cell during sequencing, two 1.5 mL microcentrifuge tubes were prepared, one containing a priming mix and the other the diluted DNA library. The priming mix consisted of 117 µL of Flow Cell Flush and 3 µL of Flow Cell Tether.
The diluted DNA library contained 15 µL of Sequencing Buffer, 10 µL of Library Beads, and the 5 µL of DNA Library.

DNA Sequencing

Prior to sequencing, the Flongle flow cell (Oxford, U.K.) was evaluated to verify the number of nanopores available for sequencing. The flow cell with the highest number of functional nanopores was chosen for sequencing. The cell was loaded by pipetting 120 µL of priming mix into the port. This was followed by 30 µL of sample mix, containing the DNA to be sequenced. The program MiniKNOW was used to interface with the flow cell and to record data.

Bioinformatic Data Processing

Following sequencing, data was compiled and processed using Linux software. A docker image, "giderk2020/junglegenomics" was utilized which contains all of the programs necessary for data preparation and analyzation. The files were then demultiplexed with the original indexing file for that sequencing run using minibar, effectively matching indexing codes with samples names. A bash script was then run on the files to generate summary information and graphs for each one (Fig. 4). A dot plot of read length versus read quality was also generated for each sample, such as that for ZL 136 - Rag1 (Fig. 5). These dot plots were used to determine the median sequence length and the range of sequence lengths for each sample. This information was plugged into NGS Species ID to create a sequence consensus for each individual sample and gene region.This consensus was then input into the BLAST database. If a sequence for the genus and species identification assigned to the sample in the field was available, that sequence was chosen and recorded. If such a sequence was not available, the closest matching sequence was selected and recorded.

NanoPlot reports

Summary statistics

General summary	
Mean read length	650.7
Mean read quality	9.7
Median read length	647.0
Median read quality	9.7
Number of reads	936.0
Read length N50	648.0
STDEV read length	74.9
Total bases	609,033.0

Figure 4: Summary of NanoPlot sequencing results for sample ZL 136 - Rag1. Image of the sequencing summary for ZL 136 - Rag1 detailing the basic sequencing information for the sample

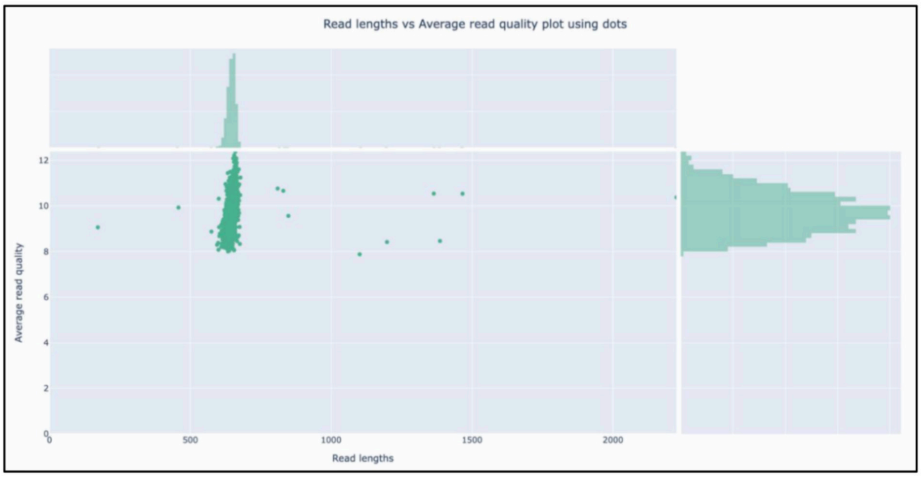


Figure 5: Dot plot of indexing reads for sample ZL 136 - Rag1. Scatter plot depicting read lengths versus average read quality. The amplicons of the sample (ZL 136 - Rag1) are shown in the large band of dots at a read length of about 600 base pairs, while the primer dimers are located to the left and to the right of the read cluster.

Results

Primer and Annealing Temperature Optimization

Throughout the DNA amplification process, primers and annealing temperatures were optimized for several different gene regions within and across the studied taxa groups. While some primer and annealing pairings were previously described in other studies, a number of combinations are first optimized and described here.

Table 1: Primer table. Primer table depicting the various gene regions and associated primers utilized for each taxa. The PCR cycling program, approximate amplicon length, and optimized annealing temperature is also detailed for each primer and taxa combination.

Taxa	Gene	Primer Name	Primer Sequence (without ONT universal tail)	PCR Program	Amplicon Length	Annealing Temp (° C)	Source
Snakes	16S	16S-SarF-O	5′-CGCCTGTTTATCAAAAACAT-3′	95°C 300s (95°C 30s 52.9°C 30s 72°C 45s) x 30 72°C 300s	~ 490 - 560 bp	51.6/52.9	This study
		16SBR	5′-CCGGTCTGAACTCAGATCACGT-3′				
Frogs	16S	16S-SarF-O	5′-CGCCTGTTTATCAAAAACAT-3′	95°C 300s (95°C 30s 52.9°C 30s 72°C 45s) x 30 72°C 300s	~ 550 bp	51.6-57.6; 63.2-66.6	This study
		16SBR	5′-CCGGTCTGAACTCAGATCACGT-3′				
	12S	12sL4E	5′-TACACATGCAAGTYTCCGC-3′	95°C 300s (95°C 30s 60°C 30s 72°C 50s) x 30 72°C 300s	~ 850 bp	60	Henicke et al. (2007)
		12sH10	5′-CACYTTCCRGTRCRYTTACCRTGTTACGACTT-3′				
	ND1	16s-frog	5′-TTACCCTRGGGATAACAGCGCAA-3′	95°C 300s (95°C 30s 60°C 30s	~ 1,400 bp	60	Wiens et al.

		tMet-frog	5′-TTGGGGTATGGGCCCAAAAGCT-3′	72°C 90s) x 30 72°C 300s			(2005)
	RAG-1	R182 R270	5′-GCCATAACTGCTGGAGCATYAT-3′ 5′-AGYAGATGTTGCCTGGGTCTTC-3′	95°C 300s (95°C 30s 55°C 30s 72°C 45s) x 30 72°C 300s	~ 650 bp	55	Henicke et al. (2007)
Tarantulas	18S/28S	18S_F4 28S_R8	5′-GGCTACCACATCYAARGAAGGCAGCAG-3′ 5′-TCGGCAGGTGAGTYGTTRCACAYTCCT-3′	95°C 600s (95°C 30s 65.2°C 30s 72°C 180s) x 30 72°C 600s	~ 3,500 bp	52.9-66.6	This study
Pseudoscorpions	18S/28S	18S_F4 28S_R8	5′-GGCTACCACATCYAARGAAGGCAGCAG-3′ 5′-TCGGCAGGTGAGTYGTTRCACAYTCCT-3′	95°C 600s (95°C 30s 65.2°C 30s 72°C 180s) x 30 72°C 600s	~ 3,500 - 3,700 bp	65.2/66.6	This study
Velvet Worm	18S/28S	18S_F4 28S_R8	5′-GGCTACCACATCYAARGAAGGCAGCAG-3′ 5′-TCGGCAGGTGAGTYGTTRCACAYTCCT-3′	95°C 600s (95°C 30s 66.6°C 30s 72°C 180s) x 30	~ 3,200	66.6	This study

				72°C 600s			
	16S	16SpotFN 16SBR	5′-GACTGTGCAAAGGTAGCATAATC-3′ 5′-CCGGTCTGAACTCAGATCACGT-3′	95°C 300s (95°C 30s 50°C 30s 72°C 60s) x 35 72°C 420s	~1,500 bp	50	Pinto-da-Rocha et al., (2014)
	12S	12SAIN 12SOP2RN	5′-AAAAACWAGGATTAGATACCCT-3′ 5′-CCCTTAAAYYTACTTTGTTACGACC-3′	95°C 300s (95°C 30s 50°C 30s 72°C 60s) x 35 72°C 420s	~ 400 bp	50	Pinto-da-Rocha et al., (2014)
	COI	LCO1490 HCOoutout	5′-GGTCAACAAATCATAAAGATATTGG-3′ 5′-GTAAATATATGRTGDGCTC-3′	95°C 300s (95°C 30s 45°C 30s 72°C 60s) x 35 72°C 420s	~ 820 bp	45	Pinto-da-Rocha et al., (2014)
Fungi	18S/28S	18S_F4 28S_R8	5′-GGCTACCACATCYAARGAAGGCAGCAG-3′ 5′-TCGGCAGGTGAGTYGTTRCACAYTCCT-3′	95°C 600s (95°C 30s 65.2°C 30s 72°C 180s) x 30 72°C 600s	~ 3,000 - 4,000 bp	63.2-66.6	This study

Sequencing Methodology Refinement

Additionally, the sequencing process was initially undertaken with both long (18S/28S) and short (16S, 16S, COI, RAG-1, ND1) amplicons included in the same library (Fig. 6). This sequencing attempt resulted in an overrepresentation of short amplicons in terms of reads. A graph depicting the distribution of reads according to read length as well as a gel showing the concentration of DNA of long and short amplicons throughout the library prep process for this sequencing run is included below (Fig. 8). As a response to this and to increase the long amplicon reads, short and long amplicons were separated for future sequencing runs (Fig. 7).

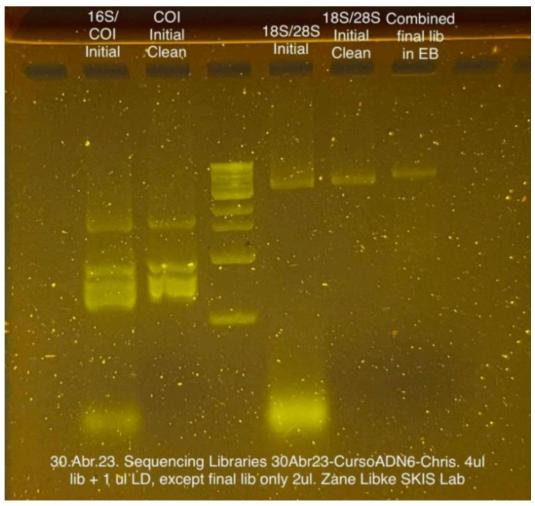


Figure 6: Library prep gel from first sequencing run. Picture of a gel that displays the initial concentrations of the long and short amplicon pools, the pools once they had undergone the first round of cleaning, and the pools after the final cleaning.

Figure 7: Library prep gel from second sequencing run. Picture of a gel that displays the initial concentration of the short amplicon pool, the pool once it had undergone the first round of cleaning, the pool once it had undergone end prep, and the pool after the final cleaning.

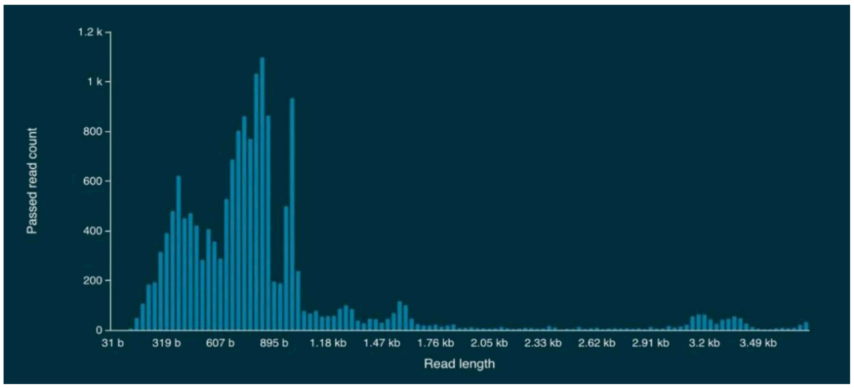


Figure 8: Sequencing read histogram. Histogram depicting the distribution of reads during the first sequence run.

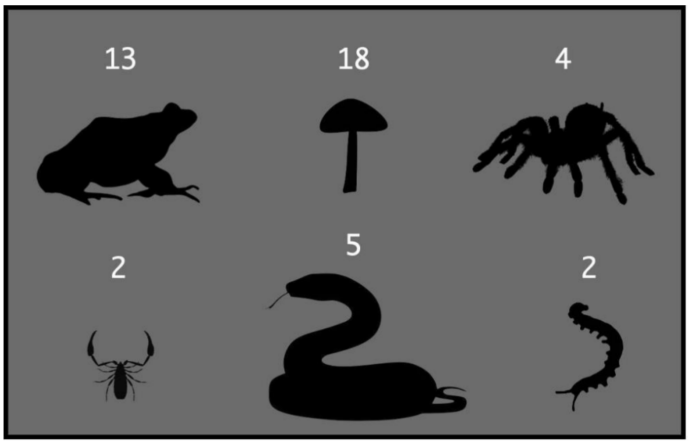


Figure 9: Taxa sequenced in this study. Picture depicting the six taxa groups investigated in this study (frogs, snakes, tarantulas, pseudoscorpions, fungi, and velvet worms) and the number of species sampled within each taxa group, included in white above the silhouette picture of the associated taxa group.

Sample Processing

Over the course of this study, 55 samples were processed, extracted from 44 putative species and six taxonomic groups (Fig. 9). 25 PCRs and 12 gels were run, resulting in three rounds of DNA sequencing. All sample numbers, the field IDs of the individuals that were sampled, and photos of gels can be found in the index. The culmination of this work resulted in the ability to compare individual genetic sequences for specific gene regions with the BLAST genetic database. The results of these sequence comparisons are detailed below.

Table 2: Sequencing Results. Table detailing all samples that were successfully sequenced. Original (field) identification (genus and species), sample code, and gene region are included for each specimen. Additionally, the closest match of the sequence in the BLAST database is shown. Underlined closest blast hits indicate a blasted sequence with a different species identification than the field ID that the sample was given. Bolded blast hits indicate those which differ from the query by 3% or more for percent identity. The Accession number, which is a unique code given to each sequence in the database is also included. Query cover, which is the percentage of alignment that occurs between the two sequences for the gene region in question, and percent identity, which is the percentage of identical base pairs between the two sequences at the aligned positions, are given for each consensus.

Identified Genus	Identified Species	Sample Code	Amplicon	Closest Blast Hit	Accession #	Query Cover	Percent Identity
Snakes							
Bothrocophias	microphthalmus	BM 135	16S	Bothrocophias microphthalmus	AY223670.1	91%	97.19%
Bothrocophias	microphthalmus	BM 136	16S	Bothrocophias microphthalmus	AY223670.1	92%	97.99%
Bothrops	atrox	AB 273	16S	Bothrops atrox	MK313329.1	97%	97.58%
Micrurus	lemniscatus	AB 274	16S	Micrurus lemniscatus	MW672260.1	91%	99.60%
Oxyrhopus	petolarius	ZL 194	16S	**Oxyrhopus formosus**	KX660249.1	97%	96.88%
Oxyrhopus	occipitalis	AB 279	16S	Oxyrhopus formosus	KX660249.1	97%	97.64%
Frogs							
Boana	almendarizae	AB 285	12S	Hypsiboas sp.	JN970478.1	95%	99.19%
Boana	almendarizae	AB 285	ND1	**Hypsiboas albopunctatus**	JN898915.1	93%	85.65%
Boana	nigra	AB 284	12S	**Boana nigra**	MN921739.1	98%	95.88%
Boana	nigra	AB 284	ND1	Boana nigra	MN921914.1	74%	98.79%
Dendropsophus	minutus	AB 286	12S	**Dendropsophus minutus**	MT503932.1	97%	91.46%
Dendropsophus	minutus	AB 286	16S	**Dendropsophus minutus**	MT503932.1	98%	88.30%
Dendropsophus	parviceps	AB 278	12S	Dendropsophus parviceps	MG041801.1	96%	98.85%
Dendropsophus	parviceps	AB 278	ND1	Dendropsophus parviceps	MG041923.1	99%	99.13%
Dendropsophus	parviceps	AB 278	Rag1	**Osteocephalus buckleyi**	MT372585.1	92%	91.07%
Oreobates	quixensis	AB 261	12S	**Oreobates quixensis**	EF493828.1	87%	96.63%
Oreobates	quixensis	AB 261	16S	Oreobates quixensis	MZ429969.1	91%	98.18%
Oreobates	quixensis	AB 261	ND1	**Ischnocnema quixensis**	AY819474.1	81%	96.92%
Oreobates	quixensis	AB 261	Rag1	Oreobates quixensis	MZ560987.	85%	98.84%

					1		
Pristimantis	conspicillatus	AB 282	16S	**Pristimantis condor**	EF493701.1	98%	90.16%
Pristimantis	conspicillatus	AB 282	ND1	**Pristimantis sp.**	OM730032.1	57%	85.47%
Pristimantis	conspicillatus	AB 282	ND1 (Consensus 2)	**Pristimantis romeroae**	MT636528.1	42%	85.04%
Pristimantis	conspicillatus	AB 282	ND1 (Consensus 3)	**Pristimantis sp.**	MK881310.1	50%	91.76%
Pristimantis	conspicillatus	AB 282	Rag1	Pristimantis conspicillatus	MW451749.1	92%	98.26%
Pristimantis	diadematus	AB 287	12S	**Pristimantis diadematus**	EU186668.1	97%	96.84%
Pristimantis	diadematus	AB 287	16S	Pristimantis diadematus	MW567354.1	92%	98.99%
Pristimantis	diadematus	AB 287	ND1	Pristimantis diadematus	MW567419.1	68%	98.66%
Pristimantis	diadematus	AB 287	Rag1	Pristimantis brevicrus	MF118745.1	93%	98.29%
Pristimantis	incomptus	AB 275	16S	**Pristimantis incomptus**	MK829659.1	94%	95.42%
Pristimantis	katoptroides	AB 288	12S	**Pristimantis wiensi**	EF493377.1	95%	90.22%

Pristimantis	katoptroides	AB 288	16S	Pristimantis katoptroides	MW567330.1	82%	98.88%
Pristimantis	katoptroides	AB 288	ND1	**Pristimantis totoroi**	MK881406.1	99%	82.54%
Pristimantis	katoptroides	AB 288	Rag1	Pristimantis katoptroides	MW451753.1	92%	98.57%
Pristimantis	lacrimosus	ZL 176	12S	**Pristimantis galdi**	EU186670.1	91%	86.03%
Pristimantis	lacrimosus	ZL 176	16S	Pristimantis lacrimosus	MT636524.1	94%	98.16%
Pristimantis	lacrimosus	ZL 176	ND1	**Pristimantis eriphus**	EU186671.1	56%	84.42%
Pristimantis	lacrimosus	ZL 176	ND1 (Consensus 2)	**Pristimantis thymelensis**	MK881392.1	46%	91.77%
Pristimantis	lacrimosus	ZL 176	ND1 (Consensus 3)	**Pristimantis lacrimosus**	MT636524.1	91%	87.90%
Pristimantis	lacrimosus	ZL 176	Rag1	Pristimantis lacrimosus	MT635629.1	91%	98.40%
Pristimantis	lacrimosus	ZL 176	Rag1 (Consensus 2)	Pristimantis lacrimosus	MT635629.1	88%	98.56%
Pristimantis	pinchaque	AB 239	12S	**Pristimantis gloria**	MT778079.1	96%	90.89%
Pristimantis	pinchaque	AB 239	16S	**Pristimantis lutzae**	MK881421.1	96%	90.73%
Pristimantis	pinchaque	AB 239	Rag1	Pristimantis tinguichaca	MK881334.1	95%	98.00%
Tarantulas							
Thrixopelma	sp.	CB 1 (60.6° C)	18S to 28S	**Aphonopelma hentzi**	AY210803.1	57%	83.96%
Thrixopelma	sp.	CB 1 (60.6° C Consensus 2)	18S to 28S	**Aphonopelma hentzi**	AY210803.1	57%	85.46%
Thrixopelma	sp.	RJL 001	18S to 28S	**Bird spider**	X13457.1	38%	92.71%
Thrixopelma	sp.	RJL 001 (Consensus 2)	18S to 28S	**Aphonopelma hentzi**	AY210803.1	58%	83.93%
Neischnocolus	sp.	AB 277	18S to 28S (63.2° C)	**Aphonopelma hentzi**	AY210803.1	58%	89.72%
Velvet Worms							
Oroperipata	sp.	ZL 178	12S	**Oroperipatus sp**.	MG973710.1	84%	85.84%
Oroperipata	sp.	ZL 178	COI	**Peripatidae gen. sp**.	KC754643.1	97%	88.85%
Fungi							

Beauveria	loucustiphila	AMAJ 16	18S to 28S	**Beauveria bassiana**	MN494090.1	99%	95.71%
Beauveria	loucustiphila	AMAJ 16 (Consensus 2)	18S to 28S	**Beauveria bassiana**	MN494089.1	99%	94.98%
Cystolepiota	sp.	AMAJ 2	18S to 28S	**Tulostoma cf. fimbriatum**	KU518981.1	56%	93.19%
Entoloma	sp.	AMAJ 10	18S to 28S	**Tricholoma squarrulosum**	MT644915.1	99%	87.52%
Mycena	sp.	AMAJ 3	18S to 28S	**Mycena inclinata**	MT644919.1	96%	92.67%
Ophiocordyceps	australis	AMAJ 4	18S to 28S	**Fusarium falciforme**	CP104054.1	94%	93.24%
Ophiocordyceps	curculionum	AMAJ 6, 13	18S to 28S	Ophiocordyceps formicarum	KY649457.1	55%	97.10%
Psilocybe	sp.	AMAJ 12	18S to 28S	**Clitocybe nebularis**	MT644916.1	100%	93.17%

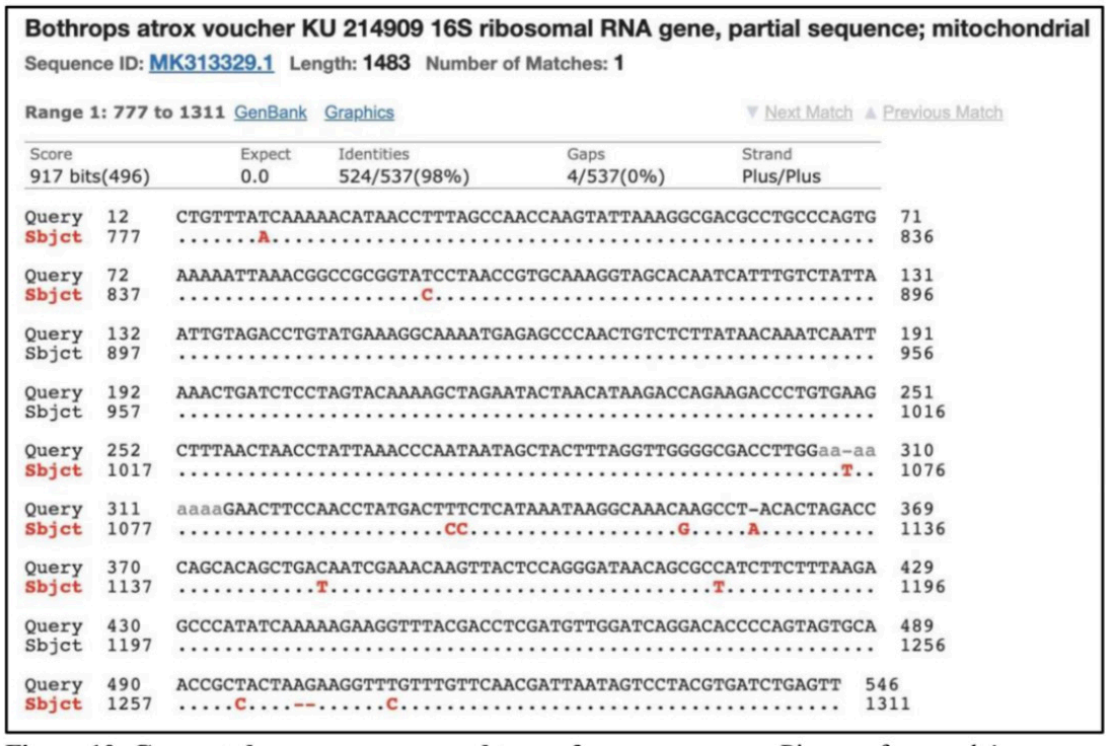


Figure 10: Generated sequence compared to a reference sequence. Picture of a sample's sequence (Bothropsatrox) being compared to a query. Dots denote matching nuecleotides, while red letters denote differences between the two sequences.

Analysis

Sequencing Methodology Refinement

Due to the novel nature of the use of long ribosomal primers to replicate larger sections of DNA at Sumak Kawsay, the sequencing process needed to be optimized for sequencing both long and short amplicons. In the first sequencing run that was undertaken as a part of this experiment, both long (18S/28S) and short (12S, 16S, COI) amplicons were sequenced simultaneously. Each pool started library prep separately, but were combined for the final pool that was used for sequencing. Clear bands for the short amplicon pool can be seen in the initial pool and after the initial cleaning, but disappear in the final library (Fig. 6). The longer amplicon pool, by comparison can be seen throughout the three processes and is the only visible band in the final pool (Fig. 6).

Figure 7 depicts the gel results from a different session of library prep for short amplicons and demonstrates what a more typical concentration of DNA throughout the process should look like. Bands of different sizes, each pertaining to a different short amplicon gene region, are clearly seen throughout, from the initial pool to the final library that is to be utilized in the sequencing run. This higher concentration of DNA is more ideal and allows for more reads to occur during sequencing.

Despite the overrepresentation of the long amplicons in the library of the combined sequencing run, as indicated by the gel, these amplicons were underrepresented during the sequencing run (Fig. 8). Several peaks of passed reads can be seen at different sections of read length, with the vast majority of reads occurring below 1,000 base pairs.

Two very small peaks can be seen between 3.2 and 3.49 kilobases. These peaks pertain to the long amplicon (18/28S) samples, whereas each peak below 1,000 basepairs pertains to a different short amplicon (12S, 16S, COI). Due to the fact that the long amplicons required more time to read, the number of reads for these samples was greatly diminished in comparison to that of the shorter amplicons. Available pores would attach to and read shorter amplicons at a much higher rate than long amplicons. This presents a problem, as the quality of sequencing data is highly dependent on the number of reads that are able to be generated for a particular sample during a given sequencing run. Due to the low read count for the long amplicons, the methodology for sequencing had to be revised.

Following this initial sequencing run, an effort was made to optimize long amplicon sequencing and the number of reads that were produced of each sample. To accomplish this, short and long amplicons 36 underwent separate library cleanups and separate sequencing runs. Long amplicons underwent library prep with the goal of removing all amplicons below 1,000 base pairs, including primers, fragmented DNA, indexing primers, etc. Through the process of eliminating these smaller primers, available pores did not become overloaded with short, quickly readable DNA sections during sequencing and a greater number of long amplicons were processed by the nanopores.

This resulted in more comprehensive data for the long amplicons that more accurately represents the 18S to 28S gene region in question.

Sample Processing

In total, 64 consensuses of various taxa and gene regions were successfully generated (Table 2). While many samples were sequenced successfully, a number of samples that underwent successful amplification during the PCR phase, as validated by gel electrophoresis, did not produce a consensus sequence. This was most often due to the fact that the sample had a low number of reads within the region of interest. Most notably, both pseudoscorpion samples, which were included in the first, combined sequencing run, did not yield high-quality sequence data. The low number of reads for these and other long amplicon samples, such as a number of the fungi, came as a result of the problems with sequencing long and short amplicons simulta-

neously, as is explained in more detail above.

Multiple consensuses were generated for certain individuals, indicating the presence of multiple possible sequences. In the case of genes from nuclear regions (12S, 16S, 18/28S) these are likely due to the diploid nature of the individual and the inheritance of distinct genes from its mother and father. Each consensus was checked against the database as additional sequence data for the individual.

Of the consensus sequences that were generated, many did not have an equivalent sequence available for that species or gene region in the database, indicated by the underlined blasted sequence name (Table 2). This could be due to lack of sequence availability for that taxa and gene region or due to taxonomic reshuffling, such as is the case with Hypsiboas albopunctatus, which was reclassified as Boana albopunctata. While still a different species than the query sequence (Boana almendarizae), the difference in phylogenetic relationship is not as great as it may initially seem. These species may be known to science, but the sequence data may not be available for them within the BLAST database. It is another possibility, like with the Boana nigra sequences, that one gene region (12S) matches up poorly with an available sequence (95.88% percent identity), while another from the same individual (ND1) is a closer match (98.79% percent identity). Therefore, these findings must be used as a guide to promote further genetic study into the organisms in question and a more thorough assessment of their phylogenetic relationship to be sure of their status as a novel species as opposed to something that has not been sequenced before. The species that did have an equivalent within the database also often differed by over 3% for percent identity (indicated by a bolded blasted sequence name without an underline), which was the standard used in this study to indicate candidate new species to science or to sequencing that suggest the need for further scientific inquiry to discern which of these possibilities may be the case. Those species that are bolded and not underlined, meaning that there is an existing sequence for the species and the query still differs by more 3% identity or more from the reference sequence, demonstrate the highest likelihood that the sample in question is a candidate new species to science, with some notable exceptions for the tarantulas and velvet worms due to the fact that nothing even remotely close to their sequence data exists within the database.

Discussion

Through the successful optimization of PCR and sequencing in the field detailed in Table 1, this study has provided the groundwork for future investigation into these taxa within the Llanganates-Sangay Ecological Corridor. This project builds on previous work done by Elinor Sterner which investigated and optimized PCR conditions for amplification of 16S, COI, and 18S/28S in frogs. Furthermore, this study represents the first use of 18S and 28S primers in the study of invertebrate and fungi taxa in the LSEC.

All methodology, from sample collection to sequencing, was completed in the field. Located in the remote upper Amazon rainforest, it is crucial that the methodology used is optimized, to reduce the use of materials and maximize the sequences that can be generated for a given timeframe and resource availability. Despite the successful optimization of these primers in addition to the modification of sequencing for long and short amplicons, further refinement of methodology is necessary to achieve greater success in producing high quality sequences. Although successful amplification of DNA was achieved for all taxa, high-quality consensus sequences were not generated for all samples and/or loci. This could be due to a number of factors including low initial concentration of DNA, failure to index, insufficient sequencing time or pore availability. In the future, prioritization must take the form of extracting higher quality DNA to ensure that there is sufficient template DNA for genetic work.

The results from sequencing show a clear need for further genetic investigation into the biota of LSEC and the continued growth and curation of genetic databases, such as NCBI. The

information that can be discerned from an individual sequence depends on the robustness of the genetic database of similar sequences available for it to be compared to. It is incredibly important to continue the documentation of genetic diversity in the region and that these sequences are added to the BLAST database. The utility of genetic data for identifying samples is only as powerful as the database to which these sequences are compared. As an example, tarantulas were particularly underrepresented in the database, as the closest sequence to many of the samples was a tarantula from Texas that had a significantly lower query coverage than most every other sample. The representation of fungi in the database is also particularly lacking, with nearly every species sequenced not having a reference sequence available for its field identified genus or species.

This study has provided evidence of a number of novel species within the LSEC and a high rate of novel species occurrence in the region for the sampling effort performed. The sequences generated from this study may contribute to the diagnosis if these are found to represent species new to science. More research needs to be done to investigate phylogenetic relationships, morphology, and other aspects within each species to formally publish a manuscript to describe them, but the sequences produced as a result of this study provide the first step in that process. Several collaborators that were instrumental in obtaining samples for genetic processing will be working to describe the species that are first reported here in detail. Specifically, there is interest in describing several novel frog, fungi, pseudoscorpion, and tarantula species. Projects such as these have already been successfully carried out within the LSEC, as a new species of dwarf boa was described within the corridor in 2022 (Ortega-Andrade et al., 2022).

This ongoing scientific inquiry and the broadening of the genetic data associated with the fauna of Sumak Kawsay in situ will draw more research interest that is located within the reserve. The number of publications detailing species new to science will attract new investigators to continue the process. The additional draw of the ability to sequence genetic information in the field and in real time will help facilitate this research and expedite species discovery. The expansion of the knowledge of biodiversity in the region as well as the possibility of seeing species that can only be found within the corridor will attract more tourism, again providing resources that can be utilized in further protecting the corridor and promoting research.

Conclusion

A variety of species found within the Llanganates Sangay Ecological Corridor were sequenced as a part of this study, revealing a large number of candidate new species to science within the region. These species await further investigation as to whether they truly represent species new to science. Additionally, the findings highlight the potential of field-based genetic identification and reveal a critical need to continue sampling effort and sequence generation given the large proportion of potential new species undescribed to science found within the region. Having investigated and optimized a number of primer sets for a variety of gene regions across six taxa groups and streamlined sequencing for short and long amplicons, future work in this region can focus on data generation rather than troubleshooting molecular methods. This investigation further illustrates the importance of the corridor as a buffer zone between highly biodiverse ecosystems and a bountiful source of endemic species.

References

Banos, S., Lentendu, G., Kopf, A., Wubet, T., Glöckner, F. O., & Reich, M. (2018). A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. *BMC Microbiology, 18*(1), 190. https://doi.org/10.1186/s12866-018-1331-4

Bartl, S. (2003). Did the molecules of adaptive immunity evolve from the innate immune system? *Integrative and Comparative Biology*, *43*(2), 338–346. https://doi.org/10.1093/icb/43.2.338

Bentley, A. G., Reyes-Puig, J. P., Carrión-Olmedo, J. C., Libke, Z., Culebras, J., & Yánez- Muñoz, M. H. (2023). Anfibios y reptiles del corredor ecológico Llanganates - Sangay. https://wwflac.awsas-sets.panda.org/downloads/anfibios_y_reptiles_cels.pdf

Beye, M., Fahsi, N., Raoult, D., & Fournier, P.-E. (2018). Careful use of 16S rRNA gene sequence similarity values for the identification of Mycobacterium species. *New Microbes and New Infections, 22*, 24–29. https://doi.org/10.1016/j.nmni.2017. 12.009

Borchsenius, F. (1997). Patterns of plant species endemism in Ecuador. *Biodiversity & Conservation, 6*(3), 379–399. https://doi.org/10.1023/A:1018312724137

Büttner, N. (2023, February). *The Chocó: Biodiversity-hotspot in Ecuador.* Un poco del Chocó. https://www.unpocodelchoco.com/choco

Chan, A. H. E., Saralamba, N., Saralamba, S., Ruangsittichai, J., & Thaenkham, U. (2022). The potential use of mitochondrial ribosomal genes (12S and 16S) in DNA barcoding and phylogenetic analysis of trematodes. *BMC Genomics, 23*(1), 104. https://doi.org/10.1186/s12864-022-08302-4

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding the kingdom Plantae: New PCR primers for ITS regions of plants with improved universality and specificity. *Molecular Ecology Resources, 16*(1), 138–149. https://doi.org/10.1111/1755-0998.12438

Chiari, Y., Van Der Meijden, A., Madsen, O., Vences, M., & Meyer, A. (2009). Base composition, selection, and phylogenetic significance of indels in the recombination activating gene-1 invertebrates. *Frontiers in Zoology, 6*(1), 32. https://doi.org/10.1186/1742-9994-6-32

Convention on Biological Diversity. (n.d.) Ecuador - Main Details. https://www.cbd.int/countries/profile/?country=ec

EF Educational Tours. (2023). *Ecosystems of Ecuador.* https://www.eftours.com/educational tour/exploring-ecuador

Elbrecht, V., Taberlet, P., Dejean, T., Valentini, A., Usseglio-Polatera, P., Beisel, J.-N., Coissac, E., Boyer, F., & Leese, F. (2016). Testing the potential of a ribosomal 16S marker for DNA metabarcoding of insects. *PeerJ, 4*, e1966. https://doi.org/10.7717/peerj.1966

Fungal Diversity Survey. (2022). *Examining your BLAST results.* https://fundis.org/component/sppagebuilder/41-examining-your-blast-results

Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E. M., & Troedsson, C. (2014). Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. *PLoS ONE, 9*(2), e87624. https://doi.org/10.1371/journal.pone.0087624

Hałakuc, P., Karnkowska, A., & Milanowski, R. (2022). Typical structure of rRNA coding genes in diplonemids points to two independent origins of the bizarre rDNA structures of euglenozoans. *BMC Ecology and Evolution, 22*(1), 59. https://doi.org/10.1186/s12862-022-02014-9

Hillis, D. M., & Dixon, M. T. (1991). Ribosomal DNA: Molecular evolution and phylogenetic inference. *The Quarterly Review of Biology, 66*(4), 411–453. https://doi.org/10.1086/417338

Initiative 20x20. (2023). *Protecting endemic species with private reserves.* Initiative 20x20. https://initiative20x20.org/restoration-projects/protecting-endemic-species-private-reserves

IUCN. (2023). IUCN red list of threatened species. https://www.iucn.org/resources/conservation tool/iucn-red-list-threatened-species

Jones, J. S., Porter, A., Muñoz-Pérez, J. P., Alarcón-Ruales, D., Galloway, T. S., Godley, B. J., Santillo, D., Vagg, J., & Lewis, C. (2021). Plastic contamination of a Galapagos Island (Ecuador) and the relative risks to native marine species. *Science of The Total Environment, 789*, 147704. https://doi.org/10.1016/j.scitotenv.2021.147704

Jurkowski, T. P. (2020). Technologies and applications for the assessment of 5-hydroxymethylcytosine. In *Epigenetics methods* (pp. 261–278). Elsevier. https://doi.org/10.1016/B978-0-12-819414-0.00013-6

Kleemann, J., Koo, H., Hensen, I., Mendieta-Leiva, G., Kahnt, B., Kurze, C., Inclan, D. J., Cuenca, P., Noh, J. K., Hoffmann, M. H., Factos, A., Lehnert, M., Lozano, P., & Fürst, C. (2022). Priorities of action and research for the protection of biodiversity and ecosystem services in continental Ecuador. *Biological Conservation*, *265*, 109404. https://doi.org/10.1016/j.biocon.2021.109404

Krehenwinkel, H., Pomerantz, A., Henderson, J. B., Kennedy, S. R., Lim, J. Y., Swamy, V., Shoobridge, J. D., Graham, N., Patel, N. H., Gillespie, R. G., & Prost, S. (2019). Nanopore sequencing of long ribosomal DNA amplicons enables portable and simple biodiversity assessments with high phylogenetic resolution across broad taxonomic scale. *GigaScience, 8*(5), giz006. https://doi.org/10.1093/gigascience/giz006

Lawson, L. P., Loader, S. P., Lyakurwa, J. V., & Liedtke, H. C. (2023). Diversification of spiny throated reed frogs (Anura: Hyperoliidae) with the description of a new, range-restricted species from the Ukaguru Mountains, Tanzania. *PLOS ONE, 18*(2), e0277535. https://doi.org/10.1371/journal.pone.0277535

Liria, J. (2022). Áreas de endemismo de Ecuador: Un análisis a partir de datos de distribución de especies de plantas, animales y hongos. *Revista Mexicana de Biodiversidad, 93*, e934031. https://doi.org/10.22201/ib.20078706e.2022.93.4031

Manes, S., Costello, M. J., Beckett, H., Debnath, A., Devenish-Nelson, E., Grey, K.-A., Jenkins, R., Khan, T. M., Kiessling, W., Krause, C., Maharaj, S. S., Midgley, G. F., Price, J., Talukdar, G., & Vale, M. M. (2021). Endemism increases species' climate change risk in areas of global biodiversity importance. *Biological Conservation, 257*, 109070. https://doi.org/10.1016/j.biocon.2021.109070

Merheb, M., Matar, R., Hodeify, R., Siddiqui, S. S., Vazhappilly, C. G., Marton, J., Azharuddin, S., & Al Zouabi, H. (2019). Mitochondrial DNA, a powerful tool to decipher ancient human civilization from domestication to music, and to uncover historical murder cases. *Cells, 8*(5), 433. https://doi.org/10.3390/cells8050433

Milan, D. T., Mendes, I. S., Damasceno, J. S., Teixeira, D. F., Sales, N. G., & Carvalho, D. C. (2020). New 12S metabarcoding primers for enhanced Neotropical freshwater fish biodiversity assessment. *Scientific Reports, 10*(1), 17966. https://doi.org/10.1038/s41598-020-74902-3

Morgan, J. A. T., & Blair, D. (1998). Mitochondrial ND1 gene sequences used to identify echinostome isolates from Australia and New Zealand. *International Journal for Parasitology, 28*(3), 493– 502. https://doi.org/10.1016/S0020-7519(97)00204-X

Murienne, J., Daniels, S. R., Buckley, T. R., Mayer, G., & Giribet, G. (2014). A living fossil tale of Pangaean biogeography. *Proceedings of the Royal Society B: Biological Sciences, 281*(1775), 20132648. https://doi.org/10.1098/rspb.2013.2648

Nirjhon, R. (2023, March 24). *BLAST in bioinformatics; its uses, application & function.* https://discover.hubpages.com/education/BLAST2

Ortega-Andrade, H. M., Bentley, A., Koch, C., Yánez-Muñoz, M. H., & Entiauspe-Neto, O. M. (2022). A time relic: A new species of dwarf boa, Tropidophis Bibron, 1840 (Serpentes: Amerophidia), from the Upper Amazon Basin. *European Journal of Taxonomy, 854*, 1–107. https://doi.org/10.5852/ejt.2022.854.2021

Price, J., & Jenkins, R. (2022, March 23). *Climate change risks extinction of endemic species.* Tyndall Centre for Climate Change Research. https://tyndall.ac.uk/news/climate-change-risks-extinction-of-endemic-species/

Pomerantz, A., Sahlin, K., Vasiljevic, N., Seah, A., Lim, M., Humble, E., Kennedy, S., Krehenwinkel, H., Winter, S., Ogden, R., & Prost, S. (2022). Rapid in situ identification of biological specimens via DNA amplicon sequencing using miniaturized laboratory equipment. *Nature Protocols 17*, 1415–1443. https://doi.org/10.1038/s41596-022-00682-x

Rubbmark, O. R., Sint, D., Horngacher, N., & Traugott, M. (2018). A broadly applicable COI

primer pair and an efficient single-tube amplicon library preparation protocol for metabarcoding. *Ecology and Evolution, 8*(24), 12335–12350. https://doi.org/10.1002/ece3.4520

Rull, V. (2022). Biodiversity crisis or sixth mass extinction?: Does the current anthropogenic biodiversity crisis really qualify as a mass extinction? *EMBO Reports, 23*(1), e54193. https://doi.org/10.15252/embr.202154193

Sterner, E. (2023). Tree to tree: Phylogenetics of diverse Anura using portable lab equipment: Primer optimization, bioinformatic pipeline, and phylogenetic analyses reveal potential new species and ability to identify evolutionary relationships in a hyper diverse anura clade using Nanopore sequencing. SIT Digital Collections. https://digitalcollections.sit.edu/isp_collection/3513/

van Uhm, D. P. (2016). The sixth mass extinction. In T*he illegal wildlife trade: Inside the world of poachers, smugglers and traders* (pp. 17–32). Springer.

Villa, A., & Notarangelo, L. D. (2019). RAG gene defects at the verge of immunodeficiency and immune dysregulation. *Immunological reviews, 287*(1), 73-90. https://doi.org/10.1111/imr.12713

Waltert, M., Bobo, K. S., Kaupa, S., Montoya, M. L., Nsanyi, M. S., & Fermon, H. (2011). Assessing conservation values: Biodiversity and endemicity in tropical land use systems. *PLoS ONE, 6*(1), e16238. https://doi.org/10.1371/journal.pone.0016238

Wang, Y., Zhao, Y., Bollas, A., Wang, Y., & Au, K. F. (2021). Nanopore sequencing technology, bioinformatics and applications. *Nature Biotechnology, 39*(11), 1348–1365. https://doi.org/10.1038/s41587-021-01108-x

World Wildlife Foundation. (n.d.). *Priority & endangered species.* https://wwf.panda.org/discover/knowledge_hub/endangered_species/

Wu, L. W., Lin, L. H., Lees, D. C., & Hsu, Y. F. (2014). Mitogenomic sequences effectively recover relationships within brush-footed butterflies (Lepidoptera: Nymphalidae). *BMC Genomics, 15*(1), 468. https://doi.org/10.1186/1471-2164-15-468

Xu, J. (2020). Fungal species concepts in the genomics era. *Genome, 63*(9), 459–468. https://doi.org/10.1139/gen-2020-0022

Yang, C. H., Wu, K. C., Chuang, L. Y., & Chang, H. W. (2019). Decision theory-based COI- SNP tagging approach for 126 scombriformes species tagging. *Frontiers in Genetics, 10,* 259. https://doi.org/10.3389/fgene.2019.00259

Yang, L., Tan, Z., Wang, D., Xue, L., Guan, M., Huang, T., & Li, R. (2014). Species identification through mitochondrial rRNA genetic analysis. *Scientific Reports, 4*(1), 4089. https://doi.org/10.1038/srep04089

Yang, R., Cao, Y., Hou, S., Peng, Q., Wang, X., Wang, F., Tseng, T. H., Yu, L., Carver, S., Convery,

I., Zhao, Z., Shen, X., Li, S., Zheng, Y., Liu, H., Gong, P., & Ma, K. (2020). Cost- effective priorities for the expansion of global terrestrial protected areas: Setting post-2020 global and national targets. *Science Advances, 6*(37), eabc3436. https://doi.org/10.1126/sciadv.abc3436

Yusnita, Y., Norsiah, M. D., & Rahman, A. J. (2010) Mutations in mitochondrial NADH dehydrogenase subunit 1 (mtND1) gene in colorectal carcinoma. *The Malaysian Journal of Pathology 32*(2), 103-110.

Zang, W., Jiang, Z., Ericson, P. G. P., Song, G., Drovetski, S. V., Saitoh, T., Lei, F., & Qu, Y. (2023). Evolutionary relationships of mitogenomes in a recently radiated Old World avian family. *Avian Research, 14*, 100097. https://doi.org/10.1016/j.avrs.2023.100097

Targeted Advertising: How Companies And Governments Are Weaponizing User Data Against The Consumer

Joseph Garneau

ABSTRACT

With the recent rise of a new digital age, many people are ditching more traditional methods of performing everyday tasks and replacing them with free and convenient online services. But as the saying goes, there is no such thing as a free lunch, and that sentiment holds true in terms of online services as well. Consumers pay for these online services with something much more valuable than money—their personal data. This paper explores how companies and governments present consumers with targeted advertisements, shows how targeted advertisements are inherently manipulative, and gives an example of one government and one company using this technology to influence the behavior of its target demographics. The paper concludes with a discussion of the ethical implications of the research, states its limitations, proposes possible solutions by providing contextual information, and concludes by arguing that the only way to change this behavior would be through legislative measures.

Introduction

After the unfortunate passing of Linday Robertson's mother in 2014, she took on the responsibility of arranging her mother's affairs (Nudson, 2020). One of those responsibilities was to figure out a price for a gravestone, and, after a quick Google search, Robertson soon found what she was looking for and ended her browsing session. However, like a restless soul with unfinished business, the haunting feeling of death still lingered — virtually. Soon Roberston's internet was cluttered with gravestones. They followed her everywhere; she could not escape (Nudson, 2020). Robertson's story is not unique. In the wild west of the internet, companies are able to harvest user data and use it to serve personalized advertisements in the form of targeted advertising. This practice is harmful to individuals, yet companies (and even governments) use these technologies because they are profitable and effective.

The Issues

Data is being collected in droves, and despite some users' attempts on limiting the sharing of this information, it is almost impossible to use online services without sharing some data (Callanan et al., 2021). For example, even if one were to disable many tracking settings and delete all cookies before switching websites, advertisers would still be able to track the user across multiple sites through information such as the user's screen resolution, what web browser they are using, what version of web browser the user has, and what operating system is installed. This information can be compiled into a profile for that given user which is used to track their activity across multiple sites (Callanan et al., 2021).

However, most people do not even bother to go through with such rigorous privacy settings. This allows advertisers to open the flood gates on a targets' information. One advertiser, Meta Platforms Inc. (formally known as Facebook) reportedly, "provides ways to target geographic locations, personal interests, characteristics and behavior, including activity on other internet services and even in physical stores" (Callanan et al., 2021, p. 157). Advertisers can use this information to build a very detailed profile of a user including "their political affiliation; how likely they are to engage with political content; whether they like to jog, hike or hunt; what kind of beer they like; and so on" (Callanan et al., 2021, p. 157).

Advertisers do not collect all this information without reason. There are many strategies advertisers use to make their ads successful on a target. Thanks to the work of many academic researchers, advertisers know exactly what mood, content, and context any given ad must contain to be successful on a given site (Voorveld et al., 2018). For example, Twitter users engage with an ad if they view it as being informative, while users on YouTube view ads more negatively when using the platform for entertainment. These incredibly detailed measurements were made from the outside looking in, as the researchers were limited to asking a relatively small number of users simple questions to get their data (Voorveld et al., 2018). The tools companies have for analyzing their internally generated data are many times more accurate, thus allowing companies to effectively maximize the use of massive amounts of data their users produce.

There is also evidence that some governments are taking advantage of targeted advertising in order to change their respective population's behavior. Such activities raise serious moral and ethical concerns. One study found that the UK government was actively using the same targeting systems companies use to encourage and discourage certain types of behavior (Collier et al., 2022). This activity ranged from targeting adolescents who looked up illegal topics with messages and warnings of punishment to showing fire safety ads to people who recently bought candles. There are even cases of the government using local community leaders in advertisements in order to better connect with targets. Understandably, this activity raises many moral and ethical questions, such as "Is it democratic for governments to be influencing behav-

ior in this top-down approach?" and "Is it ok for the government to counter disinformation or should that be the responsibility of the population?" (Collier et al., 2022).

Targeted advertising is harmful because it is exploitative. Advertising agencies have unfettered access to vast amounts of data, which they use advanced artificial intelligence (AI) to filter through and find emerging behavior patterns. These AIs are able to predict outcomes of consumer choices, predict future behavior, and even predict details about a person even if the person is not aware of it yet (Callanan et al., 2021). In a now infamous example, Target – a mainly brick and mortar retailer – collected and sourced loads of data and then compiled it into a "pregnancy prediction" score. Target used this score to predict the likelihood of a customer becoming pregnant and allowed Target to send the expecting mother coupons. Predictions were often very accurate even before the customer had set up a baby-shower registry with the company (Callanan et al., 2021). Target was even able to estimate the stage of a customer's pregnancy and thus supply them with relative coupons. These Orwellian-esque tactics are very concerning, and Target knew that. A Target executive spoke on the matter saying,

[W]e found out that as long as a pregnant woman thinks she hasn't been spied on, she'll use the coupons. She just assumes that everyone else on her block got the same mailer for diapers and cribs. As long as we don't spook her, it works. (Callanan et al., 2021, p. 160)

Other agencies like Google, were able to predict a user's mood, emotions, and sell this information off to advertisers. Meta is no better, also allowing the sale of their user's information, such as whether or not teenagers were feeling "'insecure', 'worthless', or otherwise in need of a 'confidence boost'" (Callanan et al., 2021, p. 158). It is painfully obvious that this information can be easily used to manipulate and influence vulnerable people into making decisions, and most importantly – purchases that they most likely would not have made given a better state of mind.

If advertisers and governments know this behavior is harmful, then why do these agencies keep using these tactics? This question can be answered in two parts. First, many companies have legal requirements to make their stakeholders happy. Often, this means making the stakeholders richer by any means possible (Callanan et al., 2021). Second, laws surrounding this behavior are either very vague or non-existent. Therefore, if it is not illegal to use targeted ads, and it is also very profitable; so why not use targeted advertisements (Callanan et al., 2021)? One recent notable change is the passage of the General Data Protection Regulation (GDPR) in the European Union. The GDPR includes a right to be forgotten, and a right to access, and a right to be informed when it comes to personal data (GDPR, 2018). Hopefully, these recent changes can force companies and governments to change their manipulative behavior.

Ethics

There are many ethical implications that targeted advertising produces. One of the biggest ethical issues is where we draw the line on how much personal information parties can collect. The answers society provides will shape the future of privacy focused legislation. The GDPR proposes many reasonable first steps that will inevitably radically change the way companies handle data. For instance, the GDPR creates a "right to be forgotten" (GDPR, 2018). How does a company handle this responsibility? Does a user have to request that they be forgotten or is the right exercised automatically? What about the death of a user?

Governments also provide a whole new set of ethical questions when it comes to using targeted advertising. Collier et al. (2022) argue that using these technologies for influencing behavior should not be a top-down approach. Instead these tools should be given to the wider population in order to encourage change from the bottom up. However, there are some big risks to this stance. Radical groups could also use this hypothetical technology to spread hateful rhetoric. Thus, society would need an arbiter of what is allowed to be spread. These points

require more research if we are to implement a similar system.

There is also an argument that the government should be able to use targeted advertising, as it has been very successful in the UK government. Over a six month period, a NCA campaign seems to have completely stopped all growth in the purchase of Distributed Denial of Service (DDOS) attacks (Collier et al., 2022). This is notable because during the same time period, sales of DDOS attack services rose internationally. However, since these government programs are targeted to prevent criminal behavior before it happens, it is hard to objectively show a link between the use of targeted advertising by the government and a decline in any given crime. As Collier et al. (2022) put it, "We are also aware that the effects of these campaigns may be exaggerated, misreported or have not been continued" (p. 6).

Solutions

One would assume that a majority of people are in support of regulation of this practice, and that would be a correct assumption. In fact, 91% of Americans think that companies are tracking some or all of online activity, and 77% also believe the same about the government (Auxier & Rainie, 2019). Public support is there, yet in the United States, the political scene may be less suitable for change. In recent years, political tensions have been sharply rising, thus making it a miracle even for bipartisan legislation to become law. However, the topic of regulating companies is far from by-partisan. A survey conducted by Pew Research (2019) found that 71% of Republicans (America's dominant conservative party) think Government is too involved in matters that should be left to the private sector, while 78% of Democrats (America's dominant liberal/progressive party) think the government should be doing more to solve problems. Even if there was a popular push for government regulation of targeted advertising agencies, it would inevitably be split on party lines and be very controversial.

Although it will be very challenging to pass legislation, it is the only way to make significant change a reality. This is because tech companies hold a lot of power in the United States' legal system. For example, in 2022 the lobbying group TechNet (which is partially made up of members of the "Big Four"), successfully neutered a right-to-repair bill in New York (Cunningham, 2022). However, there may still be hope. With the recent public suspicion of the very popular short form video site TikTok, it may be possible for privacy activists to carry this momentum into changing US policy. For example, during the hearings, the wider population learned details on how TikTok collected data. These strategies are not isolated to TikTok; many of the "Big Four" (Apple, Google, Microsoft, and Meta) also use these exact same strategies. It is worth noting that change is most likely going to be most influential in the United States, as that is where Apple, Google, Meta, and Microsoft (a.k.a the "Big Four") are located (Callanan et al., 2021). Since the "Big Four" control most of the targeted advertising market, any change in their host country will propagate throughout the rest of the internet and – to an extent – the rest of the Western world. Once the public is aware of this, it could potentially push lawmakers into drafting and passing more privacy focused laws.

Limitations

One major limitation of this research is the legality of the proposed legislation. This study did not look into any case law and combines research from multiple countries, each with similar, yet distinct, legal systems. A law that might work in the European Union would be unlikely to work in the United States. The European Union, however, continues to pioneer this legislation in their system, and hopefully the Americans take note. Another limitation is the fact that most of the literature around this topic only sources from Western, Educated, Industrialized, Rich, and Democratic (WEIRD) countries. As our current internet mostly ignores borders, it is

worth considering how any changes will affect those who are not of one's country.

Conclusion

In conclusion, companies and governments are collecting user data and using it to craft personalized advertisements that manipulate targets. This practice is perpetuated by outdated laws and a drive for ever-increasing profit. These behaviors raise many ethical questions that will direct the future of targeted advertising. Solutions to discussed issues will need to be implemented via law for any change to occur. The author hopes that this paper will provide a jumping off point for future research.

References

Auxier, B., & Rainie, L. (2019). *Key takeaways on Americans' views about privacy, surveillance and data-sharing*. Pew Research Center. https://www.pewresearch.org/short-reads/2019/11/15/key takeaways-on-americans-views-about-privacy-surveillance-and-data-sharing/

Callanan, G. A., Perri, D. F., & Tomkowicz, S. M. (2021). Targeting vulnerable populations: The ethical implications of data mining, automated prediction, and focused marketing. *Business and Society Review, 126*(2), 155-167. https://doi.org/10.1111/basr.12233

Collier, B., Flynn, G., Stewart, J., & Thomas, D. (2022). Influence government: Exploring practices, ethics, and power in the use of targeted advertising by the UK state. *Big Data & Society, 9*(1), 205395172210787. https://doi.org/10.1177/20539517221078756

Cunningham, A. (2022). *New York governor signs modified right-to-repair bill at the last minute.* Ars Technica. https://arstechnica.com/gadgets/2022/12/weakened-right-to-repair-bill-is-signed-into law by-new-yorks-governor/

General Data Protection Regulation (GDPR) – Legal Text. (2018). General Data Protection Regulation (GDPR). https://gdpr-info.eu/

Nudson, R. (2020, April 9). When targeted ads feel a little too targeted. *Vox*. https://www.vox.com/the goods/2020/4/9/21204425/targeted-ads-fertility-eating-disorder-coronavirus

Pew Research Center. (2019). *2. Views of government and the nation.* Pew Research Center. https://www.pewresearch.org/politics/2019/12/17/views-of-government-and-the-nation/

Voorveld, H. A. M., van Noort, G., Muntinga, D. G., & Bronner, F. (2018). Engagement with social media and social media advertising: The differentiating role of platform type. *Journal of Advertising, 47*(1), 38-54. https://doi.org/10.1080/00913367.2017.1405754

Ancient Rome and Modern Russia: A Comparative Analysis of Propaganda and Statecraft during Times of Conflict

Sydney Lynch

ABSTRACT

The careful study of trends in propaganda can provide dramatic insights into the political climate of a particular nation. Specifically, shifts in either the narrative or dissemination of propaganda during times of great conflict or domestic turmoil illuminate to outside audiences where the government feels there are possible weaknesses that could fracture and eventually destroy the security and legitimacy of their regime. Therefore, this paper investigates the Kremlin's recent methods of propaganda and disinformation compared to those of Imperial Rome, allowing for a closer examination of these techniques. Overall, the comparative analysis of Imperial Roman and current Russian propaganda and disinformation policies allows for examination of the continuities and identifies changes in political propaganda as nations face times of instability and strain.

Introduction

Politicians and their governments utilize propaganda and disinformation to secure a political regime or to maintain popular support for a specific issue. Particularly during times of unrest and uncertainty, propaganda becomes vital to maintaining stability as a nation's people may begin to question the actions taken by their government. Therefore, the perceived trust and confidence of the general populace in a leader and their government allows that leader to take action with minimal public disruption. However, confidence in a leader and support for their regime does not necessarily naturally occur. Instead, the popular perception of leaders is carefully curated through various propaganda mediums. Often relying on deception, propaganda mediums build the leader's public image, create an ideological framework to be adopted by the public, and ultimately justify the actions taken by said leader. The importance of propaganda is evident in Russia's recent invasion of Ukraine. Russian information operations and propaganda have played a central role in justifying and retaining public support for the invasion. Because of the nature of this conflict, it is difficult to closely analyze the specific details as this crisis unfolds. However, a careful analysis of historical propaganda can help modern scholars identify patterns to better understand the means and methods of Russian propaganda.

The comparative analysis of ancient and modern propaganda can provide valuable insights into the diversity of methods during perceived threat or instability. Specifically, during the Russo-Ukrainian War, a case can be made to study the parallels between Russian and Imperial Roman statecraft as the environment constantly changes. When applying these ideas to a specific case study, one could pose the question: Can the comparative analysis of ancient Rome and contemporary Russia's approaches to propaganda elucidate the methods and techniques of statecraft during times of crisis? Upon closely examining imperial Roman and modern Russian propaganda, parallels between specific thematic elements illustrate a broader connection between statecraft and political turmoil. While multiple methods of statecraft can be compared, propaganda and disinformation provide an interesting look not only into a specific regime's more comprehensive ideological agenda but also highlight areas of concern that the current government believes threaten their security and must be reinforced through this manufactured information.

Developing an Identity

Of the many essential themes or narratives touched upon in propaganda, developing a public identity or persona is central to gaining and maintaining power for both ancient and modern governments. The concept of creating a politician so widely popular that they are idolized transcends history, and the curating of a public image to garner the populace's trust while maintaining the stability of their rule can be seen even in ancient contexts. Image-building is essential during the early period of an individual coming into power and during unrest. When the population is more critical of the acting government, a politician with a solid public image appears to be a guiding hand of confidence who will lead their people through political turmoil.

While many characteristics may be over-emphasized to the public depending on the existing political climate, many parallels can be drawn between the image-building of early Roman emperors and Vladimir Putin. The emphasis on characteristics such as morality, trust, and civil responsibility is significant during transitional periods when uncertainty is high, such as the transition of Rome from Republic to Empire and Putin's ascension to the presidency following Boris Yeltsin. Therefore, creating this perfect public image early in a political career garners popular support and insulates the individual from intense domestic scrutiny during tumult. As previously mentioned, the importance of a public image becomes exceptionally apparent during unrest and conflict, which is especially true for both Imperial Rome and Russia. Since the beginning of his political career, Putin and the Kremlin have been carefully crafting

the ultimate public image of a strong, moral, and patriotic man to lead Russia to greatness.

From public relations campaigns that depicted him saving a group of journalists from a Siberian tiger to pop songs being released praising him for his strength and morality, the idea of Putin as the ideal Russian man and leader has been essential to the stability of his reign.[1] Furthermore, in the lead-up to his second election and the 2014 annexation of Crimea, calendars, magazine covers, and images of Putin during myriad physical activities were released to praise his strength and masculinity.[2] However, since the initial invasion of Ukraine and the Wagner mutiny in June 2023, the security and stability of Putin as Russia's leader has been called into question. These events placed more emphasis on reinforcing Putin's public image as one of strength and aplomb in the face of adversity. The Kremlin and state media underplayed the chaos of the Wagner mutiny even after Yevgeny Prigozhin's plane crash in August 2023. Between the release of Putin Team Russia merchandise at the Army-2023 forum and videos of his warm welcome to Derbent Dagestan in late June 2023, the media pushed a message to the public that everything is in order and that support for Putin is unwavering.[3] Because of the extensive work done to manufacture Putin's public image early in his career, and despite some political missteps, Putin's domestic approval rating, as of March 2024, is around 87%.[4]

Similarly, when Rome was transitioning from a Republic to an Empire, there was a painful growth period involving the assassination of Julius Caesar to the bloody civil conflicts that ensued to the ascension of his nephew to the new Imperial throne; and individuals such as Augustus understood the importance of a solid public image. Since the Romans were wary of another monarch-type ruler, Augustus engineered himself to appear trustworthy and pious to confirm that his rule was divinely favored. Shortly after he secured his title as Emperor and Pontifex Maximus, statues of Augustus donning robes and general regalia were erected all over Rome to demonstrate his religious virtue and military prowess to citizens.[5] Furthermore, Vergil's *Aeneid* was a careful piece of propaganda created to not only attach Augustus to the mythic founding of Rome but also to frame his rule as the divine will of the gods.[6] Through the *Aeneid*, Vergil communicates to audiences the legitimacy of Augustus' reign by tracing his bloodline to the goddess Venus and foreshadowing the prosperity that will follow for Rome and its people.[7]

As is demonstrated in the case of Augustus and the transition of Rome from a Republic to an Empire, most of the propaganda being created focused on Augustus and his legitimacy as a ruler. This promotion was aligned with the timing of Augustus's ascent to the imperial throne, which came during a period of crisis for the Roman people and when public trust in the government as an institution was low. Instead of creating the image of the best politician or government official to run Rome, Augustus focused his identity-building efforts on creating the ultimate Roman. This subtle shift in focus to the individual greatly aided Augustus's early political success because Romans liked him as a person, which then enhanced their desire for

---

1 Fiona Hill and Clifford G. Gaddy, "Who Is Mr. Putin," essay, in *Mr. Putin: Operative in the Kremlin* (Brookings Institution Press, 2013), 14-16.
2 Valerie Sperling, "Putin's Macho Personality Cult," *Communist and Post-Communist Studies* 49, no. 1 (2016): 13–23, https://doi.org/10.1016/j.postcomstud.2015.12.00., 15-16.
3 Francis Scarr, Twitter Post, June 2023, 4:33 p.m., https://twitter.com/francis_scarr/status/1674154082771107842?s=20.

4 "Approval of the Authorities," Levada-Center, accessed April 2024, https://www.levada.ru/en/ratings/.

5 Rosemarie Trentinella, "Roman Portrait Sculpture: The Stylistic Cycle," The Met's Heilbrunn Timeline of Art History, January 1, 1AD, https://www.metmuseum.org/toah/hd/ropo2/hd_ropo2.htm.

6 Sabine Grebe, "Augustus' Divine Authority and Vergil's Aeneid," *Vergilius* 50 (2004): 35–62, https://doi.org/http://www.jstor.org/stable/41587284.

7 Marie-Laure Freyburger-Galland, "Political and Religious Propaganda Between 44 and 27 BC," *Vergilius* 55 (2009): 17–30, http://www.jstor.org/stable/41561878.

him to become their ruler. Similarly, Putin emerged onto the Russian political scene during great uncertainty and change. Unhappy with President Boris Yeltsin and the state of the Russian Federation after the dissolution of the Soviet Union, Russians were weary of the traditional Moscow politician and instead desired someone they could relate to and entrust to strengthen the nation.[8] Like Augustus, Putin shifted the focus of his public identity-building to center on character rather than political efficacy. In doing so, he conditioned the Russian to like him as a person, which then, in turn, enhanced their trust in him as a president.

Overall, both Augustus, as well as other Roman emperors, and Putin see the importance of creating and maintaining an unimpeachable public image for the security of their regimes. Furthermore, by focusing on similar characteristics, both Roman emperors and Vladimir Putin have managed to transcend the status of popular politician to achieve godlike appearance for their domestic audiences. The solid popular support for these individuals, not just as leaders but also as human beings, becomes critical during tumult, such as the transition of Rome from Republic to Empire and Russia's invasion of Ukraine. Due to the general public's strong affinity for these individuals, their questionable actions could more easily be overlooked because of this accumulated public trust.

Historical Manipulation and Collective Memory

During the past few years, Russian disinformation operations and propaganda have transitioned their focus from creating Putin's impenetrable persona to manipulating perceptions of history to justify the invasion of Ukraine. In an attempt to reshape the collective memory of the Russian people and generate popular support for the war, Putin and the Kremlin have worked tirelessly to reframe and sanitize the role of the former Soviet Union during the second world war. Victory Day parades, monuments, and articles penned by Vladimir Putin himself all tell a tale of Soviet valor against the evils of Nazis and fascism.[9] Evidence of this historical manipulation spans beyond just the narratives of World War II, which is evident in state-mandated curricula at the university level and new children's books that not only skew the events of World War II to elevate the Soviet Union but also claim that Ukraine was never an independent state from Russia.[10] Furthermore, to justify and secure public support for the invasion of Ukraine, Putin has been invoking the traumatic times of the Great Patriotic War and the glory of the former Soviet Union.[11] Through speeches, articles, historical societies, and new public monuments, the adoration of "the Great Patriotic War" frames the invasion of Ukraine as Russia freeing its people from "Nazi" and "fascist" rule in Ukraine.[12]

In the case of the Roman Empire, the manipulation of history was centered around controlling public memory. Aware of the Roman people's approbation of another monarch-like figure, Augustus engineered his rule to be seen as divine and with ancient roots. Through the creation of the *Aeneid*, Vergil created an epic founding story of the Roman people that connected Augustus and his family to this empire's mythic beginnings and claimed the rule of

---

8 Valerie Sperling, "Putin's Macho Personality Cult," *Communist and Post-Communist Studie*s 49, no. 1 (2016): 13 23, https://doi.org/10.1016/j.postcomstud.2015.12.00.

9 "Vladimir Putin's Historical Disinformation," U.S. Department of State, May 6, 2022, https://www.state.gov/disarming-disinformation/vladimir-putins-historical-disinformation/.

10 Vitaly Shevchenko, "Russia's New Schoolbook Aims to Justify War on Ukraine," BBC News, August 9, 2023, https://www.bbc.com/news/world-europe-66450520.

11 "Vladimir Putin's Historical Disinformation," U.S. Department of State, May 6, 2022, https://www.state.gov/disarming-disinformation/vladimir-putins-historical-disinformation/.

12 Daniel Shultz and Christopher Jasparro, "How Does Russia Exploit History and Cultural Heritage," April 2022, https://thinktank.theantiquitiescoalition.org/.

Augustus to be divinely ordained.[13] The *Aeneid* and Augustus's divine family lineage started a trend for Roman emperors to associate themselves with essential figures or deities involved in Rome's early days. Furthermore, the Roman practice of *damnatio memoriae* (condemnation of memory) attempted to manipulate history through the complete erasure of an individual and their deeds.[14] Popularized during the Roman Empire, this practice involved removing names and faces from statues, burning books, and defacing graves, which occurred when the emperor or senate deemed an individual so evil that they must be erased from history.[15] Of course, this was rarely based on truth and instead was widely known as a fate to befall the political rivals of particularly vicious Emperors, which can be seen in the example of Caracalla, who enacted *damnatio memoriae* after killing his brother and rival Geta.[16]

Both Rome and Russia identified the importance of history and collective memory relative to the security of their rulers. Furthermore, both Imperial Rome and Russia invoked these historical narratives during times of perceived high instability. Rome manipulated history during transitional periods between forms of government and Emperors, and Russia has acted similarly to justify the invasion of Ukraine. Both nations successfully invoke collective memory by manipulating history to achieve a specific political goal.

Censorship

Finally, Russia's extensive information operations and propaganda efforts would not be as successful for its domestic audience without the onerous censorship of Russian media. For many oppressive political regimes, the most effective way of ensuring that propaganda functions as intended is by restricting other information from their people. Russia has undertaken extensive measures to ensure the isolation of the public from any international news or media that threatens Russian political ideology. While the Russian constitution bans the act of censorship, other laws regarding slander and defamation promote self-censorship for Russian media platforms that fear breaking the law.[17] To further achieve its goal of information restriction, the Russian government relies heavily on its extremism law to ban social media platforms.[18] Since the invasion of Ukraine, there has been a greater effort from the Russian government to censor social media platforms, restricting sites like Facebook, Twitter, and Instagram because these platforms were either editing Russian content or promoting extremist ideas that harm Russia.[19] The Russian government has made a disinformation ecosystem that allows propaganda to flourish by creating its own internet service, alternative social media platforms, and state-run news media, as well as banning media and publications that contradict Russia's ideological goals.[20]

Similarly, in Imperial Rome, there was also a significant shift in censorship to manipulate

---

13 Marie-Laure Freyburger-Galland, "Political and Religious Propaganda Between 44 and 27 BC," *Vergilius* 55 (2009): 17–30, http://www.jstor.org/stable/41561878, 17-18.

14 Susan Rahyab, "Censorship and Book-Burning in Imperial Rome and Egypt" (dissertation, CUNY Academic Works, 2020). 3-4.
15 Ibid.

16 Anne Leader, "A Not-so-Festive Case of Fratricide: Caracalla Murdered His Brother on 19 December 221 CE.," Italian Art Society, accessed August 24, 2023, https://www.italianartsociety.org/2015/12/a-not-so-festive-case-of fratricide-on-19-december-221-ce-caracalla-killed-his-brother-geta-in-order-to-gain-full-command-of-the-roman empire/.
17 Greg Simons, "Russian Media and Censorship: A Means or an End?," *Russian Journal of Communication* 7, no. 3 (2015): 300–312, https://doi.org/10.1080/19409419.2015.1082438, 2-3.
18 Ibid.
19 Alexander Marrow and Elizabeth Culliford, "Russia to Restrict Facebook Access for 'censoring' Its Media," Reuters, February 26, 2022, https://www.reuters.com/business/media-telecom/russia-limit-facebook-access response-media-censorship-2022-02-25/.
20 Shannon Bond and Bobby Allyn, "Russia Is Restricting Social Media. Here's What We Know," NPR, March 21, 2022, https://www.npr.org/2022/03/07/1085025672/russia-social-media-ban.

public opinion. During the Republican era, the action of censorship was primarily concerned with religious texts and practices that threatened the more significant Roman social order, an example of which can be seen through the banning of the *Bacchanalia*.[21] However, beginning with the reign of Augustus, this censorship began targeting people and works that threatened the power and political legitimacy of the emperor. During his reign, Augustus combined a law from the Twelve Tables with the laws of treason, which made written works subject to the charge of treason.[22] This effort, combined with the burning of over 2,000 prophetic texts in the year 12 BCE, illustrates both a shift in focus of censorship and Augustus's understanding of the importance of protecting his rule.[23] Throughout the Imperial age, from Augustus until the fall of the Western Roman Empire, book burning, *damnatio memoriae*, and executions were done to protect the emperor, his authority, and the information circulated to the public. This censorship illustrates the fragility of the emperor's position and the threat that criticisms or contradictory information posed to the Imperial throne.

The parallels between the Kremlin and Imperial Rome's use of censorship, to not only insulate their people but also to create an environment where certain propaganda narratives can flourish, illustrates both the understanding of the importance of the conditions necessary for disinformation to thrive and the strict security of the regime that calls for such an insular society. Furthermore, while censorship may not be considered a traditional form of propaganda, its role in repression and manipulating public memory proves to be an invaluable tool for those in power. As both Rome and Russia illustrate, censorshp plays a significant role in ensuring that propaganda and disinformation are effective, which becomes especially important in times of perceived insecurity or crisis. When examining ancient examples such as Caracalla's erasure of his brother Geta's memory and contemporary events like Russia's suppression of dissident information during the Russian invasion of Ukraine, one can discern what is being targeted by these regimes as a perceived threat.

Conclusion

The close study of trends in propaganda and disinformation can provide dramatic insights into the political climate of a particular nation. Specifically, shifts in either the narrative or dissemination of propaganda during great conflict or inner turmoil expose what other countries might perceive as weaknesses of a particular government, thus affirming these points of contention as a legitimate threat against their regime. Especially for volatile environments such as the Russo-Ukrainian War, the comparison between the Kremlin's recent methods of propaganda and disinformation and that of Imperial Rome helps identify patterns in statecraft that would otherwise be challenging to ascertain if only studying the contemporary evidence of an ongoing conflict. Ultimately, the comparative analysis of Imperial Roman and modern Russian propaganda and disinformation facilitates a unique and insightful examination of the continuities and changes in political propaganda as nations face times of instability and strain.

21 Susan Rahyab, "Censorship and Book-Burning in Imperial Rome and Egypt" (dissertation, CUNY Academic Works, 2020). 5-7.

22 Ibid, 9-10.

23 Ibid

References

"Approval of the Authorities." Levada-Center. Accessed August 23, 2023. https://www.levada.ru/en/ratings/approval-of-the-authorities/.

Bond, Shannon, and Bobby Allyn. "Russia Is Restricting Social Media. Here's What We Know." NPR, March 21, 2022. https://www.npr.org/2022/03/07/1085025672/russia-social-media-ban.

Freyburger-Galland, Marie-Laure. "Political and Religious Propaganda Between 44 and 27 BC." *Vergilius* (1959-) 55 (2009): 17–30. http://www.jstor.org/stable/41561878.

Grebe, Sabine. "Augustus' Divine Authority and Vergil's Aeneid." *Vergilius* (1959-) 50 (2004): 35–62. https://doi.org/http://www.jstor.org/stable/41587284.

Hill, Fiona, and Clifford G. Gaddy. "Who Is Mr. Putin?" In *Mr. Putin: Operative in the Kremlin,* 1–15. Washington, D. C.: Brookings Institution Press, 2013. http://www.jstor.org/stable/10.7864/j.ctt4cg8c5.4.

Leader, Anne. "A Not-so-Festive Case of Fratricide: Caracalla Murdered His Brother on 19 December 221 CE." Italian Art Society. Accessed August 24, 2023. https://www.italianartsociety.org

Marrow, Alexander, and Elizabeth Culliford. "Russia to Restrict Facebook Access for 'censoring' Its Media." *Reuters*, February 26, 2022. https://www.reuters.com/business/media-telecom/russia limit-facebook-access-response-media-censorship-2022-02-25/.

"Одежда Putin Team Представлена На Выставке 'Армия-2023.'" *Life.ru*, August 15, 2023. https://life.ru/p/1600718.

Rahyab, Susan. "Censorship and Book-Burning in Imperial Rome and Egypt." Dissertation, CUNY Academic Works, 2020.

Scarr, Francis (@francis_scarr). "State TV Reporter Pavel Zarubin Has Just Posted This Clip of Putin "Meeting the People of Derbent' after He Held a Meeting on Tourism There with Local Officials the Message His Trip to Dagestan Is Meant to Send out Is Clear: Things Are Back to Normal and the People Are with Me," Twitter, June 28, 2023, https://twitter.com/francis_scarr/status/1674154082771107842?s=20.

Shevchenko, Vitaly. "Russia's New Schoolbook Aims to Justify War on Ukraine." *BBC News,* August 9, 2023. https://www.bbc.com/news/world-europe-66450520.

Shultz, Daniel, and Christopher Jasparro. "(PDF) How Does Russia Exploit History and Cultural Heritage For ..." https://thinktank.theantiquitiescoalition.org/, April 2022. https://www.researchgate.net/publication/370631002_How_does_Russia_exploit_history_and_cultural_heritage_for_information_warfare_Recommendations_for_NATO_POLICY_BRIEF.

Simons, Greg. "Russian Media and Censorship: A Means or an End?" *Russian Journal of*

Communication 7, no. 3 (2015): 300–312. https://doi.org/10.1080/19409419.2015.1082438.

Sperling, Valerie. "Putin's Macho Personality Cult." *Communist and Post-Communist Studies 49*, no. 1 (2016): 13–23. https://doi.org/10.1016/j.postcomstud.2015.12.001.

Trentinella, Rosemarie. "Roman Portrait Sculpture: The Stylistic Cycle." The Met's Heilbrunn Timeline of Art History, January 1, 1AD. https://www.metmuseum.org/toah/hd/ropo2/hd_ropo2.htm. "Vladimir Putin's Historical Disinformation." U.S. Department of State, May 6, 2022. https://www.state.gov/disarming-disinformation/vladimir-putins-historical-disinformation

The Case Of Santiago De Chile: Pedestrian Deaths, Neo-Liberal Urbanism, And Insufficient Traffic Policy Reform

Olivia Maurer

ABSTRACT

Chile's rate of road fatalities and pedestrian deaths has remained a global outlier, even as comparable states have reduced occurrences. Santiago, Chile's capital and one of the most urbanized cities in Latin America, serves as a unique product of competing urban design ideologies put forth by democratic and authoritarian governments throughout the 20th century; and the social and economic stratification created has continued to present challenges for solving urban planning issues in modern Santiago. Recent adjustments in traffic laws have begun a reduction in road fatalities, but they still do not account for the discrepancy between Chile and other states. This is due to the failure to address the underlying problem of urban design solely shaped to create profit, which has ignored lower-income sectors of the population who rely heavily on walkability in urban areas.

Introduction: Pedestrian Deaths in Perspective

Out of all the members of the Organization for Economic Co-operation and Development (OECD), Chile has led in most road fatalities since 1998 per 10,000 vehicles, with more than five fatalities per registered 10,000 vehicles, compared to the OECD average of less than one. Furthermore, in recent years, as safety issues have become more widely addressed, other states have made significant progress in reducing their rates. Between 2001 and 2013, Germany lessened road fatalities by 50% and Spain by 70%, as Chile reduced by 10% (OECD, 2021). The long-term trend for road deaths points to stagnation in Chile. Between 2000 and 2019, the number of annual road fatalities fluctuated at around 2,000 deaths per year. In 2019, Chile registered 1,973 road deaths – a 0.9% increase from 2018. Even besides fatalities per vehicle, Chile experienced 10.5 traffic deaths per 100,000 inhabitants. The average in the European Union in 2019 was 5.1 deaths per 100,000 inhabitants (International Transport Forum, 2019). These staggering statistics show Chile to be an outlier in traffic fatalities and lead us to question the causes and policies that have created this phenomenon and how it plays out in the daily lives of Chileans.

Traffic accidents will become the fifth main cause of death by the year 2030, according to the World Health Organization (WHO) (2015). However, traffic crashes are accepted for the most part to be caused by known and preventable causes, such as speeding, distracted driving or driving under the influence, non-use of seat belts, and lack of respect for vulnerable road users such as cyclists and pedestrians (UNECE, 2009). If the correlation between these factors and traffic accidents and fatalities is agreed upon, then why has the problem stagnated in places with high levels of traffic fatalities such as Chile?

As is in many situations in both life and policy, there is a gap between knowing and doing, especially when these traffic fatalities disproportionately impact lower-class citizens who have less access to and influence in the policy-making process. Some of the most socially vulnerable individuals in traffic accidents are pedestrians, who account for a third of Chilean road deaths (International Transport Forum, 2019), as well as one-quarter of global road traffic deaths (WHO, 2013a). Though pedestrians with physical disabilities are not a high portion of the pedestrian population, they are at a particularly high risk of injury and death in traffic collisions. The pedestrian population remains vulnerable both to factors in the built environment as well as the demographic characteristics of pedestrians at-risk (Stoker et al., 2015). Some of the only research on this topic in this area has been conducted to prevent pedestrian specific traffic fatalities around bus stops and schools in Santiago, but that is because child deaths are sympathetic to the public, though the 0-14 age group is the least at risk of traffic fatalities (International Transport Forum, 2019). Outside of involving children, pedestrian deaths are primarily seen as the result of carelessness on the part of pedestrians instead of drivers. Children are absolved of the responsibility to obey street signs and only cross the street on sidewalks, but for all others, they must be obeyed without question.

In an ideal world, this mentality would be understandable, but when cities are designed to prioritize the efficiency of car travel over the needs of the pedestrian and land usage regulations allow for massive highways near businesses that are frequented by pedestrians, a disregard for street guidelines will occur. Since the early 2000s, the National Road Safety Commission *(Comisión Nacional de Seguridad de Tránsito, or CONASET)* has both proposed and implemented many policies to lessen traffic fatalities by regulating the behavior of cars, such as harsher restrictions on inappropriate speed and alcohol use, but they have not concretely addressed the underlying issues that cause pedestrian deaths in the first place. The built environment should be designed to protect and provide security for pedestrians, but that is not always the expressed intention or focus. Pedestrians face risk from a variety of factors, including urban development patterns and land usage, difficulties related to pedestrian walkability, such as poor lighting and a lack of

crosswalks, and risky car behavior. Incident-inducing characteristics of the built environment are not solely dependent on the actions of individuals, both pedestrians and vehicles, but the infrastructure can inform those actions, visibility, traffic volume, and speed. This work examines how policy designs have influenced the frequency of pedestrian deaths in Chile's capital city, Santiago, one of the most urbanized cities in Latin America. My initial research question asks why Chile has such a high level of pedestrian deaths and whether pedestrian deaths are due to poor policy decisions.

Literature Review

Urban Development Priorities: Frei Administration to Pinochet Military Junta

Santiago has undergone various eras of urban development with contradictory goals being pushed by different government administrations, creating a unique history of urbanization that provides important context to understanding its modern issues. Though the city has grown since 1541 after being founded by a group of Spanish conquistadors, the story of modern Santiago begins with the dictatorship of Augusto Pinochet in 1974, whose economic reforms led by neoliberalism were a sharp departure from the state owned industries and centrally-planned economic programs previously championed by Salvador Allende's Marxist government.

Influenced by the "Chicago Boys," a group of primarily United States-educated economists who advocated for libertarian economic policies such as privatization and widespread deregulation, urban design practitioners began to focus specifically on crafting policies that prioritized profit above all social considerations to fit within the neoliberal framework. Professor Francisco Vergara Perucich from Universidad de Las Américas states that this is a phenomenon within metropolises of the Global South, "an area where neoliberalism has reshaped cities for the sake of increasing capital, thereby fostering a sense that the development of a city is a financial investment rather than a social project" (Perucich, 2019, p. 3). This intense focus on profit margin has reduced urban development in Chile to define economic gross as the main goal of human activities and disregards the role of urban design to "involve cohesive socio-cultural values that shaped cities and maintain equilibrium based on human values" (Golany, 1995), and serve as "the discipline through which social aspirations can be realized physically" (Canniffe, 2006, p. 1).

The institution of a military dictatorship makes it obvious that an open and free state would not be entirely aligned with the administration's goals, but acknowledging the intense and expressed focus of urban design solely for economic benefit is important to note when understanding the history of urban design in Chile in the 1970s, exposing the true character of what the regime held to be the conception of socioeconomic development. This shift in the institutional framework led to a change from *Diseño Urbano Social* (Social-Oriented Urban Design) to *Diseño Urbano de Mercado* (Market-Oriented Urban Design). While practitioners under *Diseño Urbano Social* produced urbanism aiming to contest capitalist ideals and advance social justice, under the framework constraints of Diseño Urbano de Mercado, attention was focused on supply and demand and the needs of real estate investors (Perucich, 2019). In the administration of Jorge Alessandri (1958-1964), the policies in place were within the framework of the market economy but held a certain degree of protection for the country's business sector. They also modeled tax exemptions laws for builders off policies in the United States, creating the *Sistema Nacional de Ahorro y Préstamos* (National Savings and Loan System [SINAP]). These policies put pressure on the state from the public to assist low-income sectors, as they favored the middle and upper-income levels. Development continued but was still seen as insufficient with the overhanging accumulated deficit. The policies, however, did further the creation of *callampas*, the illegal construction of dwellings, and the occupation of periphery urban lands, which planted the seed for spatial segregation (Perucich, 2019). The Frei administration created the

Ministerio de Vivienda y Urbanismo (Ministry of Housing and Urban Affairs), whose objective was to curtail the housing shortage by encouraging the production of housing with a policy of popular participation and income redistribution that would also stimulate the internal market. Frei's programs showed ambition and a desire to reform the liberalism shown in the previous administration with which the business class had grown dissatisfied. While they were fairly successful in construction, the response was not the enthusiastic social mobilization to support the administration's policies that they had hoped for, and the allocation of resources had not appropriately considered inflation (Boano & Vergara-Perucich, 2017).

Allende's socialist faction gained popularity as people began to search for more extreme solutions, as the shantytown *callampas* and *conventillos* (deteriorated rental housing or tenements in the central areas of Santiago) grew by 300,000 inhabitants. Allende's administration was the best equipped of the administrations so far to deal with the housing crisis, and the ideological changes that came with his administration shifted institutional priority to that of *Diseño Urbano Social* (Social-Oriented Urban Design). However, at a certain point, the intense growing need for housing that led some to critique the administration was absorbed with the critiques of those who had political opposition to the administration due to its Marxist and socialist identity. The obfuscation of the need for resources into a mechanism to achieve political upheaval was a factor leading to the September 1973 military coup of the Allende government. Though there was significant government-led housing development, an average of 40,000 houses per year under Frei and 52,000 per year under Allende, this period revealed the capability of overcoming inherent obstacles of the urban environment, but it was jettisoned by the incoming military junta for its incompatibility with the prevailing ideology (Perucich, 2019).

The urban policies instituted under Pinochet's regime exemplified "an obsessive faith in the liberating forces of the marketplace as the motor for national growth" (Kusnetzoff, 1987, pg. 8). *Política Nacional de Desarrollo Urbano*, published in 1979, stated that urban planning development "will be aimed at making the process of urban development compatible with the global model of the country's development, creating the conditions most convenient for facilitating the operation of the urban land market" (MINVU, 1979). This negatively impacted access to housing and urban services, especially for the poorest of the poor, and made clear that the administration's chief priority was profit, not people. In 1985, the government developed a new national policy to deal with the effects of the acceleration of the informal settlements that increased due to the 1979 policy, reaching close to a million households by 1989. The 1985 modified policy was organized as a public-private agreement between state and free market agents– the *política adjusada* (adjusted policy) designed a necessity of creating planning instruments at a municipal level coordinated by local authorities. While this was seen as an improvement, the policy retained the guiding principle of urban development led by free-market rules and a set of permissive ad hoc regulations that were designed to extricate as much profit as possible from urban development projects. While this began a public-private partnership with the Chilean government, leading to urban growth seen as exemplary by those who value profitability, the uneven development of the city has created decades-long problems for modern practitioners to solve (Boano & Vergara-Perucich, 2017). The focus on market-based strategies above all planning priorities during the Pinochet dictatorship has been described by modern scholars as an "ideological black box" that worked to idealize the free market and install profit as the primary goal for not just infrastructure, but also education, health, and a variety of public sectors. This neoliberal model through policy strongly encouraged Chileans to become more consumption-oriented and to foster the idea of the entrepreneurial spirit as a way to encourage a shift away from more traditional and collective values.

Social Segmentation and Sprawl: Historical to Modern

Policies put in place in the Pinochet dictatorship encouraged a great deal of real-estate speculation that led to the middle and upper-income sectors gaining privileged locations within metropolitan Santiago, especially in the *communes* of the Oriente area, leading housing construction to reach an estimated 58% of the total urban land supply being inhabited by only 12% of the population of Santiago (Sabatini, 2000). Only a few years later, the *Ministerio de Vivienda y Urbanismo* loosened control over urban land by abolishing the urban limits of Santiago. This act, established by Decree No. 420, added 64,000 hectares to the existing 36,000 hectares which tripled the potential land market (Hechos Urbanos). This also signaled a shift in attitude that urban land is not scarce but can be expanded as much as the market desires. While this was designed to increase the supply of land to broaden accessibility to marginalized populations because of high prices, it actually drastically increased prices of this previously fringe land as well as prices for the urban interior. Arnold Harberger, an economic advisor to Pinochet, had believed that the limitation of the urban area by artificial regulatory instruments was the cause of the unbalanced differences in land values between urban land and surrounding rural land (Sabatini, 2000). The drastic expansion of urban boundaries encouraged the development of residential segregation. This sprawl of urban areas and spatial fragmentation due to social and economic stratification has persisted in modern Santiago and presents challenges for practitioners today to work to impact change and solve issues within certain sectors that reflect onto the whole city.

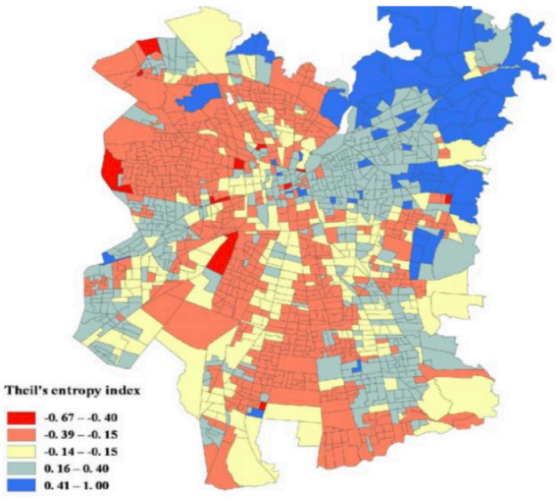
Urban Policy Today

In 1990 Chile began the transition back to a democracy, and in that period, Santiago underwent a series of transformations, including the changing lifestyles of inhabitants and the emergence of new social systems, but also a reckoning with what would be the extent of the dominance of market-oriented policies in deciding the future of urban areas and the level of flexibility available in the urban development process. The speed and shift to try to move away from repressive Pinochet policies led Santiago to become even more fragmented and uneven. Like in the transition between previous administrations, the primary strategy in Chile for responding and adjusting to changes was through urban development projects, which in this context can be interpreted as specific urban interventions that aim to resolve or relieve gaps or urban issues by working to provide a better public good (Perucich, 2019). The idea that the city's design has led to uneven development and the creation of housing without ensuring access to public goods per area has been recognized, and attempts at adjustment have been made by politicians in the 2000s. In 2012, President Sebastián Piñera gathered a board of specialists to redesign the National Policy for Urban Development, which had a greater amount of input from a variety of actors than the previous two revisions. Specifically focused on spatial segregation, this policy attempted to lessen the blatant division of economic growth in the city, which is the most segregated among OECD countries (OECD, 2013). The spatial segregation can be observed in Chart 1, from the article "Space and Social Capital: Social Contacts in a Segregated City" by Otero et al. (2022).

While spatial segregation describes the makeup of the people living in different areas, segmentation is a business term that refers to a strategy for allocating resources. Therefore, segmentation is a conscious action that divides a community into markets depending on their demands, interests, and especially their purchasing capacity. This process is inherent to the idea of a city design governed by the principles of neoliberalism, creating space for the rich and the poor, which also leads to social segregation by class, but also frequently ethnicity, race, language, etc.

Urban sprawl is associated with the fatality of pedestrian collisions, as a 2003 study found a connection where each 1 percent decrease in sprawl resulted in a 1.49 percent de-

Chart I: Spatial segregation based on quantified socioeconomic status (SES) using an adjusted Theil's entropy index.



crease in traffic-related fatalities overall, as well as between 1.47 and 3.56 percent decrease specifically in pedestrian fatality rates (Ewing, Schweiber, & Zegeer, 2003). Urban sprawl can be defined by four characteristics: "1. a widely dispersed population and low-density development; 2. the rigid separation of residential, commercial, industrial, and office uses; 3. poorly-defined activity centers; and 4. a road network typified by large blocks and poor connectivity" (Ewing, Pendall, & Chen, 2003). A 2011 study found that denser street networks with a higher number of intersections (which is the opposite of urban sprawl) had fewer crashes across all levels of severity. Vehicle-pedestrian collisions that occur in areas near schools and neighborhoods with mixed land use also had a lower rate of injury and death when compared to collisions with other development styles. Another study found that the presence of strip malls and big-box retail stores along with the high mileage of arterial roadways (another factor common in urban sprawl) are determinants of traffic injury rates (Dumbaugh & Li, 2011).

Walkability: The Culture of Transportation

To the U.S. reader, the idea of walkability may produce a variety of images based on lived experiences and perceptions of the level of "accepted" walkability in U.S. cities, and the lack of walkability supported by infrastructure in suburban and rural areas. It is important to note the cultural differences between the United States and the culture of transportation in Latin American communities.

Definitions of walkability are wide-ranging; some refer to the physical infrastructure and urban characteristics that create walkable places or the social and health-related effects that come from walkable environments, but other times it is used as a proxy term for better design in urban planning to mean increased levels of accessibility, safety, feasibility, comfort, or pleasurability (Forsyth, 2015). The majority of studies on walkability come from Europe or North America, where cars are relied on most for transportation, while many countries in the Global South, like Chile, have high levels of walking, even as cars have become more prevalent. Some believe this gap is partially due to the limited access to cars for a majority of the population, as only 40% of households in Santiago have cars (Sagaris et al., 2017). Sagaris's (2017) analysis of Santiago's 34 urban *comunas* (municipal planning areas) shows that car ownership (as well as wealth) is concentrated in only four to six *comunas*, those that are located on the city's eastern edge rising into the Andean foothills. Despite industrial pressures, economic changes, and a push for cultural changes (stretching back to the Pinochet era), walking has remained the main transport mode. Urban policies since the 1980s up until very recently have reflected the global focus on "automobility," the idea that cars are a vehicle (pun intended) of freedom, economic development, and social progress, and communities should be designed with their needs prioritized, which has led to their domination (Beckmann, 2001; Sheller & Urry, 2000).

Following the Pinchot dictatorship, Chile's subsequent period of economic growth included the development of urban highways within the country but especially in Santiago, and these were planned by the government under neoliberal policies, but constructed by private companies under Build, Operate, and Transfer (BOT) concessions. These contracts guaranteed revenues of up to 75% of the initial investments, controversial when considering that urban highway concessions from these toll-highways were $215 million in 2017, and from 2004 to 2015

seven major urban highways were built totaling 200km. These have stretched into surrounding areas and communities, creating gated communities without walkable infrastructure as well as isolating large sections of the city, particularly low-income communities that cannot afford to use them (Figueroa, Greene, & Mora, 2018; Sagaris & Landon, 2017). This car-centric attitude can be seen plainly in the distribution of investments in the built environment of Santiago between 2010 and 2016, when 30% of spending for transport projects was subsidized for urban highways and 7.7% for improvements and repairs on existing roadways, with just 2.8% investment in that period toward walking (*Coalición por un Transporte Justo*, 2016; Sagaris & Tiznado-Aitken, 2020). Thirty-two percent of sidewalks in Chile's main cities are in poor condition, according to a 2014 report (*Cámara Chilena de la Construcción*, 2014). The General Ordinance of Urbanism and Construction (*Ordenanza General de Urbanismo y Construcciones*) defines the street as a "vehicular road of any type that communicates with other roads and that includes both roads and sidewalks," the language of which makes motorized vehicles the priority and purpose of all streets, ignoring their role as public spaces for pedestrians and cyclists as well (*Ministerio de Vivienda y Urbanismo,* 2010, Article 1.1.2).

As continuous economic growth has continued into the 2000s and 2010s, car ownership has become more accessible for low-income groups, but in the same period walking trips have only decreased slightly, and not at the same level as the rise in cars, showing a persistence in walkability as a mode of transportation instead of simply a lesser alternative when cars are not available. From 2001 to 2012, the number of vehicles per household in the lower-income group rose from 0.13 to 0.17 and 1.5 to 1.65 vehicles per household for higher-income groups, a 31% growth in the lower-income group and 17% in the higher-income group (Herrera & Razmilic, 2016). The overall number of walking trips in Santiago among lower and middle-income groups at this time only decreased slightly from 36.7% to 34.5% (Herrera & Razmilic, 2016).

This persistence shows walking to be more than a transport of convenience or due to low income, but is a social and cultural part of the city and urban life. Besides the cultural reasons and economic accessibility of walking as transit, many have come to recognize the benefits of walking as a method of sustainable development. Data on the purpose of walking trips suggests that the frequency may reflect the traditional organization of urban life with high density and mixed land use (outside of segmented high income suburbs), where grocery stores are located walkable distances from residential communities (Herrmann-Lunecke, Geraldine, Mora, & Sagaris, 2020). Another factor could be the usage of public space in Latin American cultures, through events like street fairs, street festivals, and street markets; historically the street in Chile has been called "the living room of the people." The layout of Chilean streets may also be a factor in encouraging walking, as they were developed as orthogonal grids (*damero español*), which favor pedestrian movement and allow for mixed-use urban development (Herrmann Lunecke, Geraldine, Mora, & Sagaris, 2020). These cultural elements strengthen the argument that walking as transportation is more than just economics.

Despite the positives of walkability as a public health, economic, and sustainability measure, the current status of walkability in cities still presents many challenges for pedestrians, and since pedestrians tend to be women, the elderly, and low-income individuals, these challenges are disproportionately affecting these already marginalized identities. Walking comprises more than half of daily trips in Santiago's poorest *comunas*. Women account for a high percentage of walkers (56% to 77%) (Sectra, 2012), and walking has been associated with care-tasks, such as shopping, dropping off or picking others up, and health visits, accounting for 47% of daily trips and 64% of weekend trips, more than work (38%), education (10%), and recreation (6%) (Sagaris & Tiznado-Aitken, 2020). Aside from the frequency of these trips on infrastructure not always catered to pedestrians, women are particularly vulnerable due to conditions that can leave them exposed to sexual harassment, crime, or violence that would be less prevalent in vehicles (Seedat, MacKenzie, & Mohad, 2006, p. 150). While the idea of the "average com-

muter" suggests a trip straight from home to work and back, women also more frequently have multiple stops within a single trip, stopping for groceries, picking up children from school, etc.

A Review of Transportation Policy Developments and Change in Santiago

The National Road Safety Commission (*Comisiòn Nacional de Seguridad de Tránsito* or CONASET) was created on December 27, 1993, as a presidential advisory committee through Supreme Decree 223. Using the "2011 Simplified Methodology for Estimating the Social Benefits of Reducing Accidents in Interurban Road Projects" from the Road and Urban Transport Programme of the Ministry of Transport and Telecommunications and the Ministry of Social Development, CONASET determined that the estimated cost of traffic crashes in 2019 was USD 5.4 billion, equivalent to approximately 2.2% of Chile's GDP. I will be focusing on major changes implemented by CONASET between 20013 and 2019, as fatalities fluctuate with no clear trend of lessening fatalities emerging.

In 2017, Chile updated its National Road Safety Policy which serves as the general strategic guide that had originally been written in 1993. Similar to the 2012 redesign of the National Policy for Urban Development, this policy was created through a participatory process that included involvement from a variety of stakeholders, experts on road safety, citizens' groups, public and private entities, and road traffic victims associations. This rework created an updated strategic framework that would lead to a concrete action plan focused on the five strategic pillars of the United Nations' Decade of Action for Road Safety, road safety management, safer vehicles, safer road users, post-crash response, and safer driving environments. Chile's National Accord for Road Safety in 2018 created priority action items which were then used to develop the framework of the National Road Safety Strategy 2021-2030. This strategy's expressed goal contained a new target to reduce road fatalities by 30% by the year 2030 in comparison to the average number of fatalities in the period 2011-19 (International Transport Forum, 2019).

Recent changes have been focused on policing driver behaviors, specifically inappropriate speed and alcohol usage. In 2018, the Chilean Congress approved a bill to reduce the urban speed limit from 60 to 50 km/h. This initiative had been a priority of the Ministry of Transport and Telecommunications for years. This modest change in speed is significant, as when struck by a vehicle traveling 64.4 km/h (40 mph) a pedestrian has an 85 percent chance of death, but fatality does drop to 45% at 48.2 km/h (30 mph) (UK Department of Transport, 1997). However, the single most consequential intervention in reducing pedestrian fatality rates and injuries is roadway treatments such as the installation of frequent stop signs and narrowing roadways (Stoker et al., 2015). The Ministry of Transport and Telecommunications has begun to aim for Congress to allow automated speed management, but it is still in very early stages (International Transport Forum, 2019).

Drunk driving has been the topic of various pieces of legislation in the 2010s. In 2012, a new law was introduced to show a zero-tolerance policy. This set the maximum permissible blood alcohol content (BAC) at 0.3 g/l. The law defines driving under the influence of alcohol as driving with a BAC between 0.8 g/l and 0.3 g/l, while much tougher sanctions are implemented for driving while intoxicated, which is defined as driving with a BAC over 0.8 g/l. Sanctions include license suspension or annulment. Fatalities due to drunk driving declined almost 30% after the introduction of this law, from 267 in 2011 to 192 in 2012. Since the number of alcohol-related fatalities has remained around 10% of total fatalities, in 2019 9.6% of total road deaths (190) were related to alcohol. Beyond just laws related to drunk driving, a particularly high-profile law that more severely punishes drunk drivers responsible for serious injuries or fatal crashes was passed in 2014 called Emilia's Law. Exacerbating the sanctions of the 2012 zero tolerance law, a driver is subjected to at least one year of imprisonment and potentially

disqualified to drive for life. It also criminalized fleeing the scene or refusing an alcohol test.

The Chilean government has begun extremely recently showing an acknowledgment of pedestrian safety and pedestrian fatalities, but these policies have not been established and implemented long enough to determine a change, especially with the drastic change in transportation rates since the onset of the COVID-19 virus in early 2020. In 2020, The National Day of Remembrance for Road Traffic Victims was established as the third Sunday of November. CONASET and the Ministry for Transport and Telecommunications also in 2020 published a guide as a way of technical support for implementing tactical bike lanes, sidewalk extensions for walking and queuing, public transportation sanitization, and physical distancing for passengers, as a response to COVID-19, but it actually contained many of progressive planning measures that were being advocated for before the pandemic. Decree no. 71/2019, published in 2020, modified the standard road-traffic sign manual to incorporate the new design of information signs related to road traffic co-existence. This was the result of the collaboration of the advocacy working groups that came from the road coexistence law, including participation from the Ministry of Public Works, Ministry of Transport, and CONASET.

CONASET Data Analysis

I intend my research to be a successor to an article published in late 2019 by Martínez and Contreras entitled "The Effects of Chile's 2005 Traffic Law Reform and In-Country Socioeconomic Differences on Road Traffic Deaths among Children Aged 0-14 Years," which looked at those effects up until 2013. The 2005 reform introduced child restraint systems (CRS) for children under four years old, mandated seat belt use for all vehicle occupants, prohibited the use of cellphones while driving, and increased penalties for drunk driving, among others. The conclusion of the report saw a decrease in road traffic crashes during the 2002–2013 period and a significant reduction in the trends of road traffic collisions for children, but more significantly for child passengers rather than child pedestrians. During the 2002–2013 period, 34,492 road traffic collisions (RTCs) involved passengers aged 0–14 years, causing 510 deaths among that age group, while there were 21,675 road traffic collisions (RTCs) that involved pedestrians aged 0–14 years, resulting in the death of 535 people from this age group. This article also found that road traffic deaths (RTDs) of pedestrians and passengers in this age group fell during the 2002-2013 period, from 2.48 and 1.71 deaths for every 100,000 vehicles in 2002 to 0.63 and 0.89 for every 100,000 vehicles in 2013, respectively. However, as discussed in my introduction, children are not the only vulnerable population group of pedestrians; all pedestrians are vulnerable, which is why my analysis looks at changes in the overall traffic fatality rate and the pedestrian fatality rate. My analysis follows the period examined (2002-2013) to analyze the changes after the major updates of the 2010s: the reduction in speed legislation from 2018, the 2012 and 2014 (Emilia's Law) reforms related to alcohol use, and the overall updates in the National Road Safety Policy from 2017. I utilize CONASET data to determine if there is a significant change in the overall number of traffic fatalities. Though it may be too recent to examine these impacts fully, the changes from the 2005 period of reforms have stagnated which needs to be addressed. It may be too short of a time period to adequately assess the impact of these laws, but the global rate of traffic fatalities and pedestrian deaths has fallen dramatically in 2020 and 2021 due to changes in transportation rates due to COVID-19, which has made the 2020 and 2021 data outliers and not reliable enough to be considered an extension of a trend. In 2020, in Santiago the number of vehicles in traffic decreased by 65% due to changes in lifestyle and public accessibility during the pandemic (Road Safety Annual Report Chile, 2019).

In 2013, the number of traffic fatalities was 1,632. In 2019, the number of traffic fatalities was 1,617. By calculating (% increase = 100 x (final - initial)/initial), this change is only a .9%

decrease in the rate of traffic fatalities, which is not a significant change.[1] In the brief aftermath of the 2005 traffic reforms, the number of traffic fatalities fell from 1,626 to 1,652 in 2006, to 1,645 in 2007, and an almost record high of 1,782 (not reached since 1998), showing the trend to reduction as irregular then as well. The lack of significant change in Chile's overall rate of traffic fatalities between 2013 and 2019 suggests that the traffic reform policies of the 2010s have not made a meaningful impact on the rate of traffic fatalities.

However, regardless of the weak decrease in traffic fatalities, the rate of change in pedestrian fatalities between 2013-2019 was more meaningful. The rate of change in pedestrian deaths from 2013 to 2019 was 18.3%, a major difference, surprising when looking at the fairly stagnant rate of change in road fatalities. In this analysis, I have discovered that Chile's high rate of pedestrian deaths has been somewhat exaggerated due to the high rate of traffic fatalities, and though Chile still has the highest rate of pedestrian deaths among OECD countries at 3.551 per hundred thousand population, it has experienced a noteworthy reduction percentagewise. I had not foreseen this, as the high overall rates disguised the change over time in this exact period. However, with the lack of accessible multivariate data that includes the location of collisions as well as the type of road user, we are unable to ascribe whether these pedestrian deaths were due to changes in infrastructure at certain locations, which has been attributed to be a major component of lessening pedestrian deaths in other studies (Rothman et al., 2019; Schmitt, 2020).

As discussed in many sources, a more thorough evaluation and understanding of the impact of these policies will come with time, but the breadth of policy regulations shows an exciting amount of interest in road safety issues. Though there has been a decrease in pedestrian deaths, 665 in 2019 is still exorbitantly high in my opinion, especially when as we have discussed heavily, so many are preventable. Pedestrians continue to be the group the most affected by road crashes, in 2019 accounting for the largest share of road deaths, with 34% of the total, and occupants of passenger cars lagging behind at 29% (Road Safety Annual Report Chile, 2019). This problem is affecting the whole population; the 2019 article also suggested that regional socioeconomic differences are associated with higher road traffic death rates in the 0-14 age group, as well as that deaths due to road traffic collisions among children are not distributed randomly among the population. There is a clear link between these deaths and other factors of disadvantaged individuals, such as socioeconomic level, class, neighborhood, gender, and race. While these laws passed in the 2010s show the beginnings of an awareness of a problem needing to be fixed, are they getting to the root of the problem? When examining these policies, the decrease in pedestrian deaths seems to be more incidental following a less-

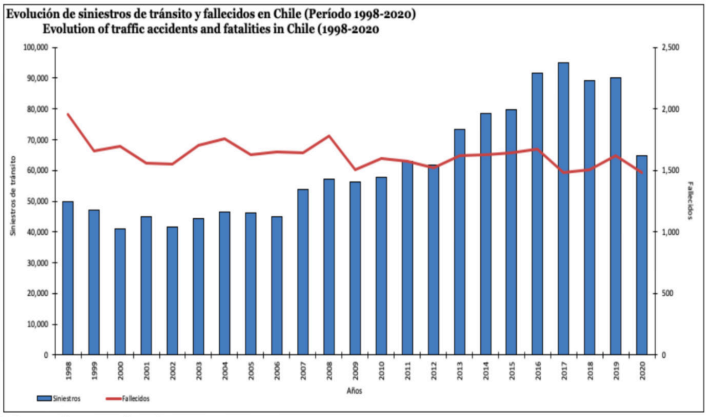


Chart II: Dataset: by age and road user

1 From 2013 to 2019 Santiago experienced a fairly consistent growth rate of between 0.66% and 0.80% each year (OECD 2021).

ening of the overall rate of road traffic fatalities, not the object of the policy reforms itself. The CONASET website includes geospatial information, using a thematic map viewer of geocoded layers of traffic accidents, which I originally thought would be helpful in understanding the prime locations of consistent pedestrian fatalities, but when we examine it further, it only has maps and filters specific to accidents involving bicycles, motorcycles, hit and runs, and critical points of traffic accidents. The data on pedestrian deaths should be just as available, but time and attention have not been given to fully understand the connections between these incidents as they are still largely seen as senseless tragedies, not the conclusion to a series of policies and apathy towards those within the pedestrian class. As mentioned before, the National Road Safety Strategy 2021-30 set a goal to decrease the number of annual road fatalities by the year 2023 to 30% of the average for 2011-2019. The average was 1,586, meaning a 30% decrease would be 1,110 deaths. And while the 2020 and 2021 statistics are lower (mostly due to COVID), I am doubtful that with the current policies in place the rate will maintain a lessening rate over time consistently enough to achieve that goal by 2030.

Aside from Chile's unique political history regarding extremely different political ideologies back to back, the implementation of active modes of transportation within Chile is different than many other nations. While more active modes of transportation were initially promoted by academics within the medical sphere and then adopted into national policy in the United States, the United Kingdom, or Australia, Chile has widely ignored the role of the physical environment when building health policy or seeing the physical environment as a potential factor in individuals' health (Ibarra & Mora, 2011; Mora, Greene & Corado, 2018). The current National Health Objectives de Chile 2012-2020 only contains two paragraphs about the role of cities and how urban planning can make people more active in a document of over 350 pages, while 87.8% of Chileans live in cities (INE, 2018).

When looking at this difference in top-down approaches, it is important to note that the Chilean institutional structure divides the national territory into regions and municipalities. The Metropolitan Area of Santiago de Chile (MAS) covers 34 municipalities, each managed at the local level of administration. There is no administrative level that functions over the entire MAS, and the 34 municipalities have 34 individual governing mayors but do not have a uniform institution or planning authority (Banzhaf et al., 2013). Therefore, developments in modernizing urban planning as well as walking persisting as a form of transit have come not from a top-down, ministry-driven initiative, but because of more people-driven behavior, bottom-up through cultural practices. Some coordination instances between the municipalities have occurred but are voluntary and depend on the political willingness of the participants, and they are not adequate to develop long-term policies. Many local initiatives that have promoted pedestrian interests have been pioneered by *comunas*, and though the 2014 Chilean National Urban Development Policy named walking a priority to meet sustainable development goals, the majority of initiatives have come from communities and citizens; and the development policy is not really in the mandate for any particular government agency, whether that be local, regional, or national. A recent example of a locally pioneered planning policy is Santiago's city center mobility plan (*Plan Centro*), which is a comprehensive policy to improve infrastructure in Santiago's original town center. It seeks to promote non-motorized mobility, by improving the walkability of a heavily used, central part of the city, where government, major retail, heritage, and other economic and cultural interests have a stake in development (de Santiago, 2015). This expanding pedestrianization covers 3.9 km of streets, complemented by a network of more than seventy pedestrian "galleries," interior walkways within usually commercial buildings. This interconnected pedestrian network has contributed significantly to making Santiago's historic center a very diverse and socially mixed quarter within a segregated city (Herrmann & Mora, 2018).

The *Plan Centro* was the first in Chile to give pedestrians and cyclists explicit priority

over private cars. On-street parking was eliminated, and car lanes were replaced by significant improvements to sidewalks, bus stops, and cycleways. Sixty percent of eight major roads were allocated for public transportation and strictly prohibited for cars during the weekday. Though there was some initial resistance, the project, which began in 2015 on Merced Street, has expanded to San Antonio Street and Santo Domingo Street and has received numerous prestigious awards, including the Sustainable Transport Award in 2017, which has gone to New York, Paris, and London among others. Though these policies are extremely successful, they are occurring in small sectors of a massive city, and the lack of an overarching administration makes the development of these projects difficult.

One change in the built landscape that could be put in place across the board would be investments in enhancing infrastructure for walkers, as currently only 2.78% of public investment in roads is directed to improving walking (Iglesias, Giraldez, Tiznado-Aitken, & Muñoz, 2019). To implement this, coordination among different sectoral planning departments (transport, urban planning, etc.), and between the national, regional, and local governments is urgently needed. Current planning regulations consistently promote and ensure the mobility of motorized traffic at all scales, so a revision of the Chilean national standards for streets and sidewalks would also be massively beneficial, as sidewalks are currently undersized by law. These regulations set rigid standards for the minimum and maximum width of vehicular lanes and sidewalks, which have made roads wide and sidewalks narrow (Herrmann, 2016). For example, two-lane roads must be 7 m wide, regardless of the speed limit, or if it is a local or trunk road (Ministerio de Vivienda y Urbanismo, 2010). In contrast, the minimum width for sidewalks is just 1.2 m for residential areas and 2 m for commercial areas. Finally, new policies at the regional level are needed to promote walking developed through close collaborations between municipal government staff and community organizations. Single *comunas* are unable to allocate the appropriate and necessary resources to undertake expansive projects, especially in deprived neighborhoods where walkable environments and public spaces have been neglected for a long period of time. Reimagining our idea of transportation infrastructure and seeing city streets as multifunctional public spaces that should be equally available to cyclists and pedestrians for social, civic, and cultural activities would have a major effect policy-wise. Redefining the street as a shared, multifunctional, and democratic space for all transport modes would create policies that give all citizens regardless of their mode of transportation a greater sense of dignity and ownership of their community.

Conclusion: The Implications of the Santiago Case

The case of Santiago and its consequences is not only relevant to Chile but is part of a larger movement to resolve the issues of how and for whom our cities globally are designed. Though Chile remains an outlier within the OECD, it ranks above numerous countries in Africa, Asia, and South America in traffic fatalities (Global Health Repository - WHO, 2021). Approximately 96% of children killed worldwide due to road traffic injuries are in low and middle-income countries and nearly all countries with the highest absolute numbers and rates of pedestrian fatalities are developing countries (Peden et al., 2004; Toroyan, 2009). Chile is the most developed nation in South America when considering its GDP, quality of life, infant mortality rate, life expectancy, and HDI in comparison to neighboring states, but it only joined the OECD in 2010. I believe that these policy alternatives are useful not only for Chile but just as much for other nations struggling with high rates of pedestrian deaths and traffic fatalities.

Latin American cities are much more similar architecturally to European cities than American ones and have a higher population density, making it possible that the Vision Zero policies may be more effective in preventing pedestrian deaths there. Chile has undergone such a significant transformation in the past three decades especially, in not only urbanization

but GDP and population growth as well. However, the state is still lower than many other OECD member countries in a number of urban-related factors related to the quality of life, such as the environment, housing, jobs, and income. Traditionally, Chile's metropolitan and urban development has been driven by sector, especially due to the structure of local and regional governments, but there is a strong need for an integrated approach to urbanism. One policy in the Netherlands is known as *woonerf*, which treats the street as a "living yard" by promoting sharing the street between all forms of transportation, but giving priority to pedestrians and cyclists over motorists. This policy is not completely out of the realm of possibility as one might think, since Sunday open street initiatives have begun gaining popularity in Latin American communities. Colombia's "*Ciclovía*" program, which closes more than 70 miles of roads for vehicular traffic in Bogotá, has been occurring since 1976, where nearly two million people participate in walking, cycling, dancing, and socializing in city streets. Santiago began a smaller version of the program called *CicloRecreoVía* which is practiced in 33 countries, involving more than 1.5 million people every week (Torres, Sarmiento, Stauber, & Zarama, 2013). The city has also provided opportunities to reduce the social segregation of the city by offering public space for recreation and community engagement (Mora, Greene, & Corado, 2018).

Other locally-grown programs like "I Love my Neighborhood" (Quiero mi Barrio), which invests in local plazas, parks, and sidewalk improvements to increase social capital in low-income neighborhoods in Santiago, and the *Programa de Barrios Comerciales*, which worked to improve high streets and made them more appealing and safe for female pedestrians, are beneficial tools for changing a community's culture surrounding walkability (Figueroa & Waintraub, 2015).

While pedestrian deaths and the other negative consequences of cities planned with a cars-firstmentality have a universal impact, they also significantly impact developing nations. The World Health Organization reports that people in developing nations account for just 1% of the world's cars, but 13% of the world's 1.3 million annual fatalities. The stratification of transportation modes by class in developing countries has prevented poorer nations from gaining stability and wealth in many instances. The World Bank estimated in 2017 that Tanzania could increase its GDP by 32% if it were able to reduce traffic injuries by 50% over a 24-year period (Schmitt, 2020). The growth of car ownership that occurs as some countries gain economic development is also cause for concern, however. Mexico has experienced a 4.2% increase in car ownership per year. Only about 15% of the trips made in Mexico are made in cars, but the cities' built infrastructure is geared towards accommodating the privileged minority that is able to affordthe social status symbol that cars represent in many developing countries (Schmitt, 2020). The problem of pedestrian deaths in Santiago, and globally, will not be solved without a recognition that there is a problem. Beginning to look at traffic fatalities and pedestrian deaths not just as random injustices but as the consequences of policy failures is so crucial to advocating for policy changes, whether they are structural improvements like narrowing streets, including more crosswalks, or more comprehensive policy plans like expanding public transportation and mixed land-use zoning.

Understanding a nation's history regarding urbanization and the varying political ideologies that formed a city's built infrastructure gives crucial context to why unequitable policies have persisted. Santiago is just one example of a city that has been designed for a minority, but by challenging the current state of affairs there is the potential to lessen the concerning rate of traffic fatalities and pedestrian deaths, not only in Chile but in other Latin American cities and those around the world.

References

Banzhaf, E., Reyes-Paecke, S., Müller, A., and Kindler, A. (2013). Do demographic and land-use changes contrast urban and suburban dynamics? A sophisticated reflection on Santiago De Chile. *Habitat International, 39*, 179-191. https://doi.org/10.1016/j.habitatint.2012.11.005

Beckmann, J. (2001). Automobility—A social problem and a theoretical concept. *Environment and Planning D: Society and Space, 19,* 593–607.

Blazquez, C. A., and Celis, M. S. (2013). A spatial and temporal analysis of child pedestrian crashes in Santiago, Chile. *Accident Analysis and Prevention, 50,* 304-311. https://doi.org/10.1016/j.aap.2012.05.001

Boano, C., and Vergara-Perucich, F. (2017). *Neoliberalism and urban development in Latin America: Thecase of Santiago. R*outledge.

Canniffe, E. 2006. *Urban Ethic: Design in the contemporary city.* Routledge.

Cámara Chilena de la Construcción. (2014). Infrastructura crítica para el Desarrollo: Bases para un Chile integrado: Estimaciones 2014-2018 Y 2014-2023. Retrieved from https://www.cchc.cl/uploads/archivos/archivos/Infraestructura-Critica-para-el-Desarrollo_2014- 2018.pdf

Coalición por un Transporte Justo. (2016). Gasto fiscal en cada modo de transporte. Retrieved from https://coaliciontransportejusto.wikidot.com/printer–friendly//wiki:gasto-fiscal-modos

Dumbaugh, E. and Li, W. (2011). Designing for the safety of pedestrians, cyclists, and motorists in the built environment. *Journal of the American Planning Association, 77*(1), 69–88. https://doi-org.umiss.idm.oclc.org/10.1080/01944363.2011.536101

Ewing, R., and Hamidi, S. (2015). Urban sprawl as a risk factor in motor vehicle occupant and pedestrian fatalities: Update and refinement. T*ransportation Research Record 2513*(1), 40-47. https://doi.org/10.3141/2513-05

Ewing, R., Pendall, R., & Chen, D. (2003). Measuring sprawl and its transportation impacts. *Transportation Research Record, 1831*(1), 175-183. https://doi.org/10.3141/1831-20

Ewing, R., Schweiber, R. A., and Zegeer, C. V.. (2003). Urban sprawl as a risk factor in motor vehicle occupant and pedestrian fatalities. *American Journal of Public Health, 93*(9), 1541–45.

Ewing, R., and Dumbaugh, E. (2009). The built environment and traffic safety: A review of empirical evidence. *Journal of Planning Literature, 23*(4), 347–67.

Fan, Y., and Song, Y. (2009). Is sprawl associated with a widening urban-suburban mortality gap? *Journal of Urban Health, 86*(5), 708-728.

Figueroa, C., Greene, M., and Mora, R. (2018). Impacto de las autopistas en la accesibilidad de los barrios, un análisis desde la sintaxis espacial. *Revista, 180*(42), 14–25. https://doi.org/10.32995/rev180.Num-42.(2018).art-578

Figueroa, C., and Waintraub, N. (2015). Movilidad femenina en Santiago de Chile: Reproducción

de inequidades en la metrópolis, el barrio y el espacio público. *Revista Brasileira de Gestão Urbana,7*(1), 48–61. https://doi.org/10.1590/2175-3369.007.001.AO03

Forsyth, A. (2015). What is a walkable place? The walkability debate in urban design. *Urban Design International, 20*(4), 274–292. https://doi.org/10.1057/udi.2015.22

Galetovic, A., and Jordan, P. (2006). Santiago ¿Dónde estamos y hacia dónde vamos?. *Estudios Publicos, 101.*

Glaeser, E. L., and Meyer, J. R. (2002). *Chile: Political economy of urban development.* Harvard University Press.

Gobierno de Chile. (2014). *Política nacional de desarrollo urbano.* Consejo Nacional de Desarrollo Urbano.

Golany, G. S. (1995). *Ethics and urban design: Culture, form, and environment.* John Wiley and Sons.

Greene, M., Rosas, J., and Valenzuela, L. (2011). *Santiago proyecto urbano.* Ediciones ARQ.

Gross, P. (1991). Santiago de Chile (1925-1990): Planificación urbana y modelos políticos. *Revista EURE-Revista de Estudios Urbano Regionales, 17*(52-53).

Hamidi, S., Ewing, R., Tatalovich, Z., Grace, J. B., and Berrigan, D. (2018). Associations between urban sprawl and life expectancy in the United States. *International Journal of Environmental Research and Public Health, 15*(5), 861.

Hauer, E. (1999). Safety in geometric design standards. Accessed January 11, 2022. https://www.researchgate.net/publication/228903642_Safety_in_geometric_design_standards

Herrmann-Lunecke, M. G., Mora, R., and Sagaris, L. (2020). Persistence of walking in Chile: Lessons for urban sustainability. *Transport Reviews, 40*(2), 135-159. https://doi.org/10.1080/01441647.2020.1712494

Herrmann-Lunecke, M. G. (2016). Instrumentos de planificación y diseño urbano para promover al peatón en las ciudades. Un estudio comparado entre Chile y Alemania. *Urbano, 34,* 48–57. https://doi.org/1022320/07183607.2016.19.34.5

Herrmann-Lunecke, M. G., and Mora, R. (2018). The layered city: Pedestrian networks in downtown Santiago and their impact on urban vitality. *Journal of Urban Design, 23*(3), 336–353. https://doi.org/10.1080/13574809.2017.1369869

Herzog, L. (2014). *Global suburbs: Urban sprawl from the Rio Grande to Rio de Janeiro.* Routledge.

Herrera, A., and Razmilic, S. (2016). Moverse en Santiago hoy: ¿Qué ha cambiado en los últimos años? Retrieved from https://www.cepchile.cl.

Iglesias, V., Giraldez, F., Tiznado-Aitken, I., and Muñoz, J. C. (2019). How uneven is the urban mobility playing field? Inequalities among socioeconomic groups in Santiago De Chile. *Transportation Research Record, 2673*(11), 59-70. https://doi.org/10.1177/0361198119849588.

Instituto Nacional de Estadísticas. (2018). Síntesis de resultados. Censo 2017. *INE*.

International Transport Forum. (2019). *Road safety annual report 2014.* OECD Publishing. Retrieved October 5, 2021, from https://www.itf-oecd.org/sites/default/files/docs/irtad-road-safety-annual-report-2019.pdf.

International Transport Forum. (2019). *Road safety annual report 2019* - United States. Retrieved October 5, 2021, from https://www.itf-oecd.org.

Jacobsen, P. L. (2003). Safety in numbers: More walkers and bicyclists, safer walking and bicycling. *Injury Prevention, 9*(3), 205–9.

Job, R. F. (1998). Pedestrians at traffic light controlled intersections: Crossing behaviour in the elderly and non-elderly. *In Proceedings of the Conference on Pedestrian Safety, 29-30 June*, edited by K. Smith, B. G. Aitken, and R. H. Grzebieta, 3–11. Australian College of Road Safety and Federal Office of Road Safety.

Kerr, J., Rosenberg, D., and Frank, L. (2012). The role of the built environment in healthy aging: Community design, physical activity, and health among older adults. J*ournal of Planning Literature 27*(1), 43–60.

Kusnetzoff, F. (1987). Urban and housing policies under Chile's military dictatorship: 1973-1985. *Latin American Perspectives, 14*(2), 157–86.

LaScala, E. A., Johnson, F. W., and Gruenewald, P. J. (2001). Neighborhood characteristics of alcohol-related pedestrian injury collisions: A geostatistical analysis. *Prevention Science, 2*(2), 123–34.

Langlois, J. A., Keyl, P. M., Guralnik, J. M., Foley, D. J., Marottoli, R. A., and Wallace, R. B. (1997). Characteristics of older pedestrians who have difficulty crossing the street. *American Journal of Public Health, 87(*3), 393–97.

Legg, S., and McFarlane, C. (2008). Ordinary urban spaces: Between postcolonialism and development. *Environment and Planning A, 40*(1), 6–14.

López Morales, E., Luco, C. A., Martínez, P. J., and Díaz, H. E. eds. (2014). C*hile Urbano hacia el siglo XXI. Investigaciones y reflexiones de política urbana desde la Universidad de Chile.* Editorial Universitaria.

Martínez, P., and Contreras, D. (2020). The effects of Chile's 2005 traffic law reform and in-country socioeconomic differences on road traffic deaths among children aged 0-14 years: A 12-year interrupted time series analysis. *Accident Analysis and Prevention, 136*, 105335-105335. https://doi.org/10.1016/j.aap.2019.105335

Martinez, S., Sanchez, R. and Yañez-Pagans, P. (2019). Road safety: Challenges and opportunities in Latin America and the Caribbean." *Latin American Economic Review, 28*(1), 17. https://doi.org/10.1186/s40503-019-0078-0

Marshall, W. E., and Garrick, N. W. (2011). Does street network design affect traffic safety? *Accident Analysis & Prevention, 43*(3), 769–81.

Mead, J., Zegeer, C., and Bushell, M. Evaluation of pedestrian-related roadway measures: A summary of available research. *P & BI Center. Chapel Hill, NC, UNC Highway safety Research Center, 115.*

Ministerio de Vivienda y Urbanismo (MINVU). (2010). *1979 Política nacional de desarrollo urbano* MINVU.

Ministerio de Vivienda y Urbanismo (MINVU). (2010). *Ordenanza general de urbanismo y construcciones.* MINVU.

Mora, R., Greene, M., and Corado, M. (2018). Implicancias en la actividad física y la salud del programa ciclo recreo vía en Chile. *Revista Médica de Chile, 146*(4), 451–459.

Munizaga, G. (2014). *Diseño urbano. Teoría y método.* Ediciones UC.

Muñoz, J. C., Batarce, M., and Hidalgo, D. (2014). Transantiago, five years after its launch. *Research in Transportation Economics, 48,* 184–193. https://doi.org/10.1016/j.retrec.2014.09.041

Mutangadura, G. B. (2002). *World health report 2002: Reducing risks, promoting healthy life: World Health Organization.* World Health Organization.

Narváez, Y. V., Sierra, V. P., Cárdenas, F. P., Ramos, L. R., González, B. Z., Vargas Martínez, J. I., and Aranda, O. M. (2019). Road risk behaviors: Pedestrian experiences. *Traffic Injury Prevention, 20*(3), 303-307.

OECD. (2013). The Chilean urban system and its challenges. In *OECD Urban Policy Reviews, Chile 2013.* OECD Publishing. https://doi.org/10.1787/9789264191808-4-en

OECD. (2016). *OECD factbook 2015-2016: Economic, environmental and social statistics*. OECD Publishing. https://doi.org/10.1787/factbook-2015-en

OECD. (2021). Road accidents: Transport. *OECDiLibrary*. https://doi.org/10.1787/8dacf707-en

OECD.Stat. (2021). Metropolitan areas. stats.oecd.org/Index.aspx?DataSetCode=CITIES

Perucich, F. V. (2019). *Urban design under neoliberalism: Theorising from Santiago, Chile.* Routledge.

Rivara, F. P., and Barber, M. (1985). Demographic analysis of childhood pedestrian injuries. *Pediatrics, 76*(3), 375–81.

Rodríguez, A., and Sugranyes, A. (2005). *Los con techo: Un desafío para la política de vivienda social.* Ediciones SUR.

Rosenbloom, S., and Plessis-Fraissard, M. (2009). Women's travel in developed and developing countries: Two versions of the same story? Paper presented at the 4th International Conference on Women's Issues in Transportation, Irvine, California. Published in *Transportation Research Board Conference Proceedings, 46,* 63–77.

Rothman, L., Howard, A. W., Camden, A., and Macarthur, C. (2012). *Pedestrian crossing location influences injury severity in urban areas. Injury Prevention, 18*(6), 365–370.

Sabatini, F. (2000). Reforma de los mercados de suelo en Santiago, Chile: Efectos sobre los

precios de la tierra y la segregación residencial. *Revista Latinoamericana de Estudios Urbano Regionales,23*(77), 49–80. https://doi.org/10.4067/S0250-71612000007700003

Sagaris, L., Tiznado-Aitken, I., and Steiniger., S. (2017). Exploring the social and spatial potential of an intermodal approach to transport planning. *International Journal of Sustainable Transportation, 11*(10), 721–736. https://doi.org/10.1080/15568318.2017.1312645

Sagaris, L., and Landon, P. (2017). Autopistas, ciudadanía y democratización: La costanera norte y el acceso sur, Santiago de Chile (1997–2007). *EURE (Santiago), 43*(128), 127–151. http://dx.doi.org/10.4067/S0250-71612017000100006

Sagaris, L., and Tiznado-Aitken, I. (2020). Walking and gender equity: Insights from Santiago, Chile. In *Urban Mobility and Equity in Latin America*, edited by D. O. Hernandez, N. Villamizar-Duarte, and A. A. Pinto. Emerald Books.

Schmitt, A. (2020). *Right of way: Race, class, and the silent epidemic of pedestrian deaths in America.* Island Press.

Schneider, R. J. (2018). "Complete streets" policies and eliminating pedestrian fatalities. *American Journal of Public Health (1971), 108*(4), 431-433.

Seedat, M., MacKenzie, S., and Mohad, D. (2006). The phenomenology of being a female pedestrian in an African and an Asian city: A qualitative investigation." *Transportation Research Part F: Traffic Psychology and Behaviour, 9(*2), 139–53.

Sheller, M., and Urry, J. (2000). The city and the car. *International Journal of Urban and Regional Research, 24*(4), 737-757.

Silva, C. (2020). The rural lands of urban sprawl: Institutional changes and suburban rurality in Santiago De Chile. *Asian Geographer, 37*(2), 117–144.

Stoker, P., Garfinkel-Castro, A., Khayesi, M., Odero, W., Mwangi, M. N., Peden, M., and Ewing, R. (2015). Pedestrian safety and the built environment: A review of the risk factors. *Journal of Planning Literature, 30*(4), 377–392.

Torres, A., Sarmiento, O. L., Stauber, C., and Zarama, R. (2013). The Ciclovia and Cicloruta Programs: Promising interventions to promote physical activity and social capital in Bogotá, Colombia. A*merican Journal of Public Health, 103*(2), 23–30. https://doi.org/10.2105/AJPH.2012.301142

United Kingdom Department of Transportation. (1997). *Killing speed and saving lives.* UK Department of Transport.

UNECE. (December 2009). Improving global road safety: Setting regional and national road traffic casualty reduction targets. United Nations Economic Commission for Europe. Retrieved October 5, 2021, from http://www.unece.org/fileadmin/DAM/trans/roadsafe/docs/Report_2009.pdf.

Watson, V. (2009). Seeing from the South: Refocusing urban planning on the globe's central

urban issues. *Urban Studies (Edinburgh, Scotland), 46*(11), 2259–2275.

Whitelegg, J. (1993). *Transport for a sustainable future: The case for Europe*. Belhaven.

World Health Organization. (2015). *Global status report on road safety.* World Health Organization.

World Health Organization. (2021). Road traffic deaths. Global Health Repository. https://apps.who.int/gho/data/view.main.51310?lang=en.

Zahabi, S. A. H., Strauss, J., Manaugh, K., and Miranda-Moreno, L. F. (2011). Estimated potential effect of speed limits, built environment, and other factors on severity of pedestrian and cyclist injuries in crashes. *Transportation Research Record, 2247, 81–90*. https://doi.org/10.3141/2247-10

WINNER

Outstanding Research Writing (Graduating Student)

Link's Sword is Mightier than the Pen: Composing and Performing in the *Super Mario* and *Legend of Zelda* Series

William Nelson

ABSTRACT

Player interaction has largely defined video games and their concomitant music. Since *Pong* (1972), players have exercised agency over both in-game events and, by extension, the sounds that accompany them. In this paper, I explore ways in which the "authorial agency" of the player is reflected musically in Nintendo's *Super Mario* (1985–) and *The Legend of Zelda* (1986–) series. Through earcons and other interactive audio, players are able to influence the musical architecture of video games. I begin by considering the indeterminacy of video games as a medium and their consequently indeterminate musical elements. I then examine earcons and the manipulation of musical modules in *Super Mario Bros.* (1985) and *The Legend of Zelda* (1986), specifically in terms of the precedent they set for later installments in each series. Using *The Legend of Zelda: The Wind Waker* (2002) as a case study, I then consider the use of earcons and the effect of player strategy on the game's musical structures. With the agency to interact with and influence game mechanics and the music and sounds that accompany them, I argue that the player is able not only to pave their own paths within the game world but also compose and perform within a given game's musical architecture.

Indeterminacy and Video Games

Video games have long represented a significant site of player agency, notably as pertains to a game's music. Ludomusicologists have always stressed the interactive nature of video games and the mutability of their music: Karen Collins has discussed the mutability of game states and player input in the shaping of dynamic audio, Elizabeth Medina-Gray has theorized various ways in which musical modules may combine within a musical architecture,[1] and Michiel Kamp has discussed the perceptions of the constructed ecologies and environmental music of video games and player reactions thereof.[2] Each of these interpretations points to the indeterminacy of video game music, especially as related to player agency and decision-making. Indeterminate music, to a greater extent than other genres, relies on the performer to realize aspects of the composition. Whereas the performer's role in genres like serial music is "entirely technical, not interpretative," composers of indeterminate music grant the performer the agency to realize and manipulate some aspects of their compositions to "preserve and extend the performer's role."[3]

In Michael Parsons' words, composer Cornelius Cardew "has taken the idea further: he regards notation more as a stimulus to the players' imagination than a blueprint for exact sound."[4] Indeterminacy forces performers to be creative and enter into conversation with (and to some extent, collaborate with) the composer.[5] In a video game, where creativity is heightened by interaction and musical notation is ordinarily absent, the responsibility of the player to realize the game's musical architecture becomes more significant.

Scholars in the field of ludomusicology have by-and-large emphasized the indeterminacy of video games as a medium and the music by which they are accompanied. Karen Collins' definition of dynamic audio in particular describes how decisions made by the player (i.e., the performer) of a video game make it impossible to replicate a playthrough of a video game.[6] In their preface to Music in Video Games, K.J. Donnelly, William Gibbons, and Neil Lerner discuss the dynamic nature of video game playthroughs in terms of interpretation and musical performance:

> [T]his framework [of games being defined by rules] is only part of what it means to play; a game with no player is no game at all. The rest of the 'expressive experience' comes from players interpreting the framework. The same freedom of choice … that differentiates every musician's version of "I Got Rhythm" (1930) renders each playthrough [of a video game] unique.[7]

---

1 Finding terms like "composition" and "soundscape" problematic, I will be using the term "musical architecture throughout this essay in reference to the amalgamation of dynamic audio and environmental music in video games and ludic events. In *Playing with Sound: A Theory of Interacting with Sound and Music in Video Games* (Cambridge, MA: MIT Press, 2013),3, Karen Collins terms this amalgamation simply as "game sound." I believe the term "architecture" more clearly suggests the extent to which a game's music and sounds are comprised of various elements that interact with each other and can be freely manipulated.

2 The pioneering work of Karen Collins, Michiel Kamp, Elizabeth Medina-Gray, Tim Summers, and Zack Whalen has provided theories for understanding video game music. Karen Collins, *Game Sound: An Introduction to the History, Theory, and Practice of Video Game Music and Sound Design* (Cambridge, MA: MIT Press, 2008); Michiel Kamp, "Musical Ecologies in Video Games." *Philosophy & Technology* 27 (2014): 235–249; Elizabeth Medina-Gray, "Modular Structure and Function in Early 21st-Century Video Game Music" (Ph.D. dissertation, Yale University, 2014), Pro-Quest Dissertations & Theses Global; Tim Summers, *Understanding Video Game Music* (Cambridge: Cambridge University Press, 2016); and Zack Whalen, "Play Along: An Approach to Videogame Music," *Game Studies: The International Journal of Computer Game Research* 4, no. 1 (2004), www. gamestudies.org/0401/whalen/. More recently, Melanie Fritsch has argued for a more nuanced understanding of performativity in video games; Steven Reale has called into question whether musical notation and music theory can adequately describe music in video games; and Tim Summers has drawn on queer theory to analyze video game music. Melanie Fritsch, "Game — Music — Performance," in Melanie Fritsch and Tim Summers, ed., T*he Cambridge Companion to Video Game Music*, 238–262; Steven Reale "Analytical Traditions in Game Music," in Fritsch and Summers, *The Cambridge Companion to Video Game Music,* 193–219; and Tim Summers, *The Queerness of Video Game Music* (Cambridge: Cambridge University Press, 2023).

3 Michael Parsons, "Sounds of Discovery," *The Musical Times* 109, no. 1503 (1968): 429.

4 Ibid.

5 Benjamin Piekut, "Indeterminacy, Free Improvisation, and the Mixed Avant-Garde: Experimental Music in London, 1965–1975," J*ournal of the American Musicological Society* 67, no. 3 (2014): 774.

6 Karen Collins, *Game Sound*, 4.

7 K.J. Donnelly, William Gibbons, and Neil Lerner, ed., Preface to Music in Video Games: Studying Play (New York and Abingdon, UK: Routledge, 2014), viii.

In the same way that no two playthroughs of *Pong* (1972) are the same, every performance of its sounds is unique because the player must constantly make decisions based on game states.

The idea of performing music in a video game is hardly new and is described at length in Roger Mosely and Aya Saiki's essay "Nintendo's Art of Musical Play." In their essay, the authors cite several games in which Nintendo places the performance of music in the forefront. Of particular interest is *Donkey Konga* (2003), in which players perform rhythms on the DK Bongo controller—a pair of bongos that act as a video game controller—along with famous tunes such as Richard Berry's "Louie Louie," Queen's "We Will Rock You," and Brahms' *Hungarian Dance No. 5*.[8] The bongo controller was used again in *Donkey Kong Jungle Beat* (2004), where instead of performing prescribed rhythms, the player controls the titular character's movements by playing the bongos. As they play *Jungle Beat*, the player improvises unique rhythmic compositions using the DK Bongo controller.

Moreover, instrumental performance and composition have been discussed in virtual ocarina performances in *The Legend of Zelda: Ocarina of Time* (1998). As Tim Summers explains in *Understanding Video Game Music,* the player learns and performs several melodies with specific game functions in *Ocarina of Time*. In addition, the player is encouraged to "exert their own musical agency where the player is challenged to compose a new eight-note melody which becomes part of the game's song repertoire" in order to summon the music-loving scarecrow, Pierre.[9] Figure 1 below shows Link composing a melody for Pierre's scarecrow friend Bonooru. While all the functional melodies in *Ocarina of Time* utilize only five pitches, the player isn't limited to these five pitches if they choose to improvise melodies for fun. With the left analog stick, they may also bend each of the five pitches within a whole tone and compose with even more liberty.

Figure 1. Screenshot from *The Legend of Zelda: Ocarina of Time* (Nintendo 64). As Link composes an eight-note melody on the ocarina, the scarecrow Bonooru dances. Later in the game, Pierre is summoned by this same melody.

8 Roger Mosely and Aya Saiki, "Nintendo's Art of Musical Play," in Donnelly, Gibbons, and Lerner, *Music in Video Games,* 62.
9 Tim Summers, *Understanding Video Game Music*, 178–79.

The freedom to compose can also be observed in Nintendo's *Animal Crossing* series (2001–). Each game presents the player with the opportunity to compose a tune to represent the town in which their avatar lives. This tune is then played any time the player speaks with one of their anthropomorphic neighbors. In *Animal Crossing* (2001), the player can change the town tune by interacting with the "Melody" billboard in front of the town post office. Upon reading the billboard, a compositional interface is presented to the player, as depicted in Figure 2. The interface relies on pitch names and iconic notation—fitting for the game's intended young audience.[10] Within the interface, players can change pitches, sustain pitches, create rests, and even introduce their own indeterminacy by calling for random pitches.

Figure 2. Screenshot from *Animal Crossing* (Nintendo GameCube). Compositional interface for creating a town tune. Frogs are used to represent each beat and note names within their mouths represent pitches. Dashes indicate the previous pitch is sustained, question marks indicate random pitches, and sleeping frogs indicate rests. The melody shown is the default town tune.

In the examples of *Jungle Beat, Ocarina of Time,* and *Animal Crossing*, the connection between gameplay (i.e., ludic performance) and musical performance is clear due to the nature of the DK Bongo controller and the ocarina interface. In these games, players are encouraged to compose within and outside of normal gameplay. Parallels may also be drawn between instrumental performance and pressing buttons on typical controllers and their consequent in-game sound effects. Sound designer Toshio Iwai noticed this parallel while playing *Super Mario Bros*. (1985); he "started playing around and producing sounds by making Mario jump, which made me feel like I was playing instruments while playing the game."[11] Iwai's revelation supports Richard Stevens' assessment that, as musical events respond to ludic events triggered by the player, "these musical associations will likely be ascribed to their actions. You (the player) act, superhero music plays, you are the superhero."[12] By interpreting and instigating game and musical events, the player acts as both a listener and performer of a game's musical architecture as well as a collaborator with the game's composers. Video game music blurs the lines between conventional roles in music-making and consumption. In *Understanding Video Game Music,* Summers says

> [...] we are playing with games and complicating the divisions of roles that have traditionally held sway in musical thought—unlike the image of music in the concert hall, here we become the performers, listeners and analysts of the music, all at once. In causing the assembly of the musical material, we also gain authorial agency, if not becoming composers in the strictest sense of the term. [...] This human agency is writ large in games through the performative nature of play and musical investigation.[13]

In this paper, I will argue further that video game players do in fact become co-composers when they interact with and explore sound effects in video games. By playing with sound

10 Later installments in the *Animal Crossing* series—namely *Animal Crossing: City Folk* (2008) and *Animal Crossing: New Horizons* (2020)—place the frog icons on three-lined musical staves, immersing the player more deeply in a common-practice period compositional process.
11 Toshio Iwai, *Iwai Toshio no shigoto to shūhen* (Tokyo: Rikuyosha, 2000), 64, as translated in Mosely and Saiki, "Nintendo's Art of Musical Play," in Donnelly, Gibbons, and Lerner, *Music in Video Games,* 58.
12 Richard Stevens, "The Inherent Conflicts of Musical Interactivity in Video Games," in *The Cambridge Companion to Video Game Music,* ed. Melanie Fritsch and Tim Summers (Cambridge: Cambridge University Press, 2021), 76.
13 Tim Summers, *Understanding Video Game Music*, 32.

effects, players construct the building blocks of a game's musical architecture and fully realize unique performances of the game's music.

Pong and Player-Directed Sound Effects

Since *Pong* (1972), a player's inputs have had an impact on a game's overall musical architecture. In the absence of a dedicated sound chip and streaming audio capabilities, the *Pong* arcade machine was only able to produce sounds through electrical pulses in its circuitry. The game has no environmental music—that is, music that accompanies the in-game environment—but features sound effects that constitute its musical structure. The sound effects are synchronized with *Pong's* three primary ludic elements: hitting the ball with the paddle, the resulting bounce of the ball off the top or bottom of the screen, and scoring a point. By controlling these elements, the player's actions "give sonic voice to the ludic events" of *Pong* using only three possible sounds.[14] The player has the power—by striking the single-pixel table tennis ball— to "create in [*Pong's*] soundtrack a minimalistic accompaniment."[15] Karen Collins corroborates this, claiming that the player is not a "passive 'receiver'" of a game's music, but rather an active participant in its musical structure.[16] Actively interacting with *Pong*'s sounds gives the player the agency to simultaneously create and perform a form of indeterminate music. The ability to create and perform music using sound effects remains present in many games but is heavily featured in the majority of Nintendo's franchises, particularly the *Super Mario* (1985–) and *The Legend of Zelda* (1986–) series.

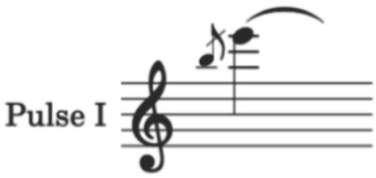
Earcons and Modular Systems in Super Mario Bros. and The Legend of Zelda

Released in 1983, the Nintendo Entertainment System (NES) was among the first home consoles to include a sound chip capable of producing continuous music and sound effects through the generation of waveforms.[17] The NES sound chip (the RP2A0X) consists of four waveform-producing sound channels—two pulse voices, one triangle, and one noise (only capable of percussion-like sounds)—that create the sound chip's unique sound.[18] The limitation of four channels presents unique challenges and limitations for game composers and sound designers. Even with the limitations of the NES sound chip, it is not always necessary to omit sections of musical material in favor of sound effects. For instance, some sound designers use only three channels for their musical accompaniment, reserving the fourth for sound effects.[19] Koji Kondo, composer of *Super Mario Bros.*, however, uses all four channels to add textural complexity to the game's soundtrack. Kondo consequently allows sound effects to override the Pulse I channel, which plays the environmental music's primary melody, whenever they occur.[20] Compensating for the absence of this melody, sound effects—such as those which accompany Mario collecting a coin—are comprised of pitches that conform to the key of the environmental music. As such, James Newman notes, "[t]he harmonic integrity of the soundscape (if not the melody) is preserved and the sound effect is integrated into the music, joining the visual, sonic, haptic and

14 Tim Summers, *Understanding Video Game Music*, 199.
15 Neil Lerner, "Mario's Dynamic Leaps: Musical Innovations (and the Specter of Early Cinema) in Donkey Kong and Super Mario Bros," in *Music in Video Games: Studying Play,* ed. K.J. Donnelly, William Gibbons, and Neil Lerner (New York and Abingdon, UK: Routledge, 2014), 1.
16 Karen Collins, *Game Sound*, 3.
17 James Newman, "Before Red Book: Early Video Game Music and Technology," in *The Cambridge Companion to Video Game Music*, ed. Melanie Fritsch and Tim Summers (Cambridge: Cambridge University Press, 2021), 14.
18 Andrew Schartmann, *Koji Kondo's* Super Mario Bros. *Soundtrack* (New York: Bloomsbury, 2015), 37–42; and Newman, "Before Red Book," 20.
19 In Newman, "Before Red Book," 25, the author notes that the Commodore 64 game *The Human Race* does just this with the console's three sound channels, reserving two for environmental music and one for sound effects.
20 Ibid., 24–25.

ludic aspects of the game into one experiential whole."[21] As the player controls Mario's jumps and collects coins, mushrooms, and fire flowers, they produce sounds that contribute to and interact with the overall musical architecture, at times causing Kondo's melody to cease and allowing the player's own melody to take the forefront. By synchronously interacting with ludic elements, sound effects, and environmental music, players are not merely observers but active participants in the video game and collaborators in its dynamic musical architecture.

As with *Pong*, ludic elements in Nintendo's *Super Mario Bros*. (1985) are voiced musically. Spontaneous actions—such as jumping or collecting coins—are accompanied by sound effects categorized by Eve Hoggan and Stephen Brewster as earcons. In their definition, earcons are "abstract, synthetic tones in structured combinations to create auditory messages."[22] As applied to video games, earcons act as the musical voice of interactive game components. In *Super Mario Bros.*, these earcons are largely Mickey-Moused—a term borrowed from film that describes the synchronization of musical and visual elements.[23] For example, when the player collects a coin, an earcon resembling the *cha-ching*! of a cash register sounds (see Example 1).[24]



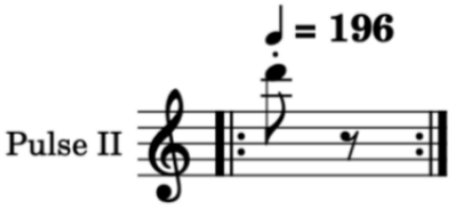
Example 1. *Super Mario Bros.*, coin earcon.[25]

Earcons may also be more heavily integrated into the modular system of the game's soundtrack.As defined by Medina-Gray, a modular system describes the layering and sequence of modules—"discrete chunks of music" that undergo "a process of assembly to yield the overall sounding result."[26] An assembly of modules may be measured by its smoothness, the degree to which the combination of modules "yields cohesion and continuity among modules in real-time soundtracks, and [avoids] producing sounds that might be jarring or unpleasant."[27]

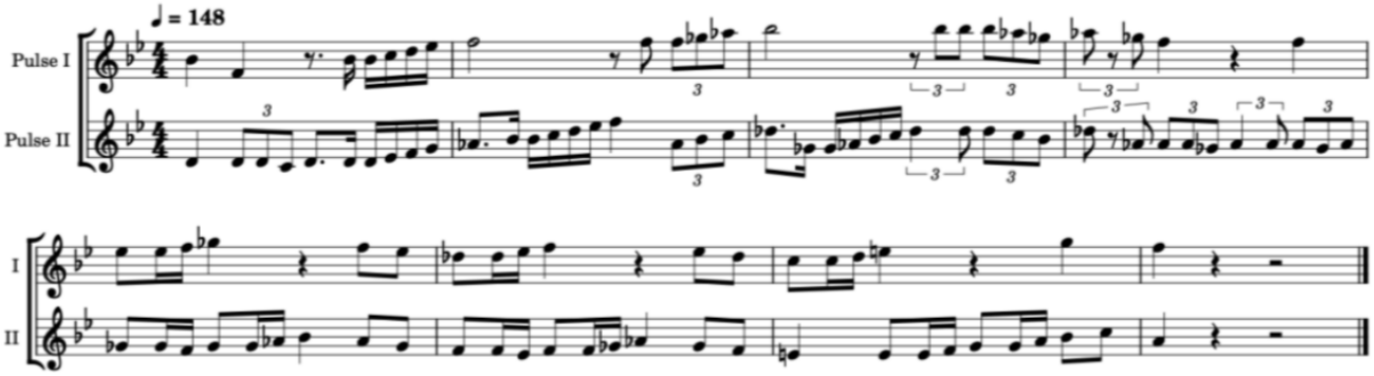
The Legend of Zelda (1986) features a continuously sounding heartbeat-like earcon when the player character Link is low on health points (see Example 2). The heartbeat completely usurps the NES sound chip's Pulse II channel, responsible for the environmental music's countermelody. The earcon fits smoothly with the timbre of the Pulse I channel (both generating the same waveform) and within the keys of the musical themes that accompany the overworld and the many dungeons (see Examples 3a and 3b). The pitch frequency and the tempo at which the earcon repeats, however, are in disjunction with both themes. The incessant and disjunct chirping encourages the player to restore Link's health points, thereby ending the earcon and

21 Ibid., 25.
22 Eve Hoggan and Stephen Brewster, "Nonspeech Auditory and Crossmodal Output," in *The Human-Computer Interaction Handbook: Fundamentals, Evolving Technologies, and Emerging Applications*, ed. Julie A. Jacko, 3rd ed. (Boca Raton, FL: CRC Press, 2012), 222.
23 Schartmann, *Koji Kondo's* Super Mario Bros. *Soundtrack*, 103–108; Lea Jacobs, "Mickey Mousing Reconsidered," in *Film Rhythm after Sound: Technology, Music, and Performance,* 1st ed. (Oakland, CA: University of California Press, 2015), 58.
24 Schartmann, *Koji Kondo's* Super Mario Bros. *Soundtrack*, 111.
25 All transcriptions have been made by the author. They are also all inherently limited by the system of notation used. The transcriptions represent a partial rendering of the music and sound effects found in their respective games and cannot adequately account for variance in playthroughs, tone quality, and timbre. In spite of these limitations, my transcriptions will prove useful in conceptualizing sound effects not only as sounds that coincide with environmental game music but also as sounds that interact with all components of a given game's musical architecture.
26 Elizabeth Medina-Gray, "Meaningful Modular Combinations: Simultaneous Harp and Environmental Music in Two *Zelda* Games," in Donnelly, Gibbons, and Lerner, *Music in Video Games,* 105.
27 Ibid. For an in-depth analysis of smoothness between horizontally organized modules, see Elizabeth Medina-Gray, "Analyzing Modular Smoothness in Video Game Music," *Music Theory Online* 25, no. 3 (2019), under "A Method for Analyzing Smoothness at Modular Seams" and "Analysis of Ocean Combat in *The Legend of Zelda: The Wind Waker*."

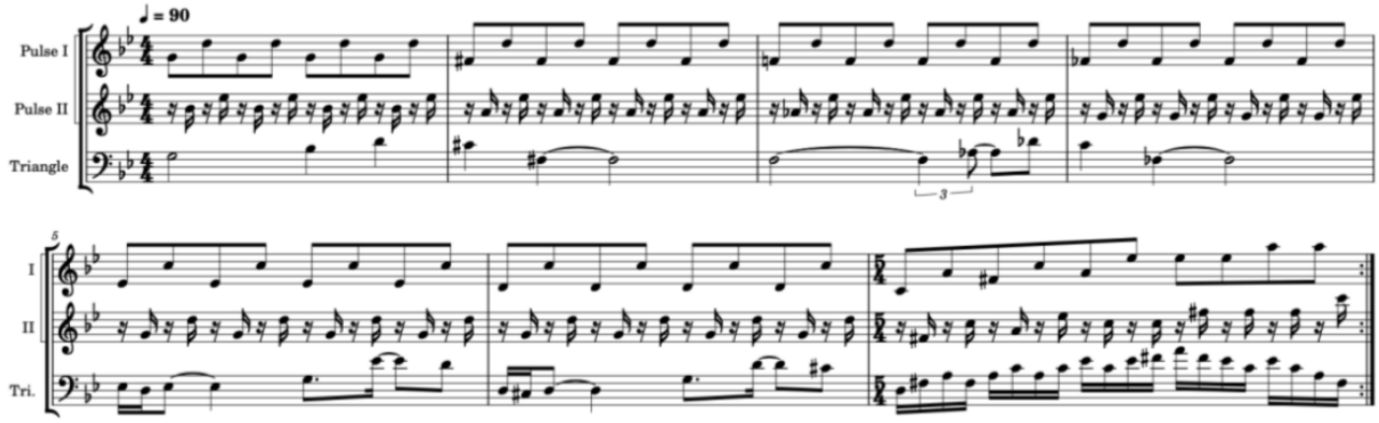
resuming the countermelody pulse channel. By rejuvenating Link, the player has the power to alter the modular layering and the overall musical architecture.



Example 2. *The Legend of Zelda* heartbeat earcon. The earcon repeats in a continuous loop while Link has low health.



Example 3a. Excerpt from *The Legend of Zelda* overworld theme.



Example 3b. *The Legend of Zelda* dungeon theme.

After *Zelda II: The Adventure of Link* (1987), Nintendo's sound designers took advantage of hardware improvements made to the NES's successors to devote separate sound channels or audio tracks to the heartbeat earcon.[28] Although the relentless nature of the earcon is maintained in each installment of the *Zelda* series, The *Legend of Zelda: Breath of the Wild* (2017) and its direct sequel *Tears of the Kingdom* (2023) minimize the disjunction between the heartbeat and environmental music by reducing its volume and pitch frequency. Regardless, by restoring Link's health, the player has the ability to discontinue the heartbeat in every *Zelda* game and affect the structure of the modular system and the interactions of individual modules.

Every game in both the *Super Mario* and *Legend of Zelda* series uses earcons and modular systems to some extent to amplify the actual agency of the player.[29] Until now, I have primarily discussed games from the late 1980s that have relied on sound chips for sound production. Since the Nintendo 64 (1996), all of Nintendo's home consoles have streamed audio from pre-recorded tracks. Streaming audio from its graphics processing unit, the GameCube (2001) has greater capability than the NES to layer and combine musical modules. Following is a discussion of three musical architectures related to battles in *The Legend of Zelda: The Wind Waker* (2002; hereafter *Wind Waker*), the first *Zelda* game on the GameCube. In these battles, the use of earcons in the game's many combat episodes augments the player's control over the game mechanics and soundtrack. As with striking the ball in *Pong*, the earcons in *Wind Waker*'s battles give the player the power to perform and participate in composing the game's dynamic musical architecture.

The Legend of Zelda: The Wind Waker

The story of *Wind Waker* takes place in a vast ocean and on the islands that rise from it. The player controls the green-clad hero, Link, on his seafaring quest to rescue his sister, and subsequently Princess Zelda and the world, from the evil Ganondorf. *Wind Waker* is distinguished from other three-dimensional entries in the *Zelda* series by its cel-shaded graphics and simple art style, resulting in an overall cartoon visual aesthetic. The cartoon visuals are closely intertwined with the game's sound effects, which largely rely on Mickey-Mousing effects that interact with the game's visuals. Throughout *Wind Waker,* Link wields and performs with the eponymous Wind Waker, a magical conductor's baton that allows him to lead an invisible chorus, direct the wind, teleport across the ocean, change the time of day, and even take control of certain secondary characters. The player's ability to perform with the Wind Waker baton affords them with many opportunities for musical exploration within the Wind Waker's interface (see Figure 3). The game includes six songs with specific functions in relation to the game's mechanics and plot, but the player may also decide to compose and perform with the baton outside of its narrative, resembling the role of the conductor in Charles Ives' *The Unanswered Question* (1903, rev. 1930–35), which "allows the conductor to cue instrumental groups freely."[30] As with the ocarina in *Ocarina of Time*, Wind Waker follows a tradition in the *Zelda* franchise in which the player

28 Nintendo continued to use analog sound chips in the Super Nintendo Entertainment System home console and Game Boy handheld consoles. The Nintendo DS (2004) is the last system to utilize a dedicated sound chip. Technical advancements displayed in the Nintendo 64 (1996) allowed the console's graphics processing unit to stream digital audio stored in a game's cartridge or disc. Rather than producing sound in real-time through channels, streaming audio accesses and plays pre-recorded tracks.

29 The term *actual agency* is derived from Robert S. Hatten, *A Theory of Virtual Agency for Western Art Music* (Bloomington: Indiana University Press, 2018), 8. In Tim Summers, *The Queerness of Video Game Music* (Cambridge: Cambridge University Press, 2023), Summers ascribes Hatten's theory of virtual agency to musical events in video games. I argue in addition, that by interpreting and controlling the virtual agency of video game characters—and thereby their action's repercussions—they exhibit actual agency over aspects of the game's musical structure and mechanics.

30 Jonathan De Souza, "Reassessing the Emergence of Indeterminate Music," *British Postgraduate Musicology* 9 (2008), http://britishpostgraduatemusicology.org/bpm9/desouza.html.

(through Link) is given the ability to perform and compose with a musical instrument.[31] While the *Wind Waker* presents the player with a significant source for musical performance and creativity, the earcons that accompany Link's sword attacks afford the player the same ability within Wind Waker's various combat episodes.

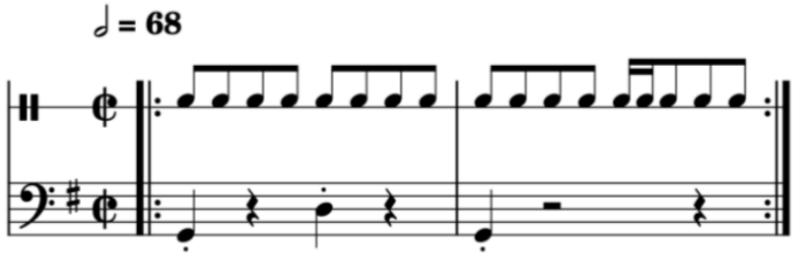
Figure 3. Screenshot from *The Legend of Zelda: The Wind Waker* HD (Wii U). Link conducts the "Wind's Requiem," allowing him to change the direction of the wind. In the Wind Waker interface, the player controls conducting gestures, meter, and dynamics using the analog sticks.

Sword Training with Master Orca

Before leaving his home on Outset Island and embarking on his dangerous adventure, Link must first obtain a sword and learn how to use it. Luckily for Link and the player, Outset Island is home to a former swordmaster named Orca who runs a sword-training dojo. Upon entering Orca's dojo, Orca gifts Link the Hero's Sword and offers to teach him the basics of sword fighting—and by proxy, to teach the player *Wind Waker*'s basic combat controls (see Figure 4). As individual combat techniques are taught, Link must demonstrate them against Orca. After eight successful demonstrations, Orca introduces another technique until the player has learned all of the game's combat mechanics. The music that accompanies Link's sword training is inherently elementary, reflecting the rudimentary sword skills being taught in Orca's dojo. In its most basic form, the sword training music consists only of a bass ostinato oscillating between the tonic and dominant and a rhythmic ostinato maintaining the subdivision (see Example 4a). By landing a successful blow against Orca, the player triggers a strong chordal earcon similar to the Mickey-Moused musical stingers that complement the POWs!, BAMs!, and KAPOWs! which accompany Batman's punches in William Dozier's *Batman* TV series (1966–68).

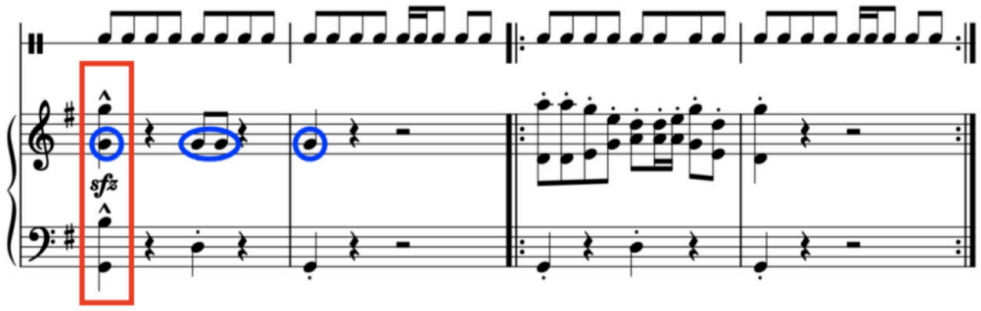
Figure 4. *The Legend of Zelda: The Wind Waker* HD. Master Orca awaits Link's demonstration of a horizontal slice.

31 The player is able to make music with a multitude of instruments throughout the series, including a flute in *The Legend of Zelda* and *Zelda II*; horns, drums, and a skeletal guitar in *Majora's Mask* (2000); a harp in *Skyward Sword* (2011); and even wolf vocals in *Twilight Princess* (2006).



Example 4a. The rhythmic and bass ostinati that constitute the sword training music.

Each successful demonstration of a sword technique is synchronized with its own chordal earcon and shifts the music into new modules. The chord structure of the earcons outlines a basic tonic-predominant-dominant phrase model, ascending through the first five notes of a diatonic scale (see Example 4b). While the player learns the basics of *Wind Waker*'s combat mechanics, they are also implicitly learning the basics of tonal music composition and performance.



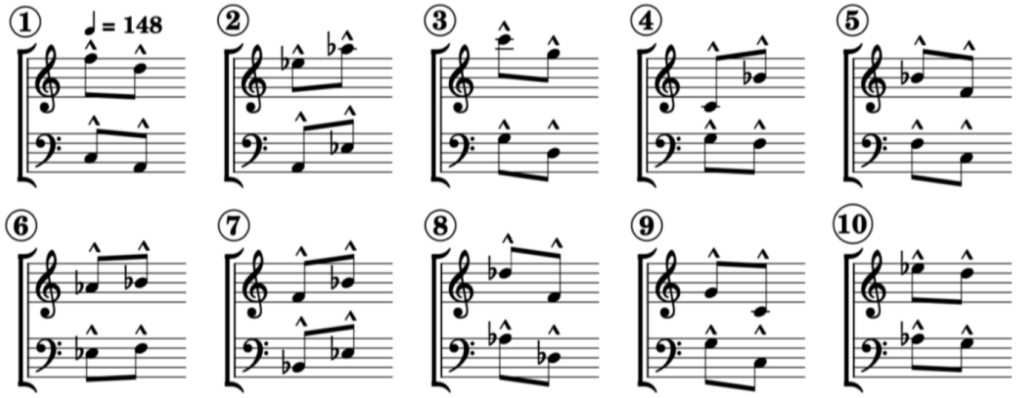
Example 4b. When striking Orca, the earcon (boxed) sounds and begins the following module. The circled notes ascend stepwise from the tonic to dominant upon each successful strike and outline a basic T-PD-D phrase model.

Normal Battles

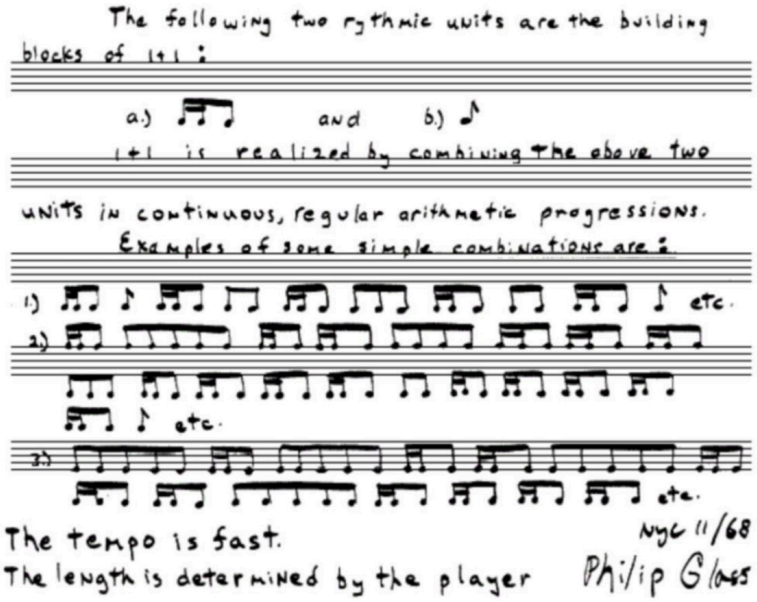
The vastness of *Wind Waker*'s oceanic world gives the player a great amount of agency to explore, discover new islands, and solve puzzles. Episodes of combat, however, are considerably less vast. In normal battles (i.e., those against ordinary enemies), the player's attention is taken away from the world around Link and is focused instead on their opponent(s). When the precedent for this kind of focus was set in *The Legend of Zelda: Ocarina of Time* (1998), developers found it necessary to compose music to accompany normal battle events and heighten their inherent drama. In *Ocarina of Time*, normal battle music consists of a rhythmic ostinato, quick half-step oscillations in the bass, and a dark brass melody.The music that accompanies normal battles in *Wind Waker* maintains a rhythmic ostinato and includes a more scalar bass track. Missing from *Wind Waker*'s battle music, however, is any sort of pre-composed melody. Because the game's composers did not write a melody for the battle music, the onus is on the player to create it for them.

As in Orca's dojo, Link's sword strikes produce earcons that interact with the battle music's architecture. There are ten possible sword earcons in the normal battle music (outlined in Example 5a) that sound after consecutive hits on individual enemies. These ten earcons be-

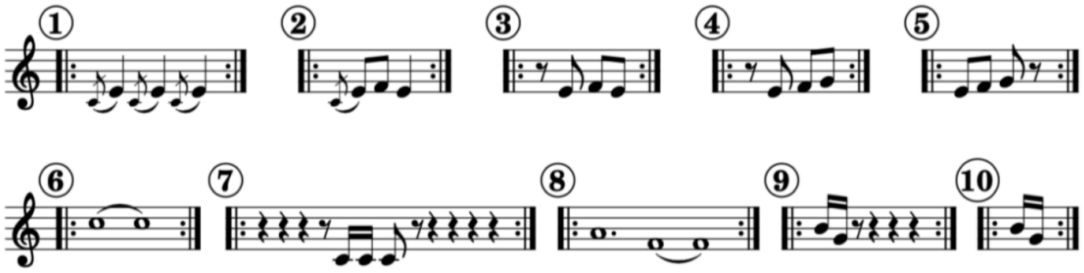
come the player's compositional lexicon, not dissimilar to the rhythmic units of Philip Glass's *1+1* (1968) or the melodic fragments of Terry Riley's *In C* (1966) shown in Examples 5b and 5c. In these examples, the decisions of the performer result in unique constructions of the works' predetermined musical building blocks. In the same way, the player's dynamic combat decisions in *Wind Waker* have great effects on the performance of the battle music: if a player likes to take battles slowly, then the music will be less densely paced. Conversely, if a player is more animated, the battle music will follow suit. These decisions transform Link's sword slashes into pen strokes, writing unique and unrepeatable melodies for every battle. Every first strike against an enemy sounds Earcon 1, but the combinations of the earcons thereafter are practically limitless. As Link battles several enemies at once, builds large attack combos, and rests between attacks, the architecture of the earcons and the environmental music becomes truly dynamic with each battle.



Example 5a. The ten earcons synchronized with sword attacks in *Wind Waker*'s battle music.



Example 5b. Excerpt from *1+1* (Philip Glass, 1968) showing the piece's two rhythmic units and possible combinations of them.



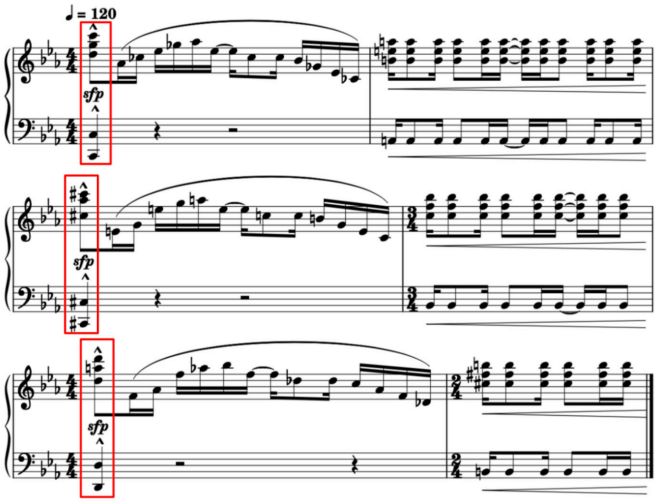
Example 5c. The first ten melodic fragments from *In C* (Terry Riley, 1966).

The Battle Against Phantom Ganon

Halfway through the game, after Link has obtained the legendary Master Sword, he is able to launch an attack on Ganondorf's Forsaken Fortress. Upon invading the fortress, Link is greeted by Phantom Ganon, a puppet and spiritual manifestation of Ganondorf's evil. A battle ensues between the two as a magical approximation of a tennis match using a ball of dark energy and swords instead of rackets—a long-standing trope in the *Zelda* series known as the "Dead Man's Volley" (see Figure 5). Link successfully wins a volley if Phantom Ganon is unable to return the dark energy and is damaged by it. As the battle progresses, the rate at which Link must return the energy increases until Link has finally vanquished his ghastly foe.

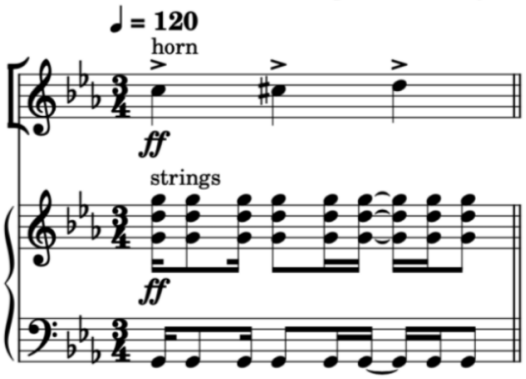
Figure 5. *The Legend of Zelda: The Wind Waker* HD. Link engages in the Dead Man's Volley, returning the ball of dark energy toward Phantom Ganon.

The Phantom Ganon battle serves as a musical recapitulation of Orca's training sequence. As with Link's demonstrations of combat techniques before, successfully returning the dark energy to Phantom Ganon sounds an earcon and shifts the battle music into a new module. The music for this battle is composed primarily of diminished seventh chords and supported by quartal harmony, obscuring any clear tonal center and standing in stark contrast to the simplicity of the elementary tonality of Orca's battle music. The more complex harmony demands greater complexity in sword strike earcons. Rather than rising diatonically, successive earcons and the modules that they introduce ascend chromatically as Link successfully returns the ball of dark energy (see Example 6).



Example 6. A possible rendering of the Phantom Ganon modular sequence. The earcons (in boxes) may occur at any point due to Link's distance from Phantom Ganon in addition to the increasing speed of the dark energy ball.

The battle represents a significant test of Link's swordsmanship and the player's implied growth as a composer since acquiring their combat skills and musical vocabulary from Orca. In contrast to training with Orca, the player can lose against Phantom Ganon, whose virtual agency gives him the power to fight back against Link, thus manipulating the musical structure and challenging the musical architecture being constructed by the player. If Link takes damage from the dark energy, a cue sounds that suggests the player's failure. The player's failure resets the earcon to its original pitch and Link must rise through the scale again. When Link successfully wounds Phantom Ganon, a triumphant module consisting of a G major triad (the dominant of the implied key of C minor) sounds (see Example 7). The tonality of the module hearkens back to the beginning of Link's sword training and implied musical training and legitimizes Link's success in both his mastery of swordplay and his compositional skills.



Example 7. The triumphant module that marks Link's success in the Dead Man's Volley. The module occurs any time Link successfully wins a volley.

Conclusions

Wind Waker is a model example of Nintendo's sound designers granting the player the agency to perform and create musical architectures using earcons. I have also shown that indeterminate techniques have also been employed by Nintendo's sound designers of the 1980s and '90s. Players have taken notice of the use of earcons in more recent Nintendo titles, such as *Super Mario Odyssey* (2017).[32] In *Odyssey*, players have the ability to "capture" (effectively a form of corporeal possession) certain creatures and ordinarily inanimate objects by throwing Mario's magical hat, Cappy, at them. When capturing electricity and moving through a power line, a module consisting of an Alberti bass begins to accompany the environmental music. When possessing a caterpillar-like creature known as a Wiggler, the player has the ability to stretch and contract the Wiggler. As the Wiggler stretches, an accordion-like instrument plays a rising figure. Lastly, each of Mario's footsteps sounds distinct pitches in certain levels. All of these sounds fit with a high level of vertical smoothness in the key and chord progressions of whatever environmental music they accompany and allow the player to interact with and create inside the game's various musical architectures.

While I have focused on earcons as a mode of composition in video games, they should not be the only sound effects to be considered musical. If "[m]usic is sounds, sounds around us whether we're in or out of concert halls: cf. Thoreau,"[33] then auditory icons must also be considered as elements that contribute to a video game's musical architecture. As "natural, everyday sounds that can be used torepresent actions and objects within an interface,"[34] auditory icons resemble sounds heard in the actual world. The sound of Link swinging his sword is like that of the movement of air when swinging a sword in real life. Like earcons, auditory icons are woven by the player into the sonic fabric of a video game. If the chordal stingers that accompany Link returning dark energy to Phantom Ganon in *Wind Waker* are musical, so too should we view the more naturalistic sounds that accompany the Dead Man's Volley at the climax of *Ocarina of Time* as musical. In this way, video game players perform and create within a given game's musical architecture by means of any gameplay that produces sound effects.

32 JalopesTL, "Sounds in *Super Mario Odyssey* Harmonize with the Background Music," YouTube Video (November 4, 2017), 0:43–3:28. https://youtu.be/U5-YDxH6It8?si=NM5u6gUSNkJuhdlH.

33 John Cage, as quoted in R. Murray Schafer, *The New Soundscape* (Scarborough, ON: Berandol Music Limited, (1969), 1.

34 Hoggan and Brewster, "Nonspeech Auditory and Crossmodal Output," in Jacko, *The Human-Computer Interaction Handbook*, 220.

References

Collins, Karen. *Game Sound: An Introduction to the History, Theory, and Practice of Video Game Music and Sound Design*. Cambridge, MA: MIT Press, 2008.

———. *Playing with Sound: A Theory of Interacting with Sound and Music in Video Games.* Cambridge, MA: MIT Press, 2013.

Custodis, Michael. "Playing with Music." In *Music & Game: Perspectives on a Popular Alliance,* edited by Peter Moorman, 159–70. Wiesbaden: Springer, 2013.

De Souza, Jonathan. "Reassessing the Emergence of Indeterminate Music." *British Postgraduate Musicology 9* (2008). http://britishpostgraduatemusicology.org/bpm9/desouza.html.

Donnelly, K.J., William Gibbons, and Neil Lerner, eds. *Music in Video Games: Studying Play.* New York and Abingdon, UK: Routledge, 2014.

Fritsch, Melanie. "Game — Music — Performance." In *The Cambridge Companion to Video Game Music,* edited by Melanie Fritsch and Tim Summers, 238–62. Cambridge: Cambridge University Press, 2021.

Fritsch, Melanie, and Tim Summers, eds. *The Cambridge Companion to Video Game Music* Cambridge: Cambridge University Press, 2021.

Hart, Iain. "Semiotics in Game Music." In *The Cambridge Companion to Video Game Music,* edited by Melanie Fritsch and Tim Summers, 220–37. Cambridge: Cambridge University Press, 2021.

Hatten, Robert S. *A Theory of Virtual Agency for Western Art Music.* Bloomington: Indiana University Press, 2018.

Hoggan, Eve, and Stephen Brewster. "Nonspeech Auditory and Crossmodal Output." In *The Human-Computer Interaction Handbook: Fundamentals, Evolving Technologies, and Emerging Applications*, 3rd ed., edited by Julie A. Jacko, 211–35. Boca Raton, FL: CRC Press, 2012.

Iwai, Toshio. *Iwai Toshio no shigoto to shūhen.* Tokyo: Rikuyosha, 2000.

Jacobs, Lea. "Mickey Mousing Reconsidered." *In Film Rhythm after Sound: Technology, Music, and Performance*, 1st ed., 58–108. Oakland, CA: University of California Press, 2015.

JalopesTL. "Sounds in *Super Mario Odyssey* Harmonize with the Background Music." YouTube Video. November 4, 2017. 3:49. https://youtu.be/U5-YDxH6It8?si=NM5u6gUSNkJuhdlH.

Kamp, Michiel. "Autoethnography, Phenomenology, and Hermeneutics." In *The Cambridge Companion to Video Game Music*, edited by Melanie Fritsch and Tim Summers, 159–75. Cambridge: Cambridge University Press, 2021.

———. "Musical Ecologies in Video Games." *Philosophy & Technology 27* (2014): 235–49.

Lerner, Neil. "Mario's Dynamic Leaps." *In Music in Video Games: Studying Play,* edited by K.J. Donnelly, William Gibbons, and Neil Lerner, 1–29. New York and Abingdon, UK: Routledge, 2014.

Liebe, Michael. "Interactivity and Music in Computer Games." In *Music & Game: Perspectives on a Popular Alliance,* edited by Peter Moorman, 41–62. Wiesbaden: Springer, 2013.

Medina-Gray, Elizabeth. "Analyzing Modular Smoothness in Video Game Music." *Music Theory Online 25*, no. 3 (2019). https://doi.org/10.30535/mto.25.3.2.

———. "Interacting with Soundscapes." In *The Cambridge Companion to Video Game Music*, edited by Melanie Fritsch and Tim Summers, 176–92. Cambridge: Cambridge University Press, 2021.

———. "Meaningful Modular Combinations." In *Music in Video Games: Studying Play,* edited by K.J. Donnelly, William Gibbons, and Neil Lerner, 104–21. New York and Abingdon, UK: Routledge, 2014.

———. "Modular Structure and Function in Early 21st-Century Video Game Music." Ph.D. dissertation, Yale University, 2014. ProQuest Dissertations & Theses Global.

Moseley, Roger, and Aya Saiki. "Nintendo's Art of Musical Play." In *Music in Video Games: Studying Play*, edited by K.J. Donnelly, William Gibbons, and Neil Lerner, 51–76. New York and Abingdon, UK: Routledge, 2014.

Newman, James. “Before Red Book: Early Video Game Music and Technology.” In *The Cambridge Companion to Video Game Music,* edited by Melanie Fritsch and Tim Summers, 12–31. Cambridge: Cambridge University Press, 2021.
Parsons, Michael. “Sounds of Discovery.” *The Musical Times 109*, no. 1503 (1968): 429–30.
Piekut, Benjamin. “Indeterminacy, Free Improvisation, and the Mixed Avant-Garde: Experimental Music in London, 1965–1975.” *Journal of the American Musicological Society 67*, no. 3 (2014): 769–824.
Plank, Dana. “Audio and the Experience of Gaming.” In *The Cambridge Companion to Video Game Music*, edited by Melanie Fritsch and Tim Summers, 284–301. Cambridge: Cambridge University Press, 2021.
Reale, Steven. “Analytical Traditions in Game Music.” In T*he Cambridge Companion to Video Game Music*, edited by Melanie Fritsch and Tim Summers, 193–219. Cambridge: Cambridge University Press, 2021.
Schafer, R. Murray. *The New Soundscape*. Scarborough, ON: Berandol Music Limited, 1969.
Schartmann, Andrew. *Koji Kondo’s* Super Mario Bros. *Soundtrack*. New York: Bloomsbury, 2015.
Stevens, Richard. “The Inherent Conflicts of Musical Interactivity in Video Games.” In *The Cambridge Companion to Video Game Music*, edited by Melanie Fritsch and Tim Summers, 74–93. Cambridge: Cambridge University Press, 2021.
Summers, Tim. *Understanding Video Game Music.* Cambridge: Cambridge University Press, 2016.
———. *The Queerness of Video Game Music.* Cambridge: Cambridge University Press, 2023.
Whalen, Zack. “Play Along: An Approach to Videogame Music.” *Game Studies: The International Journal of Computer Game Research 4*, no. 1 (2004). www.gamestudies.org/0401/whalen/.

Ludography

Atari. *Pong*. Arcade. Sounds programmed by Allan Alcorn. Atari and Atari Japan, 1972.
Namco. *Donkey Konga.* Nintendo GameCube. Music composed by Junko Ozawa and Jesahm. Nintendo, 2003.
Nintendo EAD. *Animal Crossing*. Nintendo GameCube. Music composed by Kazumi Totaka, Kenta Nagata, Toru Minegishi, and Shinobu Tanaka. Nintendo, 2001.
Nintendo EAD. *Animal Crossing: City Folk.* Wii. Music composed by Kazumi Totaka, Manaka Kataoka, and Shiho Fujii. Nintendo, 2008.
Nintendo EAD. *The Legend of Zelda: Majora’s Mask*. Nintendo 64. Music composed by Koji Kondo. Nintendo, 2000.
Nintendo EAD. *The Legend of Zelda: Ocarina of Time.* Nintendo 64. Music composed by Koji Kondo. Nintendo, 1998.
Nintendo EAD. *The Legend of Zelda: The Wind Waker*. Nintendo GameCube. Music composed by Kenta Nagata, Hajime Wakai, Toru Minegishi, and Koji Kondo. Nintendo, 2002.
Nintendo EAD. *The Legend of Zelda: Twilight Princess.* Nintendo GameCube/Wii. Music composed by Toru Minegishi and Asuka Hayazaki. Nintendo, 2006.
Nintendo EAD Tokyo. *Donkey Kong Jungle Beat.* Nintendo GameCube. Music composed by Mahito Yokota. Nintendo, 2004.
Nintendo EAD Tokyo. *Super Mario Galaxy.* Wii. Music composed by Mahito Yokota and Koji Kondo. Nintendo, 2007.
Nintendo EAD and HexaDrive. *The Legend of Zelda: The Wind Waker* HD. Wii U. Music composed by Kenta Nagata, Hajime Wakai, Toru Minegishi, Koji Kondo, and Atsuko Asahi. Nintendo, 2013.
Nintendo EPD. *Animal Crossing: New Horizons.* Nintendo Switch. Music composed by Kazumi Totaka, Yasuaki Iwata, Yumi Takahashi, Shinobu Nagata, Sayako Doi, and Masato Ohashi. Nintendo, 2020.

Nintendo EPD. *Super Mario Odyssey.* Nintendo Switch. Music composed by Naoto Kubo, Shiho Fuji,and Koji Kondo. Nintendo, 2017.
Nintendo EPD. *The Legend of Zelda: Breath of the Wild*. Nintendo Switch. Music composed by Manaka Kataoka, Yasuaki Iwata, and Hajime Wakai. Nintendo, 2023.
Nintendo EPD. *The Legend of Zelda: Tears of the Kingdom.* Nintendo Switch. Music composed by Manaka Kataoka, Maasa Miyoshi, Masato Ohashi, and Tsukasa Usui. Nintendo, 2023.
Nintendo R&D4. *Super Mario Bros*. Arcade/Nintendo Entertainment System. Music composed by Koji Kondo. Nintendo, 1985.
Nintendo R&D4. *The Legend of Zelda*. Nintendo Entertainment System. Music composed by Koji Kondo. Nintendo, 1986.
Nintendo R&D4. *Zelda II: The Adventure of Link*. Nintendo Entertainment System. Music composed by Akito Nakatsuka. Nintendo, 1987.

Cultural Imperialism in the Roman Empire: a Give and Take Relationship between Imperial Rome and Provincial/External Cultures

Hailey Smith

ABSTRACT

The Roman Empire is often credited with being highly culturally influential both in its time and in later cultural contexts. However, the cultural aspects of art, politics, militarism, etc. for which the Roman Empire is known are the result of interactions between the imperial regime and external entities. The history of the Roman Empire is well documented, but few historians have analyzed the role of conquered cultures and foreign parties on the cultural evolution of Rome. To address these overlooked dynamics between existing cultural groups and their influences on each other, I explore the concepts of ethnicity and framework provided by Issawi (1989) for cultural imprinting in Imperial Rome. After examining scholarship surrounding specific aspects of culture (economics, religion, and politics), I argue that the culture of the Roman Empire was a culmination of Roman creation and influence from provincial and external cultures. These findings highlight the contribution to popular culture from cultural groups with less recognition and fame than their counterparts, and we must apply these findings to modern times in order to emphasize the fluctuating dynamics of domination and submission in the current geopolitical sphere.

Introduction

A question that recently circulated on social media and plagued many users was, "How often do you think about the Roman Empire?" The consequent resurgence in popularity of the Roman Empire among younger generations has inspired the following analysis of the cultural interactions between the imperial regime and the external entities that it conquered or interacted with. It is a common belief in the West that the Roman Empire set the precedent for successful political entities. However, whether or not society owes the state of politics, militarism, literature, art, economics, and theology solely to Roman Latin Culture must be analyzed. Using scholarship surrounding the Roman Empire to understand various cultural dynamics, I argue that the cultural characteristics of the infamous imperial regime, the legacy of which so many celebrate, are the result of dynamic cultural imperialism between the Roman Empire and external cultures, often depicted as less dominant.

Categorizing Ethnicity in the Roman Empire

To understand the role of the Roman Empire in global cultural imperialism, I analyze the cultural differences between the Roman world and their foreign counterparts. In "The Romans and Us: Strabo's 'Geography' and the Construction of Ethnicity," Edward Van der Vliet (2003) analyzes the first century BCE book *Geography* by Strabo that details the various geographical and political features of different locales within and surrounding the Roman Empire. Van der Vliet (2003) states that Strabo's worldview allows him to construct his categories of ethnicity (the social classification of people based on shared cultural traditions or landscapes) by using geography to order the identities of various political entities (258). These categorizations of ethnicity distinguish different ethnic groups based on various facets of culture, such as "language, dress, behaviour, and symbolic expressions" (Van der Vliet 2003, 258-259) as well as "tradition and political unity" (Van der Vliet 2003, 265). By analyzing Strabo's interpretation as an insider to Roman culture, we can identify aspects of culture that differ between the Roman ethnic faction and their counterparts as well as political conditions that shed light on the cultural imperialism in practice.

Van der Vliet (2003) exposes Strabo's binary worldview that aligns individuals between only two ethnic groups: the "civilized" and the "uncivilized." From Strabo's perspective, the qualification for "uncivilized" people, or barbarians, is participation in "a wretched existence and savagery," with a nature "characterized by a lack of capacity to civilise themselves, and a lack of self-control and moderation" (Van der Vliet 2003, 263). The description of this ethnic group is antonymous with the description of the wisdom and success of the Romans who demonstrated moderation and careful planning in the construction of their cities and the welfare of their citizens (Van der Vliet 2003, 268). Moreover, Strabo praised the Romans' abilities in military duty and administration (Van der Vliet 2003, 268) that directly contradicts the "lack of proper means of existence" evidenced by uncivilized people (Van der Vliet 2003, 265). These two comparisons demonstrate the binary characteristics of the civilized people and uncivilized people as they neatly fit into two distinct ethnic groups based on their dissimilar cultures and political organizations. Van der Vliet (2003) exposes the dichotomy presented in Strabo's Geography, between the Romans, who exemplify civilization and a proper way of living, and uncivilized people, who lack a good quality of life and do not have the means to improve it.

After neatly categorizing the two types of people into separate ethnic groups, we can analyze the interactions between the "civilized" and "uncivilized" to identify the nature of their cultural influence on each other. Strabo's *Geography* demonstrates a sense of Roman superiority through his high praises of Roman political wisdom and elite comparisons to other cities (Van der Vliet 2003, 268). These sentiments of Roman superiority directly translate into the relation-

ship between the civilized Roman Empire and the uncivilized entities that Rome conquered. Van der Vliet (2003) describes how the Romans "brought civilization" to their ethnic counterparts by establishing new lines of communication and building infrastructure, such as roads, to enable additional contact between peoples (268). Although the new forms of communication and interaction could have facilitated equal opportunities for each group to culturally influence the other, the Romans subjugated any threats to the empire presented by other political organizations (Van der Vliet 2003, 268). Therefore, the new lines of communication built under Roman control would only serve Roman interests. The political hegemony of the Roman Empire eliminated the likelihood that outside entities would overcome this power imbalance and dictate cultural interactions between the two parties. Thus, this political domination indicates that the power dynamic concerning their cultural influence on each other heavily favored Roman imperialism.

Understanding the Framework for Cultural Imperialism

Aside from the subjective and anecdotal account from Strabo, we can also understand the domination of Roman culture over its subjects using a more theoretical framework. According to a 1989 article by Charles Issawi, "Empire Builders, Culture Makers, and Culture Imprinters," there are three conditions that enable the successful and long-lasting cultural imprinting of one ethnic group onto another: 1) be the dominant culture of an empire with a societal framework that allows the diffusion of culture; 2) have a constant and large volume of culture-bearers migrating to provinces; and 3) identify with a religion that easily or actively admits converts (184). Satisfying these conditions enables one group to culturally imperialize the ethnic groups they conquer, and the Roman Empire met all three of these conditions. First, the Roman Empire conquered many lands and peoples, and the Latinized dominant culture spread due to the political hegemony of the imperial regime over the local provinces (Van der Vliet 2003, 268). Second, life in the Roman Empire provided various reasons for migration, such as pursuits of economic prosperity, military mobilization, or slavery, all of which facilitated the interaction between Romans and other ethnic groups under imperial rule (Price 2012, 5). In fact, Issawi (1989) goes as far as to suggest that the migration of "culture-bearers from the core to the outlying parts… was probably in the hundreds of thousands" in the Roman area (184). Third, the state imperial cult, as well as Christianity (after Emperor Constantine's 313 AD Edict of Milan), promoted the unification of several ethnic factions within the empire under religious pretenses that, consequently, helped promote Roman cultural imperialism (Price 2012, 6). Therefore, the Roman Empire was qualified to overlay the existing cultures belonging to the various ethnic groups that were conquered by the imperial regime.

Furthermore, Issawi (1989) states that the widespread practice of Christianity helped increase the chances of successful cultural imprinting for Romans in three additional ways. The first way Christianity helped promote the cultural imperialism of the Romans was by deifying the Latin language. Issawi (1989) argues that "…in addition to being the language of administration and culture, Latin became the language of religion and salvation" (185). This allowed Roman Culture to be more widely accepted, as adopting the language was a prerequisite for practicing Christianity. Additionally, Christianity facilitating the dissemination of Roman culture to the masses as religion was more accessible than the elitist aspects of Roman high culture, such as "law, literature, philosophy, the natural and human sciences, music, and the visual arts" (Issawi 1989, 180). Lastly, religion not only promoted cultural imperialism but it also increased the longevity of cultural imprinting by making society more resilient to cultural shocks, like those of the barbarian invasions in the Western Roman Empire (Issawi 1989, 184). The use of religion as a tool to disseminate the Latinized culture of imperial Rome helped facilitate cultural imperialization onto provincial subjects. The theoretical framework that determines the neces-

sary conditions for cultural imprinting, as explained by Issawi (1989), indicates that the Romans met all of the requirements to successfully imperialize the cultures of their subjects who originally belonged to separate ethnic groups.

Economic Influence on the Roman Empire

Alternatively, it cannot be said that the Roman Empire was never the *recipient* of cultural influence from other cultures or political entities. Trade in the eastern hemisphere influenced the cultural imprinting dynamic between the Roman Empire and successful, external trade networks. In Matthew Fitzpatrick's 2011 article, "Provincializing Rome: The Indian Ocean Trade Network and Roman Imperialism," he describes the interaction between Rome and various trading entities that all contributed to the global economy. Roman elites were often on the receiving end of goods that pertained to high culture, as Issawi (1989) calls it, for they imported many "ostentatious commodities" (Fitzpatrick 2011, 32). This constant and disproportional influx of foreign, luxury goods challenged the traditional culture of stoicism for the elite within the empire (Fitzpatrick 2011, 32). The popularization of luxurious eastern items symbolizes a shift in the cultural values of Roman citizens from practical to materialistic, which Pliny the Elder, author of *Natural History*, claimed was deleterious to Rome both morally and financially. Fitzpatrick (2011) explains that Pliny's high estimate of the trade deficit, 100 million sesterces per year, from the Roman economy to eastern trade holds some credibility, but the estimate accounted for such a small percentage of the total GDP of Rome, 10 billion sesterces, that it was sustainable and largely unworrying (31). Therefore, the impact of foreign trade on Roman culture was noticeable despite evidence that it had a negligible impact on the economy.

However, this lack of concern for the deficit did not translate to a lack of interest in eastern prospects. Fitzpatrick (2011) recounts various military campaigns in Asia, Arabia, and Africa where Rome attempted to conquer various prosperous trading kingdoms. Trajan, the Roman governor of Syria, led the successful campaign to take control of the Nabataean trade kingdom, which gave Rome reign over two cities that were necessary for an eventual campaign to control Parthia, Rome's "military and commercial rival" (Fitzpatrick 2011, 39). On the other hand, Rome, under the command of Aelius Gallus, failed to secure Arabia and, under the command of Cornelius Gallus, failed to conquer Adulis of the African Axumite trade network (Fitzpatrick 2011, 51-52). Perhaps shockingly, the incredibly wealthy India was never a target of Roman conquest despite previously successful leaders, like Trajan, being available for military action (Fitzpatrick 2011, 40). Leading to both successes and failures, the influence of economic prosperity from other groups guided the politics and, consequently, military action of the imperial regime that sought control over these prosperous entities.

While the empire could not dominate all external trade networks that piqued its interest, aristocratic individuals from Rome financially benefited from the allure of those prosperous regions, which created the opportunity for ethnic outsiders to culturally influence Romans. Fitzpatrick (2011) details the endeavors of money-lending imperial aristocrats who increased their wealth by collecting interest from Romans wishing to travel to various trading centers (40). The fact that funding expeditions to external locales was so profitable indicates that these voyages were widespread and potentially reached far and wide if they needed to source such large funds; so Roman citizens and subjects were most likely bearing witness to a variety of different cultures and interacting with diverse ethnic groups while on their journeys. Thus, the Romans imported many commodities from other locales that influenced the high culture of the elites and aristocrats; and the economic prospects of eastern networks, confirmed by Roman political and military interest, attracted Roman travelers, which led to additional interaction with foreign cultures. Thus, despite the propensity to imprint on different ethnic factions, the Romans were also the receivers of some aspects of cultural influence by others, such as the people in the

East participating in the Indian, African, and Arabian trade networks.

Religion Evidences Cultural Coexistence

Some facets of culture were extremely resilient to imperialization, and neither the Romans nor their cultural counterparts could influence the other. Religion presents one such example, and "Religious Mobility in the Roman Empire" by Simon Price (2012) explains the unique dynamic between religious cults and different crowds. After successful colonization, imperial expansion enabled a network of movement and migration for Roman citizens and subjects alike. Price (2012) states that the reasons for migration included commercial pursuits, military mobilization, and enslavement (5), and the resulting movement put various ethnic groups in closer proximity to each other, which increased the amount of cultural interaction between them. For example, Roman citizens who represented the Roman cults established "coloniae" in imperial provinces and maintained their traditional practices and rites after moving, to emphasize their Roman heritage and values of nationalism (Price 2012, 3-4). Similarly, Jewish communities founded in different regions of the Roman Empire due to economic pursuits or enslavement presented an "adherence to eastern origins" surrounding their religious practices, evidenced through "both language and iconography" (Price 2012, 4). The framework for mobility provided by the imperial networks increased the range of people and their native cultures throughout the empire.

The resulting migration of various types of culture-bearers facilitated the diffusion of culture into different regions of the empire and gave rise to the opportunity for cultural imprinting, but neither Roman nor outsider dominated in these cultural interactions. The Romans took this opportunity by imposing their Latinized religion onto the imperial subjects from different cultures. Price (2012) describes how Roman rule subjugated the pre-Roman cults by transforming the nomenclature of the local, ancestral cults to include the names of gods from the Roman pantheon (6). However, the practitioners of these ancestral or elective cults in turn used this reference to Roman tradition to legitimize their native cults. The Cult of Jupiter Dolichenus expanded upon the new nomenclature and became the Cult of "Jupiter Optimus Maximus Dolichenus" and "implicitly asserted the over-arching position of the deities of Doliche, perhaps in competition with the Roman state cult" (Price 2012, 16). Though this suggests that Roman subjects protected their ancestral religions from imperial suppression, some subjects had to adapt their practices to conform to Roman cultural standards. Elective cults were said to lack tradition and heritage and, therefore, virtue (Price 2012, 17), and Christianity was a victim of this Roman discrimination against elective cults. In response, some followers portrayed Christianity as an ethnic religion to gain social acceptance in their new environment while maintaining their personal religious values (Price 2012, 17). Although pressure from Roman culture influenced action from ethnic and elective cults, the followers maintained their autonomy and preserved their cultures against Roman imperialism. Alternatively, followers of the Roman state cult and other local cults displayed this same resilience when practitioners of different religions worked to diffuse their beliefs across the empire as well. Therefore, the Roman Empire did not culturally imprint upon their subjects in terms of religion. The Roman subjects did not fully adopt the religious culture of Rome nor did they abandon their ancestral or elective cults, and the local cults did not manage to extensively expand their influence to the Romans until the rise of Christianity much later in the chronology of the empire.

Roman Influence on Politics and Social Mobility

On the other hand, one aspect of culture that the Romans did completely dominate was politics. Alexander Skinner (2013) analyzes the opportunities for Roman citizens and subjects

to transcend the social hierarchy in his work, "Political Mobility in the Later Roman Empire." The first argument that Skinner (2013) makes is that the terminology for economic and career mobility needs to be qualified as political as opposed to social, for the mobility did not occur between social classes but inside them (19- 20). The patterns of this mobility thus indicate that there was not a workplace-facilitated interaction between members of each social class as peers in society, for one's career depended on their social identity. Thomas Erikson (2010) gives significance to this social dynamic in his book, *Ethnicity and Nationalism: Anthropological Perspectives*. To expand on the ethnic identity of the Romans as presented by Van der Vliet (2003), Erikson (2010) explains that among members of the same ethnic group, there are criteria that define further categorizations of ethnicity in horizontal and vertical dimensions. For the situation regarding Roman citizens and subjects, we need to examine the vertical scale of ethnicity as we are examining power relations in one society (Eriksen 2010, 58). Van der Vliet (2003) provides the framework used amongst the Romans to further distinguish the differences among members of the same ethnic classification. He states that the additional distinctions for identity are dependent on one's proximity to "the center of power," which is the emperor (Van der Vliet 2003, 271). This arrangement defines the division between Roman citizens and subjects alike based solely on their social class and labor contribution, which dictates proximity to the emperor. The new ethnic divisions theorized by Erikson (2010), and exemplified by Van der Vliet (2003), show that new groups are created under the same ethnic category based on economic and political positioning in society.

Thus, any cultural differences among the vertical scale of ethnicity understood in the Roman Empire indicate that there are new interacting factions that can be subjected to cultural imperialism by another. Based on the evidence from Van der Vliet (2003), we know that the new interacting groups were the different social classes, and we can determine cultural differences between them that would allow for cultural interaction. One example of cultural differences between the social classes is how the elites versus the commoners valued stoicism. As previously evidenced through Pliny's sentiments, members of the lower social strata disdainfully viewed the Roman elites' inflated consumption of luxurious Eastern commodities (Fitzpatrick 2011, 32). The indicated difference in consumption habits and accompanying sentiments illustrates a disconnect in cultural values between those belonging to the wealthy and to the lower classes. Additionally, the success of a Cult of Mithras, due to the collaboration and shared theology of Roman citizens identifying with the same social positioning, indicates that there were shared cultural values among the group. Price (2012) describes the successful recruitment for the Cult of Mithras in the city of Virunum based on the efforts and personal contacts of the followers, and he explains that the cult followers were all "modestly successful" as they collectively funded the renovation of their 'temple' as a group without the benefaction of any particular individuals (9-10). This example shows that different Roman citizens of the same social class exhibited similar cultural values, for they all subscribed to the Cult of Mithras and worked together to accomplish their collective goals of renovation and recruitment. These examples indicate that there were distinct aspects of culture differentiating the social classes in the Roman Empire, which allowed for the possibility of cultural imperialism among the various groups.

The opportunity for cultural imperialism among the different social classes was taken by the aristocratic imperial regime who determined the political culture for all in the empire. As previously stated, Skinner (2013) claims that the opportunity for political mobility was limited to the highest stratum of Roman society as this mobility was an "internal oligarchic phenomenon" (32). Skinner (2013) supports his argument by refuting the claims of other scholars who argue that social mobility can be witnessed in the Eastern Empire by discussing the examples of Quirinus, Pelagius, Domitius, and Eumolpius (45-47), all of whom depict mobility that was occurring within their original social class, the curial stratum. This sentiment regarding the internal phenomenon is further endorsed by the recruitment of provincial aristocrats and mem-

bers of curial families to fill in the political positions opened by outgoing imperial aristocrats. Skinner (2013) states, "...it was from the wealthy magnate families at the apex of provincial life that easter senators in general were recruited" (28), and he substantiates this claim with the historical evidence of "persistent regulation" of the "recruitment of persons from curial families into imperial service" presented by the Theodosian Code (38-42). The recruitment of provincial elites indicates that the political mobility of aristocratic subjects was more important to the imperial regime than the social mobility of Roman citizens from the lower classes.

The political hegemony of the highest social class had real political impacts, which translated into cultural imprinting as the ruling class determined their political stance based on their prospects for financial gain and maintaining their status. Fitzpatrick (2011) details how even the most austere politicians, like Cato the Elder (234-149 BCE), relied on the Roman trade and commerce policies for personal wealth and resisted political measures to reduce the greed of the senatorial class (35). Thus, the domination of politics by one social class dictated the course of Roman politics, an important facet of culture, which was enforced for Roman citizens and subjects of all social classes even if they did not share the same views. The analysis of political mobility by Skinner (2013) proves how the dominant Roman culture dictated by the elite was imprinted onto all of the imperial citizens and provincial subjects, displaying the clear cultural imperialization of the Roman Empire regarding politics.

Conclusion

Therefore, after examining the distinctions between ethnic groups and the framework for cultural imperialism, it is evident that the Roman Empire as we remember it today was the result of dynamic cultural influence from and onto external entities. This assessment of the cultural imprint both on and of the Roman Empire highlights the important but often neglected dynamic of influence on a dominant culture from a less dominant one. The theories of identity and ethnicity allowed us to understand how the conditions for cultural imprinting were fulfilled by the power dynamics between the different interacting factions, Romans and outsiders, to enable cultural imperialism. Moreover, the scholarship on trade, religious mobility, and political stratification informs our understanding of the results of cultural interaction between the Roman Empire and their provincial subjects as well as neighboring entities.

Recognizing this pattern can allow us to properly appreciate and remember the contributions to larger geopolitical interactions from societies without as much geographic or historical expansiveness as the Roman Empire. Further, I believe that this kind of research will be necessary to improve our understanding of contemporary geopolitics by emphasizing the contribution to the current state of popular culture from minority and otherwise oppressed cultural groups. Moreover, the analysis of culture based on fluctuating relationships of domination and submission will further our understanding of contemporary power dynamics between interacting entities, which will better equip us to promote equality and equity in modern times.

References

Eriksen, Thomas Hylland. 2010. *Ethnicity and Nationalism: Anthropological Perspectives*. London: Pluto Press.

Fitzpatrick, Matthew P. 2011. "Provincializing Rome: The Indian Ocean Trade Network and Roman Imperialism." *Journal of World History 22*(1): 27–54. https://doi.org/10.1353/jwh.2011.0016.

Issawi, Charles. 1989. "Empire Builders, Culture Makers, and Culture Imprinters." *The Journal of Interdisciplinary History 20(*2):177–96. https://doi.org/10.2307/204831.

Price, Simon. 2012. "Religious Mobility in the Roman Empire." *The Journal of Roman Studies 102*: 1–19. https://doi.org/10.1017/S0075435812000056.

Skinner, Alexander. 2013 "Political Mobility in the Later Roman Empire." *Past & Present 218*: 17–53. https://doi.org/10.1093/pastj/gts044.

Van der Vliet, Edward Ch. L. 2003. "The Romans and Us: Strabo's Geography and the Construction of Ethnicity." *Mnemosyne 56(*3): 257–72. https://doi.org/10.1163/156852503768181031.