FAURJ

Florida Atlantic Undergraduate Research Journal

Volume 14 | Spring 2025
www.fau.edu/ouri

ACKNOWLEDGEMENTS

FAURJ would like to recognize the numerous volunteer faculty reviewers who helped make the journal possible. We sincerely thank you for your time and service.

We would like to thank the Office of Undergraduate Research and Inquiry, the Division of Research, and the University Libraries for their support.

FAURJ

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LETTER FROM THE EDITOR

This fourteenth edition of the *Florida Atlantic Undergraduate Research Journal* (FAURJ) is truly special, as we bring you the largest volume yet.

We sincerely thank the students who contributed their manuscripts for this edition. Their intellectual curiosity and exceptional research efforts make this volume truly remarkable. A special thanks goes to our university's faculty, whose generosity and expertise have been invaluable in peer-reviewing each submission. Their commitment to scholarly excellence has ensured the academic rigor and quality of this edition.

We invite you to explore the intellectual depth within these pages. This edition reflects the vibrant research culture at Florida Atlantic University, and we hope it serves as a testament to the talent and determination of our undergraduate researchers.

May it inspire a deeper appreciation for both academics and research exploration.

Sincerely,
Ana Tyulmenkova

COVER DESIGN ARTIST STATEMENT

"Serenity in Spots"

This close-up photograph captures a cheetah in a state of deep sleep, providing invaluable insight into its resting behavior in a controlled environment. Its sleek, spotted fur is in sharp focus, with black spots beautifully contrasted against the light ochre coat. This behavioral study focused on the resting habits of predators like those of cheetahs to examine how their sleep patterns reflect their stress levels and adaptability with respect to the environment. Observing it in its most vulnerable state reminds us that even the fastest animal on the planet must pause, rest, and adapt—just as nature itself finds balance in moments of stillness and motion.

Shreyansh Patel
Charles E. Schmidt College of Science

Financial Literacy: An Inside Look on Americans' Investing Habits

College of Business, Florida Atlantic University
Alexander Georgiev, Mr. Eric Levy (Faculty Advisor)

Abstract

There has been a large influx of investment platforms and increasing opportunities over the last century, with investment becoming much more widespread and heavily advertised. It is important for Americans to be knowledgeable about different investing opportunities; thus, this study aims to reveal the different factors that may affect someone's investing choices. It was anticipated that those with more education and younger people would be more comfortable in and likely to invest and save for retirement. Using an 18-question survey, this study was sent out to United States citizens over the age of 18 through Amazon Mechanical Turk. Receiving 202 responses, the data was then analyzed using SPSS. These findings can be important when addressing the need to focus on teaching older Americans about investing and financial literacy. They also can show that there needs to be more of a focus on informing those with less financial education about the importance of retirement savings.

Introduction

Financial literacy is defined as the knowledge one has to make good financial decisions by effectively using different financial skills and concepts. Financial literacy is only required to be taught in 25 of 50 states and has presented itself to be a major problem among the American population especially within the younger generation (Chen & Volpe, 1998). Without basic financial literacy knowledge, people struggle to make good decisions when investing and planning their savings for retirement, as they tend to turn to friends and family for advice instead of financial advisors (Mitchell & Moore, 1998). The lack of education in financial literacy leads to potential overconfidence when investing and eventually a troublesome retirement, as it has not been planned for effectively (Munnel et al., 2014). Even if young people and high schoolers are exposed to financial literacy courses, it is seen that they are ineffective, and there must be a greater focus

on improving the financial education (Mandell & Klein, 2009). This study aims to find the link between the answers to investing assumptions and which demographic profile each assumption correlates with in order to create a better system for teaching financial literacy in the future. This study tries to correlate the different findings of previous studies and find the connection between the results by linking retirement savings with education level to provide a reason for "overconfidence", which is being addressed with the other question regarding age and confidence levels.

Financial security, the confidence that one's personal finances are set up for the future, has led many people to partake in investing. Investing has grown in popularity over recent decades due to the increased accessibility and number of platforms that it is available on, such as Robinhood, Fidelity, Acorns, and SoFi. The growth of other investment pieces, such as NFTs and cryptocurrencies, has captivated the younger generation and made it extremely easy and accessible for first-time young users. The idea that everyone can invest freely and have the potential to make money and set themselves up for the future is very appealing to people, which in turn leads to overconfidence (Manan et al., 2023). Such overconfidence stems from a lack of knowledge when it comes to personal financial literacy. Overconfidence can prove devastating for many, as investing money without the proper knowledge about what one is doing can and usually leads to big losses of money (Pikulina et al., 2017). This study expects to find many people confident, especially the younger generation, in their investing abilities when, in reality, they lack financial knowledge to back up investment decisions, demonstrating overconfidence in their decisions.

The importance of saving for retirement has also been overlooked more and more by younger generations. This study aims to answer questions regarding retirement savings by age group to find a correlation between the assumed overconfidence of younger generations and retirement savings. A study by Munnel et al. (2014) recommends the typical household save about 15% of their earnings if they begin at the age of 25 and can retire at the age of 62. However, this rate almost doubles to 24% of income if started at the age of 35 and 44% of income if started at the age of 45. Currently, about half of households are not saving enough, and an increasing amount of Americans do not have adequate savings to live a comfortable life after retirement (Munnel et al., 2014). Based on this literature, the expected results are to find that many people surveyed are going to be below what the recommended savings amount for retirement is. This study anticipates seeing that

young Americans lack financial knowledge and awareness in planning and saving for retirement early, which can end up as a costly mistake (Navickas et al., 2014).

Materials

An 18-item multiple choice questionnaire was administered in which six demographic questions were included to gain information on gender, age, race/ethnicity, political affiliation, household income, and level of education. The other 12 questions focused on assessing people's comfort/knowledge about investing in stocks and cryptocurrencies (e.g., "How comfortable do you feel investing considering your financial situation?"), retirement plans/savings, and the amount they had saved for retirement (e.g., "How much money do you have saved for retirement?").

Questions focused on comfort and knowledge used a three-point Likert scale of *very*, *somewhat*, and *not very* (e.g. *very comfortable*, *somewhat comfortable*, *not very comfortable*). Questions focused on retirement savings used ranges (e.g. *$0-$50,000*; *$50,001-$100,000*; *$100,001-$150,000*; *$150,001+*). Amazon Mechanical Turk was used to distribute these questionnaires.

Procedure

After creating the questionnaire, the survey was distributed to people across the United States over the age of 18, who earned a small compensation for completing the questionnaire. After receiving 202 responses to the survey, the results were analyzed through IBM SPSS. Through SPSS, the data was weighted to reflect the American population's demographics and provide a representative survey of the United States based on the 2020 Census (U.S. Census Bureau, 2020).

Figure 1
Sample Questionnaire

Sample Questions Administered
How knowledgeable are you about the stock market?
Do you have investments in stocks, bonds or mutual funds excluding those held in an IRA (Individual Retirement Account) or 401K (profit-sharing plan that allows employees to contribute a portion of their wages to individual accounts)?
Do you have a financial advisor?
Do you have a 401k plan, IRA (Individual Retirement Account) or a similar kind of retirement account?
How comfortable do you feel investing in stocks considering your financial situation?
How knowledgeable are you about cryptocurrencies?
Do you own any cryptocurrencies?
How comfortable would you feel accepting your wage in cryptocurrencies if your employer offered?
How comfortable do you feel investing in cryptocurrencies considering your financial situation?
Do you invest outside of stocks/crypto (e.g., Real Estate, Non-Fungible Tokens, Savings Account, Bonds etc.)
How much money do you have saved for retirement?
When considering investing, how much risk are you willing to accept?

Notes. The 12 questions that were distributed through Amazon Mechanical Turk focused on assessing people's comfort/knowledge investing in stocks and cryptocurrencies.

Results

We focus on two questions from the survey in our analysis: "How comfortable do you feel investing in stocks considering your financial situation?", and "How much money do you have saved for retirement?". Both questions yielded statistically significant results (p = .01) after doing crosstabulation analysis.

The survey received 42 responses from 18 to 29-year-olds, 68 responses from 30 to 49-year-olds, and 92 responses from 50 to 64-year-olds. When asked about comfort when considering their financial situation, 37% of respondents reported being very comfortable while 62% reported being only somewhat comfortable. However, when

looking at this question compared to age, 69% of 18- to 24-year-olds reported being very comfortable, while only 31% of them reported being somewhat comfortable. The 50- to 64-year-olds, on the other hand, only had 17.2% reporting they were very comfortable and 82.8% reporting being somewhat comfortable.

Figure 2

Frequency Results of the Question "How comfortable do you feel investing in stocks considering your financial situation?"

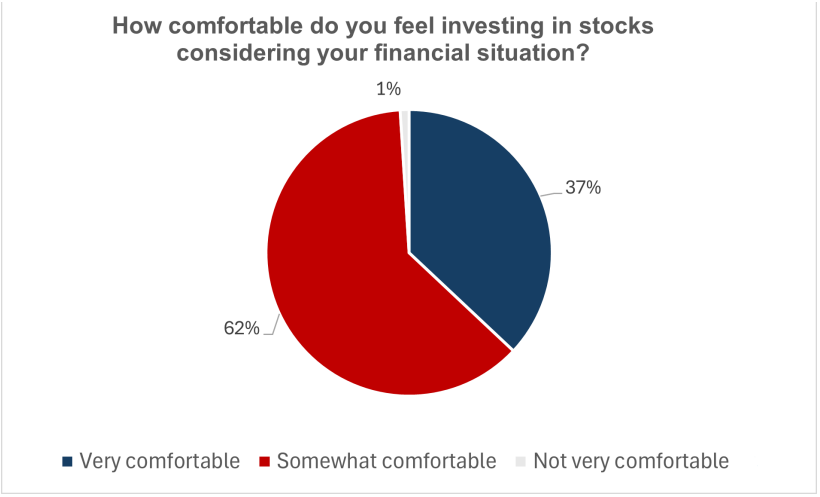

Figure 3

Crosstabulation Results between "How comfortable do you feel investing in stocks considering your financial situation?" and the Age of the Respondent

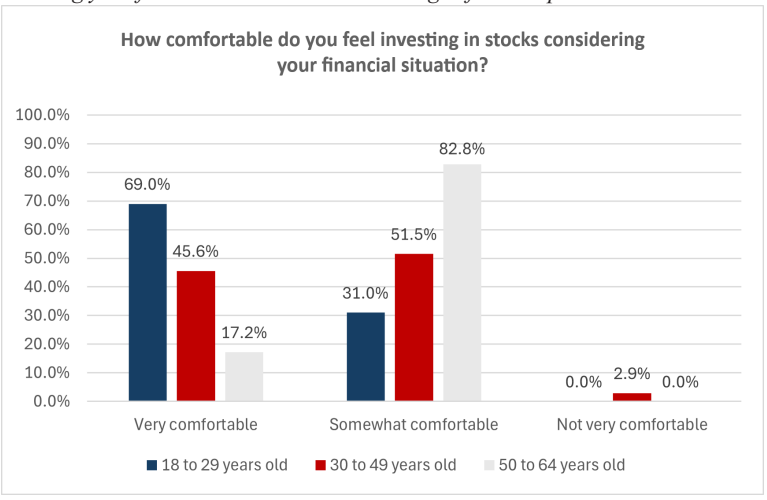

Table 1
Numerical Results from the Crosstabulation between "How comfortable do you feel investing in stocks considering your financial situation?" and the Age Range of the Respondent

			Comfort level investing in stocks			
			Very comfortable	Somewhat comfortable	Not very comfortable	**Total**
Age range	18 to 29 years	Count	29	13	0	42
			69.0%	31.0%	0.0%	100.0%
	30 to 49 years	Count	31	35	2	68
			45.6%	51.5%	2.9%	100.0%
	50 to 64 years	Count	16	77	0	93
			17.2%	82.8%	0.0%	100.0%
Total		Count	76	125	2	203
			37.4%	61.6%	1.0%	100.0%

The survey received 11 responses from people who have done only some college, 147 responses from people who have a college degree, and 42 responses from people who have a postgraduate degree or higher. When asked "How much money do you have saved for retirement", 30% of respondents reported having $0 to $50,000; 49% of respondents reported having $50,001 to $100,000; 20% of respondents reported having $100,001 to $150,000; and only 1% of respondents reported having over $150,000 saved for retirement. However, when looking at this question compared to age, all of the respondents who attended only some college reported having $0 to $100,000; and 81.2% of them reported having only between $0 to $50,000 saved for retirement. Respondents who obtained a postgraduate degree or higher education, however, had largely different responses. Each respondent with a postgraduate degree or higher reported having over $50,000 saved for retirement and 23.8% of them reported having over $100,001.

Figure 4
Frequency Results of the Question "How much money do you have saved for retirement?"

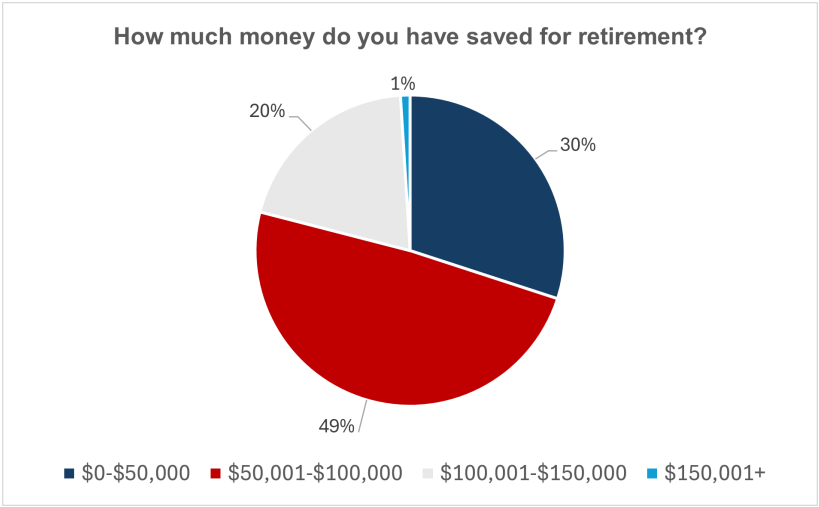

Figure 5
Crosstabulation Results between "How much money do you have saved for retirement?" and the Education Level of the Respondent

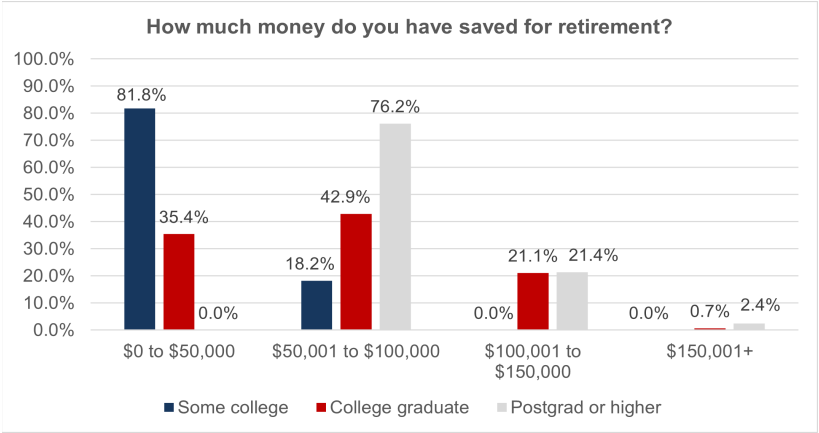

Table 2
Numerical Results from the Crosstabulation between "How much money do you have saved for retirement?" and the Education Level of Respondents

			Range of retirement savings					
			$0 to 50,000	$50,001 to 100,001	$100,001 to 150,000	$150,001+	I don't know	**Total**
Education level	Some college	Count	9	2	0	0	0	11
			81.8%	18.2%	0.0%	0.0%	0.0%	100.0%
	College graduate	Count	52	63	31	1	0	147
			35.4%	42.9%	21.1%	0.7%	0.0%	100.0%
	Postgrad or Higher	Count	0	32	9	1	0	42
			0.0%	76.2%	21.4%	2.4%	0.0%	100.0%
Total		Count	61	97	40	2	0	200
			30.5%	48.5%	20.0%	1.0%	0.0%	100.0%

Discussion

This study is important when looking at future financial education as it shows the value of financial literacy education and the dangers that may come about if one is not making educated decisions with their money. For example, studies have suggested that people with lower financial literacy (higher demand for financial education) tend to rely more on informal moneylenders, or rather, predatory practices (Valenzuela et al., 2023).

The study finds that an overwhelming majority of young people feel confident in their investing abilities when considering their financial situation compared to the older generation. This can be attributed to three factors: 1.) Overconfidence in the younger generation, 2.) Frugal spending by the older generation as they near retirement, and 3.) The older generation is less financially aware and financially educated than the younger generation. When looking at overconfidence in the younger generation, it becomes apparent that they are much more willing to take risks and invest their money due to feelings of confidence. As Manan et al. (2023) explains, "investment is a way to become rich faster," (p. 141). and these promising words captivate a young audience into believing that the reward will outweigh the risk. Even if their knowledge of investments is not particularly great, the dream of getting rich quickly to live in luxury blinds younger generations and plays a large role in their confidence levels (Manan et

al., 2023). One place many people look is social media accounts and websites that promote investing and different "strategies" to invest and get rich quick, however many other people also look to friends and family instead of to trained professionals (Mitchell & Moore, 1998). These sources of information tend to be unreliable, but very attractive to the younger generation who are willing to take the risk to invest in cryptocurrencies, NFTs, and stocks, even though they have no prior financial education informing their investment decisions.

The second factor that pertains to the question "How confident are you investing in stocks considering your financial situation?", is the idea that the older generations may be more frugal with their money as they approach the age of retirement. As people approach retirement age, naturally they will begin to take on less risk with the money that they have so that they have enough to last through their time in retirement (Mitchell & Moore, 1998). Lastly, it is possible that the older generation simply does not feel as confident as the younger generation investing due to lower standards of financial education. The Financial Literacy and Education Commission, for example, was only created in 2003 with the aim of bringing more financial literacy to the United States. Based on the education between generations, the younger generation is more likely to have had experience in financial literacy and therefore feel more confident.

When looking at the second question analyzed "How much do you have saved for retirement?", and comparing it with education level, it was evident that an overwhelming majority of those with more education (graduate degree or higher) had significantly higher amounts saved for retirement than those with some college or less. The idea that on average that those with more education tend to earn higher salaries than those with less education can lead to these results when saving for retirement. However, when looking at the recommended amount of savings, it is seen that few respondents, regardless of educational attainment, were even nearly close to the recommended savings. On "average workers need to accumulate investments of $538,000," (Munnel et al., 2014, p. 4–5). When looking at how much people responded having saved, few people fall close to $538,000 with 79% of all respondents reporting having only between $0 to $100,000. Furthermore, with few people being in the range of a "comfortable retirement", this can explain why older people seemed to be more "frugal" and "uncomfortable" with their investing. Had they accumulated more savings, the situation may have been different,

and the older generation may not have to had worried to worry about being frugal.

Overall, this study looks into understanding investing habits between different groups of people and what may inform these habits and tendencies. It is important for people to be financially literate and aware of their capabilities and situation. In the future, continuations of this research can include further analysis of what specific types of education people have received and why people make the decisions they do regarding their financials.

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The Relationship Between Self-Reported and Observer-Rated Maternal Anxiety During Mother-Infant Interactions

Charles E. Schmidt College of Science, Florida Atlantic University
Annalisa Tran, Jacqueline Hammack,
Dr. Teresa Wilcox (Faculty Advisor)

Abstract

Research pertaining to maternal anxiety and mother-infant interactions is a rapidly growing field. The purpose of this study is to investigate the relationship between self-reported maternal anxiety ratings and observer-rated maternal anxiety scores. Thirty-nine mother-infant dyads participated in a four-minute free-play session via Zoom. The Parent Anxiety measure from the Coding Interactive Behavior scale was used to measure observed maternal anxiety. The General Anxiety Disorder-7 scale was used to measure self-reported maternal anxiety. No significant association was found between self-reported maternal anxiety and observed maternal anxiety. An exploratory analysis was conducted to compare self-reported maternal anxiety and observed maternal sensitivity. A trending association between maternal sensitivity and an infant's age was identified, but a strong association cannot be confirmed without further research. These findings highlight the complexity of anxiety and its role in mother-child interactions and emphasize the importance of contributing to this growing field.

Introduction

Mother-infant interactions are integral to child development, as they strengthen the mother-infant bond and create a stimulating environment for the infant to grow in (Reck et al., 2018). Maternal anxiety has been shown to influence the quality of mother-infant interactions. Despite there being fewer studies surrounding maternal anxiety when compared with other psychosocial maternal factors such as depression, studies about maternal anxiety in relation to mother-infant interactions are a growing field (Lemus et al., 2022). There is

great concern about the future behavioral and developmental outcomes of children of anxious mothers (Hakanen et al., 2019). In terms of behavioral outcomes, it has been observed that infants (4 months of age) of anxious mothers exhibited greater negative reactivity when assessing behavioral reactivity to novelty (Davis et al., 2004). With regards to neuroendocrine systems, maternal anxiety can alter the activity of the maternal hypothalamus-pituitary-adrenocortical (HPA) axis, and infants (between 1 to 20 weeks in age) who were exposed to greater levels of maternal HPA axis hormones, such as cortisol, during fetal development displayed greater temperamental variation, such as fussing or difficult behavior (Davis & Sandman, 2006; de Weerth et al., 2003). When assessing anxiety disorder development risk in adolescents (14 to 17 years in age), adolescents of anxious mothers showed greater risk of developing an anxiety disorder compared to adolescents of control-group mothers (Schreier et al., 2008). In terms of developmental outcomes, longitudinal studies show that infants (6 to 30 months of age as the studies progressed) of anxious mothers displayed greater feeding and sleeping problems compared to infants of control-group mothers (O'Connor et al., 2007; Petzoldt et al., 2015). These studies used different measures for maternal anxiety and still observed similar results, further supporting the concern for the behavioral and developmental outcomes of children of anxious mothers.

Maternal sensitivity and maternal intrusiveness are commonly observed qualities of maternal behaviors during mother-infant interactions and have been shown to predict future behavioral and developmental outcomes in children. Maternal sensitivity can be defined as "the mother's warm and responsive style that provides the species-specific set of maternal behaviors, including the expression of positive affect, constant gaze, warm vocalizations, and affectionate contact, in a manner that is predictable and suited to the infant's moment-by-moment signals" (Feldman, 2010, p. 174). Maternal sensitivity has been shown to predict social adaptation, social maturity, and emotion regulation in children as they progress into adolescence and adulthood (Feldman, 2010). Maternal intrusiveness can be defined as "the mother's controlling style that overstimulates the child and imposes the maternal agenda" (Feldman, 2010, p. 175). Maternal intrusiveness has been shown to predict maladaptive behavioral outcomes in children and adolescents, such as higher risk for substance abuse, feeding disorders, low cognitive competence, and impaired ability to interact socially with strangers (Feldman, 2010).

Thus, when studying the influence of maternal anxiety on mother-infant interactions, maternal sensitivity and intrusiveness are often measured.

Currently, the literature shows conflicting findings about the influence of maternal anxiety on mother-infant interactions and maternal behaviors. Some studies show that maternal anxiety is associated with more maternal intrusiveness and less maternal sensitivity during object-play mother-infant interactions with infants at 6 months of age (Wijnroks, 1999). This may be due to anxious mothers being too active, protective, or controlling during the mother-infant interactions, which can result in the mother missing or misinterpreting infant cues. Another study utilized the face-to-face still-face paradigm to study interactions between anxious or control-group mothers with their infants at 4 months of age. This paradigm is a procedure that consists of three phases: 1) the initial play phase, where the parent interacts and plays with the child normally; 2) the still-face phase, where the parent is instructed to maintain a still and unresponsive face and to not interact with the child; and 3) the reunion phase, where the parent is instructed to resume interacting and playing with the child normally (Asselman et al., 2018). The authors found that infants of anxious mothers displayed negative interactive behaviors such as distancing and self- or object-touch more often than infants of control group mothers (Asselman et al., 2018). This study concluded that infants of anxious mothers may have exhibited more negative interactive behaviors during the still-face phase compared to the initial-play phase because they noticed a change in their mother's behavior. Since anxious mothers tend to be overactive and overly enthusiastic, these infants may have been distressed or confused during the still-face phase of the experiment in which the mothers were required to abruptly display a still, unsmiling face.

Conversely, there have been studies that concluded that maternal anxiety is associated with greater maternal sensitivity and even greater behavioral synchrony during mother-infant interactions. Another study that used the face-to-face still-face paradigm observed that infants (at 6 months of age) of anxious mothers fared better during the reunion phase compared to infants of control group mothers (Kaitz et al., 2010). Since anxious mothers tend to show inconsistent affect, this results in infants who are accustomed to frequent changes in behavior. Therefore, when the anxious mothers abruptly changed from being engaged and interactive to becoming still and unsmiling, this change did not affect the infants as much as the other infants from

the control group. Furthermore, the anxious mothers would tend to overcompensate during the reunion phase to make up for perceived loss of behavioral synchrony during the still face phase.

Due to these mixed findings, some studies have noticeably shifted from comparing anxious mothers with control group mothers. Some researchers argue that anxiety is more nuanced than originally thought; rather than just considering a mother as being "anxious" or "not anxious," it may be that anxiety exists as a spectrum. Thus, studies began investigating severity of maternal anxiety. One study compared the quality of mother-infant interactions between infants at 3 months of age and mothers with low, moderate, or high anxiety. Interactions between mothers with moderate anxiety and their infants portrayed greater behavioral synchrony compared to interactions between infants and mothers of low or high anxiety (Lemus et al., 2022). The authors concluded that moderately anxious mothers were more likely to adequately maintain awareness of infant cues to achieve greater reciprocity. High-anxiety mothers would hyper-fixate on infant cues to the point of excessiveness while low-anxiety mothers would display discontinuous awareness of infant cues. These results were similar to the outcomes found in Nicol-Harper et al. (2007), which found that low-trait anxiety mothers displayed less intrusiveness compared to high-trait anxiety mothers during mother-infant interactions with infants aged 10 to 14 months. Other researchers have conducted studies observing how the timing of developing anxiety symptoms influences mother-infant interactions. When comparing mothers who developed anxiety symptoms prenatally to those who developed anxiety symptoms postnatally, mothers with prenatal anxiety, specifically developed during the third trimester, portrayed greater maternal intrusiveness during mother-infant interactions with infants at 8 months of age, while no relationships were found between postnatal anxiety and maternal intrusiveness (Hakanen et al., 2019). Toepfer et al. (2019) were cited by Hakanen et al. (2019) to explain this finding. During the third trimester, changes in oxytocin promoter DNA methylation are potential biomarkers for changes in maternal behavior and were associated with greater maternal intrusiveness (Toepfer et al., 2019).

The purpose of the current study is to investigate if there is a relationship between self-reported maternal anxiety ratings and observer-rated maternal anxiety scores. The mixed findings about the influence of maternal anxiety in mother-infant interactions could be due to the diverse methods of measuring maternal anxiety

throughout the past literature. Therefore, investigating the relationship between observed and self-reported maternal anxiety scores may identify the reliability of these measures and their ability to predict mother-infant synchrony. In the current study, we hypothesize that mothers will be able to accurately rate their anxiety and that trained behavioral coders will be able to accurately rate observed maternal anxiety; and that there will be an association between self-reported maternal anxiety ratings and observer-rated maternal anxiety scores. In Nath et al. (2019), when mothers were asked to rate their perceived ability to bond with their infant at 3 months of age, anxious mothers were more likely to score themselves as having greater difficulty bonding with their infant. To our knowledge, this study will fill a gap within the literature by revealing inner and outer perceptions of anxiety behaviors during mother-infant interactions, and contribute to the growing understanding and study of maternal anxiety in mother-infant interactions.

Methods

Fifty-four mother-infant dyads were recruited for the experiment through word-of-mouth and social media advertisements. Dyads were excluded if the infant was born preterm (<35 weeks gestation, n = 2), had a low birth weight (<2500 grams, n = 3), had a protocol deviation (n = 3) or scored at risk on a developmental screening questionnaire (n = 7). A final sample of 39 dyads (N_{male} = 15) between the ages of 6 and 24 months (M = 14.77, SD = 6.03, range = 6.41 to 24.21) were assessed. The mother-infant dyads were asked to engage in a four-minute free-play session within the comfort of their own homes. Prior to this free-play session, mothers were asked to complete a demographic questionnaire. The free-play session was conducted and monitored using Zoom video conferencing software and was hosted by an undergraduate research assistant or a graduate student. During the free-play session, the mother and infant were told to "play as you normally would." After the session, mothers completed a developmental screening questionnaire (*Ages & Stages Questionnaire®, Third Edition*). Each *Ages & Stages* questionnaire is specific to an infant's age in months and the mothers were asked about their infants' communication, motor, problem-solving, and personal-social developmental milestones. When asked if her infant exhibited a developmental milestone, a mother would choose either *Yes*, *Sometimes*, or *Not Yet*. These responses are converted into scores, where *Yes* = 10, *Sometimes* = 5, and *Not Yet* = 0. The total response scores fall into a range between 0 and 60 for each milestone category (Squires & Bricker, 2009).

To obtain the observed maternal anxiety data, trained behavioral coders (average percent agreement = 89%, range = 73% to 100%) were instructed to watch the recorded Zoom videos and utilize the Coding Interactive Behaviors scale (CIB), which is a rating system consisting of a set of 44 scales that measure the various interactive behaviors between a mother and her infant (Feldman, 1998). The CIB scale is a well-validated scale with good psychometrics, including construct and predictive validity, measurement invariance, and test-retest reliability (Leclère et al., 2016; Stuart et al., 2023). The CIB scale has shown individual stability from infancy to adolescence and associations with social-emotional outcomes, hormonal and brain activation patterns, and physiological responses (Endevelt-Shapira & Feldman, 2023; Leclère et al., 2016). It has been widely used across multiple interactive partners and different cultures (Fahrer et al., 2024; Leclère et al., 2016). To our knowledge, the CIB scale has not yet been validated against other maternal self-report measures. The Infant Cognition Lab is one of the few labs that collects data in person and remotely. We have data demonstrating successful implementation of CIB coding to videos collected remotely, while also reporting some of the challenges (Hammack et al., 2023). The lab is currently working on a project that compares CIB and other types of behavioral coding across the two settings. The CIB scale that was utilized in this study was the Parent Anxiety scale, which measures the frequency or intensity of various anxiety behaviors that parents may display, such as excessive and inappropriate enthusiasm, fidgeting with fingers and hair, nervously looking to the camera, idiosyncratic or inconsistent behavior, long periods of silence, or shifts in emotional states (Feldman, 1998). The trained behavioral coders look for these behaviors and rate the interaction with a one-time global measure on a scale from 1 to 5, where 1 indicates that no anxiety behaviors were observed and 5 indicates that high frequency or intensity of anxiety behaviors were observed during the interaction.

To obtain the self-reported anxiety data, the mothers were asked to complete the General Anxiety Disorder-7 (GAD-7) within a week of participating in the free-play session on Zoom. The GAD-7 is a seven-item questionnaire that asks mothers about the frequency of self-perceived anxiety behaviors within the last two weeks prior to completing the GAD-7 questionnaire (see Table 1). Mothers would rate themselves from 0 to 3, where 0 indicates never experiencing a behavior and 3 indicates experiencing a behavior nearly every day. From the seven questions, the individual scores are compiled together

to calculate a total self-reported anxiety score ranging between 0 and 21, where 0 to 4 indicates minimal anxiety, 5 to 9 indicates mild anxiety, 10 to 14 indicates moderate anxiety, and 15 to 21 indicates severe anxiety (Spitzer et al., 2006).

Table 1
General Anxiety Disorder-7 (GAD-7) Questionnaire

Over the last two weeks, how often have you been bothered by the following problems?	Not at all	Several days	More than half the days	Every day
1. Feeling nervous, anxious, or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it is hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid, as if something awful might happen	0	1	2	3

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS). A multiple linear regression was conducted to examine the relationship between self-reported and observer-rated maternal anxiety ratings.

Results

A multiple linear regression was conducted to predict CIB-rated anxiety scores (M = 1.32, SD = 0.57) from self-reported GAD-7 anxiety levels (M = 1.59, SD = .91) while accounting for infant age (M = 14.77, SD = 6.03; see Figure 1). The overall model was not significant (r^2 = .061, F(2,36) = 1.174, p = .321). Neither GAD-7 scores (β = -.176, t = -1.080, p = .287) nor infant age (β = .151, t = .925, p = .361) were significant predictors.

An exploratory analysis was conducted to compare self-reported maternal anxiety ratings and observed maternal sensitivity ratings to

determine if the results would support the suggestion in the literature that there is a possible relationship between maternal anxiety and maternal sensitivity. Observed maternal sensitivity was measured using a composite score that was obtained from multiple CIB scales such as acknowledging, imitating, elaborating, parent gaze/joint attention, positive affect, appropriate range of affect, vocal appropriateness, resourcefulness, affectionate touch, praising, and parent supportive presence. The composite score was rated from 1 to 5, where 1 indicates no sensitivity behaviors were observed and 5 indicates high frequency or quality of sensitivity behaviors were observed. A multiple linear regression was conducted to predict maternal sensitivity (M = 3.84, SD = .405) from self-reported GAD-7 anxiety levels (see Figure 2). The overall model was trending (r^2 = .137, F(2,36) = 2.865, p = .070). GAD-7 scores (β = .114, t = 1.647, p = .108) were not a significant predictor of maternal sensitivity, but infant age (β = .305, t = 1.951, p = .059) was borderline significant.

Figure 1
Relationship between Self-reported Maternal Anxiety Ratings and Observer-rated Maternal Anxiety Ratings

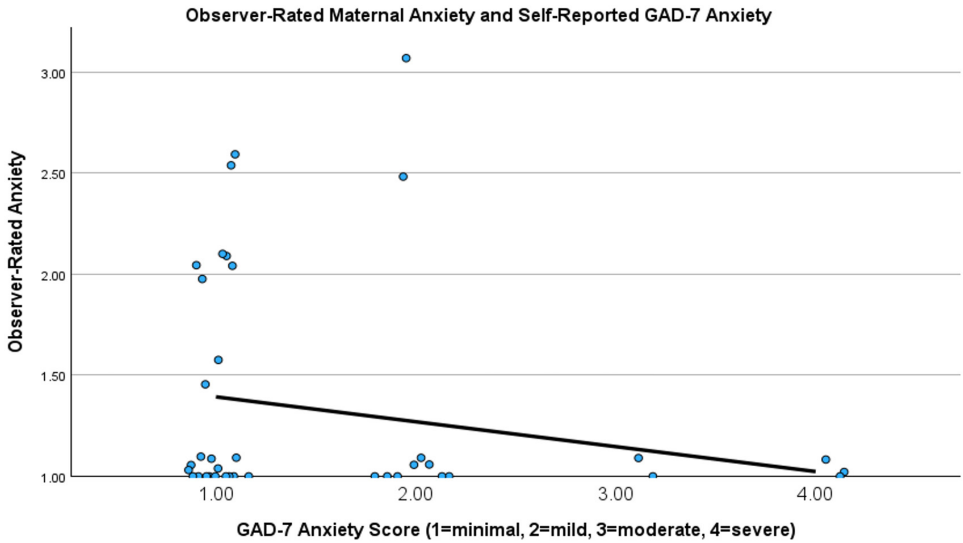
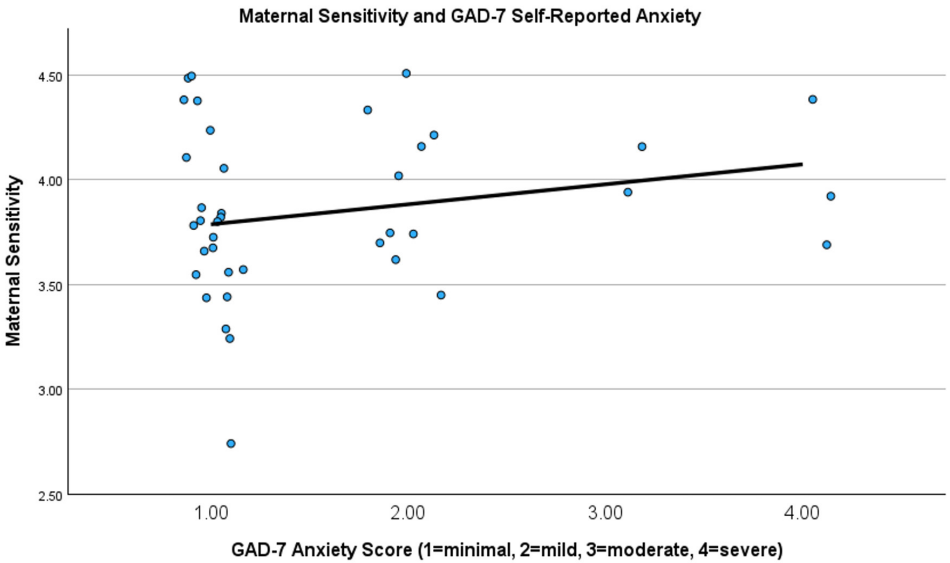


Figure 2
Relationship between Self-reported Maternal Anxiety Ratings and Observer-rated Maternal Sensitivity Ratings



Discussion

When examining the relationship between self-reported maternal anxiety ratings and observer-rated maternal anxiety scores, it was expected that there would be a positive association. It was hypothesized that as self-reported maternal anxiety ratings increased, observer-rated maternal anxiety scores would also increase. No significant association was observed between these variables. Mothers who rated themselves with minimal to mild anxiety were scored with a wide range of observer-rated maternal anxiety scores, but mothers who rated themselves with moderate to severe anxiety were given low observer-rated maternal anxiety scores. Therefore, mothers who perceived themselves to be highly anxious internally were not exhibiting frequent or intense external anxiety behaviors as predicted. It is plausible that the observed anxiety scale that was used was not nuanced enough to measure maternal anxiety specifically. The Coding Interactive Behavior (CIB) scale consists of 44 interactive behavior scales but only one of them measures parent anxiety. Within that one scale, all possible anxiety

behaviors are measured broadly and simultaneously. Using a different scale that measures the various observed anxiety behaviors in different subscales with individual scores that could be compiled into a composite parent anxiety score may have yielded a significant association between self-reported maternal anxiety scores and observer-rated maternal anxiety scores. Furthermore, while the GAD-7 questionnaire measures the internal states of generalized anxiety disorder and can be used for clinical diagnosis, it is unclear if the CIB Anxiety scale would also be appropriate for measuring generalized anxiety disorder since the CIB Anxiety scale measures external traits and behaviors. Currently, the literature shows attempts to better align self-reported and observer-rated anxiety measures. The Mark-Sheehan anxiety scales measure both self-reported and observed anxiety and match the number, content, and scaling of items (Albus et al., 1990). Further investigations can be conducted to determine if the CIB Anxiety scale and the GAD-7 can be used in conjunction in other contexts or, if not, what other scales could be used when comparing self-reported and observed anxiety.

When hypothesizing other possibilities as to why the highly anxious mothers were not scored with higher observed maternal anxiety ratings, it was proposed, given the previous literature, that the anxious mothers were not observed to be anxious because they were possibly observed to be sensitive instead (Lemus et al. 2022; Kaitz et al., 2010). An exploratory analysis was conducted to examine the relationship between self-reported maternal anxiety ratings and observer-rated maternal sensitivity scores. Mothers who rated themselves with minimal anxiety were given a wide range of observed maternal sensitivity scores while mothers who rated themselves with severe anxiety were given high observed maternal sensitivity scores. It is noted that a strong significant association between maternal anxiety and maternal sensitivity cannot be definitively concluded in this study due to sample size and composition. In this study, out of the 39 mothers recruited for participation, only 2 rated themselves with moderate anxiety and only 3 rated themselves with severe anxiety, while the other 34 mothers ranged from minimal to mild anxiety. Further studies with larger sample sizes that have wider ranges of diverse anxiety scores are necessary to support a relationship between maternal anxiety and maternal sensitivity.

The model is trending and approaching significance for infant age, meaning that as infant age increased, so did maternal sensitivity. This could possibly be because, as mothers gain more experience

as parents, they also could gain or improve the skills necessary to express maternal sensitivity. Asselman et al. (2018) found that infants at four months of age with anxious mothers displayed more negative interactive behaviors during the still-face paradigm, while Kaitz et al. (2010) found that infants at six months of age with anxious mothers displayed less negative affect during the still-face paradigm, indicating that older infants may have anxious mothers who are more sensitive due to being mothers for a longer period of time compared to the anxious mothers of the younger infants. Conversely, Lemus et al. (2022) studied infants at three months of age with low-, moderate-, or high-anxiety mothers and found that mothers with moderate anxiety showed maternal sensitivity and achieved behavioral synchrony during mother-infant interactions, indicating that infant age and parent experience may be nonsignificant. These varied findings in the literature indicate that infant age and its influence in mother-infant interactions is nuanced, similarly to the varied findings in the study of maternal anxiety and its influences on mother-infant interactions.

While this current study was not able to find a correlation between self-reported and observer-reported maternal anxiety measures, this does not imply that one measure is more valid than the other. Multiple measures exist because each measure has its own limitations and strengths, depending on the context in which the measure is used and the question an investigator is attempting to answer. Therefore, these results encourage further investigations to understand how to use these measures and to learn more about how anxiety influences parent-child interactions. This study can be expanded upon to study other factors that influence anxiety and parent-child interactions. While observed maternal sensitivity was compared against self-reported maternal anxiety in this study, we did not compare observed maternal sensitivity against observed maternal anxiety and this is a potential topic for further exploration. Future studies can investigate if changes in parent gender, parent age, child gender, or child age reveal any significant associations between self-reported and observed parent anxiety. Ultimately, this study contributes to the ongoing investigation of anxiety and highlights how the complexity of anxiety experiences and perceptions results in varied observed behaviors during parent-child interactions, emphasizing the importance of expanding upon this field of research and revealing more about anxiety and its role in parent-child interactions.

Acknowledgements

I would like to thank the Office of Undergraduate Research and Inquiry for providing funding for my research. I am grateful for the research assistants and the behavioral coders of the Infant Cognition Lab for helping me obtain and code the data for my project.

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Mock Battles: A Time and Place for Conflict

Harriet L. Wilkes Honors College, Florida Atlantic University
Benjamin Crawford, Dr. Rachel Corr (Faculty Advisor)

Abstract

Mock battles are a category of ritual widely recognized by cultural anthropologists, and yet the term as used stands as a wide umbrella covering an incredibly disparate range of human behaviors. Ethnographers have applied the label of "mock battle" both to events incorporating actual bodily harm—most famously, the Andean *Tinku*—and to non-violent theatrical performances such as the *Moros* dances popular throughout Spain and Mexico. To better understand the particular social functions of these rituals, I distinguish between two separate but interrelated "genres" of mock battle: the *Civil War* type, in which communities schedule limited outbursts of cathartic physical violence; and the *Conquest Reenactment* type, in which communities stage performances narrating the transition out of previous conflict into contemporary peace.

Introduction

Ritual conflict occurs across many cultures in a diversity of forms, and on its largest scale, can engulf entire communities in battles which dissipate as quickly as they have begun. These ritual or "mock" battles feature stark variations in their structure and content; they may contain real or staged violence, predetermined winners or no winners at all. Their variations follow directly from the social function of these rituals—communities adopt different forms of mock battle rituals dependent on the types of social cleavages which threaten their cohesion. To highlight how the content and structure of mock battle rituals follow from their function, I propose their categorization into two broad classes: *Civil Wars*, which feature eruptions of actual physical violence between social equals as a controlled release valve during times of community stress, and *Conquest Reenactments*, which are theatrical productions of simulated violence reaffirming transformed relationships between dominant and subjugated groups.

Background

In order to explore the outlined categories, it is first important to provide a unifying definition which places these disparate expressions of ritual violence under one roof. Mock battles are rituals which feature as their main component a contained hostile encounter between at least two clearly delineated groups. "Contained" in this definition refers both to regulations which limit the extent of the violence and to the fact that while animosity between involved groups may persist beyond the boundaries of the ritual, the violence that occurs within it is understood to exist in special context "outside of" ordinary time and space. "Containment" manifests within mock battles with the use of liminal spaces existing in-between the zones of ordinary social life, and special bookending rites which symbolically mark the initiation and/or diffusion of conflict. The form that bookending rites take are extremely revealing as to the social function of mock battles, following patterns distinguished by my prior typification. While games and sporting events do share certain traits with mock battle rituals, they are unalike in that their rules contain a structured, objective adjudication of victory or defeat, whereas prestige is conferred in mock battles more loosely on the informal basis of social participation. As such, and in the interest of not overstretching the scope of this article, I will not be discussing sports.

To illustrate the traits and social functions of my two categories of mock battle, I draw from a sample of six well-documented rituals: three from each of my two categories. I have chosen to highlight these examples due to the availability of richly detailed studies which enable close analysis of their proceedings.

The Andean *Tinku*, Venetian *Guerra Dei Pugni*, and Korean *sŏkchŏn* or "Stone Fight" are all civil war type rituals in which real violence occurs between participants. Of these, two are now extinct due to government opposition. The *Guerra Dei Pugni,* raucous fistfights held across the bridges of Venice, lasted from the late 16th century until 1705, when it was banned by the ruling Council of Ten for inciting public disorder. *Sŏkchŏn*, an annual tradition of throwing stones between residents of rural villages, persisted considerably longer, and remained active in parts of the Korean Peninsula until shortly after the end of Japanese occupation. Only the *Tinku*, a pre-Columbian Andean tradition of brawling to spill blood in sacrifice to the earth, survives today—albeit under considerable legal scrutiny. Even so, in spite of fines of up to $300 levied against those who participate

in the fighting, Martin (2002) notes that "signs of the *encuentro* are still visible: bloody faces, bruises, and swollen eyes" (p. 396). Such injuries are non-negotiable, even necessary components of the ritual. Urton's (1993) informants uniformly agreed that "somebody will get hurt in the [*Tinku*] fight, blood will be shed—it 'always' happens that way" (p. 122). The same held true for the *Tinku*'s extinct counterparts. Aficionados of the *Pugni* expected to come home with "scratches, sprains, teeth knocked out, dislocated jaws, gouged eyes, and finally smashed ribs and crippled legs" (Davis, 1994, p. 78), and in the case of the most raucous battles, that "some would almost inevitably be killed as well" (Davis, 1994, p. 13). So too in *sŏkchŏn* did participants expect "serious injuries, sometimes even death" (Siegmund, 2018, p. 124). The ban of these rituals has much to do with their disruption of the state's monopoly on violence; they represent a far older mode of conflict resolution, and demand a degree of temporary social rupture intolerable to government authority.

The choreographed, bloodless mock battles I typify as conquest reenactments, in contrast to civil war-type rituals, enjoy direct support from modern governments. The two best known examples, the Wild West shows of the United States and *Moros y Cristianos* dances in Mexico and Spain, are intrinsically bound up in the mythology of empire and its conquered subjects—Native Americans in the United States and Moors implicitly standing in for them in Mexico. My third example, the Pig-Kill invasion dance recorded in Papua New Guinea, represents retributive raids between villages rather than imperial domination, but is still reflective of hegemonic colonial power—the dances were encouraged by Australian governors to replace earlier forms of tribal warfare that they criminalized (Schwoerer, 2020, p. 757). These rituals are characterized by their general lack of injury. In modern *morismas* "no human blood is intentionally shed, and the Red Cross is on hand to care for any real wounds" (Harris, 2000, p. 16). Even before modern safety standards, a casualty at a Wild West show was considered exceptional enough to make headline news (William F. Cody Archive, 1888). And though dancers at the Pig-Kill brandish actual weapons, warrior exuberance is always constrained from "tipping over into actual combat" (Schecher, 2003, p. 69). The distinction between performed and actual violence, and the containment of that violence to the confines of the ritual, are significant components that typify mock battles as a whole and which I will explore in relation to my two categories.

Containment in Civil War Mock Battles

Containment is especially critical for civil war-style mock battles. Because they are held between equals committing real violence against each other, there is considerably greater risk of said violence giving rise to lasting grievances that "spill over" out of the boundaries of the ritual.

One ritual element which aids in discouraging this is the choice of battlefield. Common to civil war mock battles is their use of preexisting liminal spaces for neutral arenas. The *Guerra Dei Pugni*, for example, was strongly associated with bridges to the point they were also called "*battagliole sui ponti*, the 'little battles on the bridges'" (Davis, 1994, p. 13). In Renaissance Venice, bridges were viewed as "a kind of social no-man's-land that was neither land nor water, not clearly belonging to one parish or the other" (Davis, 1994, p. 17). The Peruvian *Tinku* also makes use of urban liminal space. It takes place in public squares: neutral ground which belongs to neither the upper nor lower part of the village. The square is transformed into a contested battleground for ritual with the addition of two potted chachakuma trees, one male and one female, which symbolize the "dualism of [...] ritual interactions between an upper moiety, female tree in opposition to a lower moiety, male tree" (Urton, 1993, p. 126). The Bolivian version of the *Tinku*, as well as Korean *sŏkchŏn*, are both predominantly rural rituals, customarily hosted outside villages in neutral natural spaces. Traditional *sŏkchŏn* are described as taking place on stony riverbeds outside the village where the community drew their water from. Much like bridges in Venice and town squares in Peru, the riverbed was a public space that belonged to nobody and saw traffic from everybody, avoiding the friction potentially caused by a violent ritual intruding on a clan's property (Siegmund, 2018, pp. 126–127). The *Tinku* in Bolivia is also held in a no-man's-land—this one literal. The mountains that host the *Tinku* are sacred spaces "inhabited by forces that transcend [mortals]" where "a 'different atmosphere' predominates" (Martin, 2002, p. 396). The high mountains are the territory of spirits, not humans, and so serve as a neutral zone for the ritual.

In addition to liminal battlefields, civil war mock battles also feature special bookending rites to "seal" them away from normal life. In both the *Tinku* and *Guerra Dei Pugni*, this takes the form of a symbolic embrace between opponents. Champions of opposing factions in the Venetian *Pugni* first initiated their *mostre*, or boxing duels, "by exchanging an embrace and a 'fraternal kiss'—gestures that many repeated at the end of a match as well" (Davis, 1994, p. 70).

These fraternal kisses were overseen by *i vecchi venerandi* (revered old ones), respected veterans of the *Pugni*, and intended both to mark the special circumstances of the champions' duels as well as to ensure that what occurred during the fights would not influence their future interactions, whether in daily life or during future *pugni*: "the kiss that introduced combat served to indicate that both duelists welcomed the fight, but also that previous grudges and rancors should not be allowed to intrude. [...] The closing kiss sealed the violence that had passed, so that the duelists could leave behind the injustices they had given and received at the *arengo* [ring] without turning them into a later vendetta" (Davis, 1994, p. 70). Similarly, Urton (1993) records that the headmen of the *ayllus* (extended family groups) participating in a *Tinku*, upon ending their fight, "embraced each other in an exaggerated, almost theatrical display of comradeship and began serving each other cups of chicha provided by their respective female allies" (p. 127). In both examples, mock battles are abruptly terminated with highly dramatic shows of friendship between elders. These shows serve as deliberate representative examples—almost morality plays—in which the exceptional violence of the ritual smoothly transitions back to expected social cohesion. Civil war mock battles are as much ritual modelling of conflict resolution as they are cathartic expressions of antipathy. This is further evidenced by the fact that these mock battles are held between sub-groups within communities who are traditional rivals. Korean *sŏkchŏn* fights were commonly staged around harvest time to "resolve political differences between [two-clan] villages" whose binary rivalries were often compared by stationed American servicemen to frontier feuds: "the Kims against the Paks instead of the Hatfields and the McCoys" (Siegmund, 2018, p. 134). The wider context of the harvest is important—harvest time was a stressful economic period during which tension between these clans would have been elevated. So, too, are *Tinku* battles fought between the rival *ayllu* of the upper and lower parts of the village (Urton, 1993, p. 121), and held at harvesttime to help resolve the inevitable tensions that "arise during the annual redistribution of the communally-owned potato lands" (Urton, 1993, p. 129). Harvesttime battles may well be a form of preemptive stress release anticipating enhanced risk of conflict. The *Pugni* served an equivalent role relieving tension during times of community stress—bridge battles were held on work holidays to keep idle (and often drunk) young men from causing trouble where it might do real harm. As with the other two rituals, the *Pugni* were

organized based on preexisting internal cleavages which dated "back to the original settlement of the Rialtine islands" (Davis, 1994, p. 14). Rather than allow animosities to stew unresolved, these communities set a time and place for them without sacrificing public order. Attitudes around *sŏkchŏn* highlight the double awareness this created:

> The relatives who threw stones say: 'I would not dare to throw stones at my relatives. It was in battle that I threw stones.' The neighbours who threw stones say: 'I would not dare to throw stones at my neighbours. It was only in battle that I threw stones.' [...] The relatives and neighbours also say: 'They would not dare to throw stones at me. It was only in battle. We ourselves have thrown stones at our relatives and at our neighbours.' This is because it has been practised for a long time and it has become a tradition since long ago. So, they all consider this the normal state of things. The correct social order is destroyed, public morale is violated, and they do not consider this abnormal. Alas! (Siegmund, 2018, pp. 156–157)

While the official in question intended his comment as a Confucian criticism of *sŏkchŏn*, taken from another perspective, what he observed is a strong indication of ritual success. Through *sŏkchŏn*, participants were able to maintain adherence in the obligate community harmony of the Confucian world by constructing a separate one to house its ritual violation—"I would not dare" except "in battle." The "correct social order" that the fraught official fears for was never destroyed, because it and the stone fight were never in any contact. This successful isolation of cathartic violence from social upheaval is predicated on the ability of ritual to create and maintain liminal divisions. Through ritual, participants of mock civil wars envision contradictory relationships of enemy and neighbor such that they overlap but do not intersect.

Containment in Conquest Reenactment Mock Battles

Because all of the fighting involved is only pantomime, there is negligible risk of violent spillover from conquest reenactment-type mock battles. However, bookending containment rites remain key to the message conveyed by the ritual. Both types of mock battle rituals outline a transformation from friend to enemy and back again, but

in conquest reenactments, participants do not re-emerge as equals. Conquest reenactments conclude by restating the existing hierarchy between conquered and conquerors, often utilizing a symbolic transition of space to convey the social transformation out of enmity into a magnanimous victor's peace. In her case study of Wild West Shows, McNenly (2014) found this "foe-to-friend" narrative transformed "the 'savage and vanishing Indian' to the 'civilised and tamed Indian' [which was] necessary to maintain the story of a successful conquest" (p. 147). Nineteenth Century Wild West shows typically saw an opening "reenactment of a famous battle such as the Battle of the Little Bighorn," and then led the audience to explore "Indian-themed vignettes" in teepee villages erected on the former battlefield (McNenly, 2014, p. 143). The effect of this two-act structure was wholly intentional—Wild West showmen used their performances as platforms to communicate the new relationship between Americans and their former enemies, now turned gracious hosts (Wilson, 1998, p. 318). Bill Cody, perhaps the most famous and influential of all Wild West showmen, saw his role as one of ambassadorship as much as entertainment:

> By the 1890s, three decades of Indian Wars had largely eliminated Indian tribes as an impediment to the settlement in the West. While white Americans had once characterized Indians as blood-thirsty savages, they increasingly viewed them as noble in defeat. [...] Indeed, Cody himself understood that the Indian Wars were over, that the winners and losers were decided, and that both sides had fought valiantly. For this reason, in 1893, he included a ritual of Anglo-Indian reconciliation in the Wild West. (Haddad, 2008, p. 24)

The 1893 ritual refers to the Chicago World's Fair. Cody took advantage of a walkthrough attraction to enact his show's foe-to-friend message on a grander scale. The layout of his American Indian Exposition walked visitors through a mock battlefield between cavalrymen and native warriors at the edge of the fair (its "frontier") and into "a frontier schoolhouse full of 'civilised and assimilated Indian students'" (McNenly, 2014, p. 154). At the exposition, Cody was famously recorded shaking hands with Sioux leader Sitting Bull in a much-reproduced photograph captioned "Enemies in '76, Friends in '85" (McNenly, 2014, p. 148). Cody's World's Fair ritual

of reconciliation literally walked audiences through a narrative of conquest into peaceful assimilation allegedly for the natives' own good. Spanish colonialism featured a similar "civilising mission" to transform conquered enemies into brethren. In *Moros y Cristianos* festivals, mock battles give way to a "denouement of conversion and peace" (Harris, 2000, p. 38), in which the beaten Moors accept baptism and are welcomed as fellow Christians with a "kiss of peace" from a performer playing the Spanish king (Harris, 2000, p. 40). Again, fraternal embrace serves as an important bookending rite to transition from ritual violence to peace, with the subjugated enemy transformed into friends. Harris argues that this kiss symbolically enables the battle to resolve into mutual celebration by both winners and losers by remaking the enemy into a friend:

> The dramaticised Christian goal is not, to use a phrase from modern U.S. sports and warfare, 'to kick butt,' but to incorporate and to live at peace with one who is an enemy no longer. The kiss of peace affirms the royal acceptance of the Moors' profession of faith and authorises the dance that celebrates their incorporations into the company of Christians. (Harris, 2000, p. 39)

Much like Cody's World's Fair expo, historic *Moros y Cristianos* used symbolic transformations of ritual space to reflect the transformation from enemies to friends. A 1462 festival commemorating a military victory began with a jousting battle against a procession of false Moors invading the governor's palace at Castille. Upon their defeat, the Moors were escorted to a recently captured mosque near the city limits, where they were playfully "baptised" in its hammam pool and swore fealty to Spain by kissing the governor's hand (Harris, 2000, pp. 17–58). The opening "invasion" of Castille transformed it back into an embattled Muslim space as it had been in recent memory, while the subsequent baptism at the mosque symbolically restored a Christian-dominated victor's peace. Contemporary festivals enact a similar framework of invasion and counterinvasion, but erect an "artificial castle" for the Spanish to conquer, thus confining the ritual to a constructed liminal space (Harris, 2000, p. 39). When Spanish conquest moved on from Iberia to the Americas, they recycled the same rituals; New World *Moros y Cristianos* festivals had indigenous play the Moors, some of whom even received their first baptisms as part of the ritual (Harris, 2000, p. 39). In this way, the mock battle

facilitated the actual legal transformation of indigenous enemy into Christian subject.

While most mock battles end with a reinforcement of hierarchal colonial relationships, such is the flexibility of the ritual that it can also serve to explain newly egalitarian relationships as well. The Papuan Pig-Kill ritual reenacts historical tribal warfare, with one party acting as raiding headhunters and the other as village defenders. The Pig-Kill is hosted on neutral "council grounds, a no man's land" (Schecher, 2003, p. 70), and only moves back into the defenders' host-village proper for a roast pig feast "once the invaders [have] merged with their 'enemies' forming one whooping, chanting, dancing doughnut of warriors" (Schecher, 2003, p. 70). The Pig-Kill follows the same structure discussed in other rituals of transitioning from a battlefield into the defeated enemy's space to celebrate peaceful assimilation. In this case, the conquest being celebrated is twofold: on one layer, the victory of the raiding party, but on another, the domination of the colonial Australian government, which imposed peace between the tribes and replaced actual raids with the Pig-Kill dances (Schecher, 2003, p. 69). Even when a conquest reenactment does not directly reflect the permanent subordination of its losers, it still represents a transformation of relationships under an imperial authority—the new identities of the tribesmen as mutually subordinated colonial subjects. Just like mock civil wars, the core of the ritual lies in people coming to terms with their neighbors.

Privilege, Glory, and Shame as Participation Incentives

In addition to serving vital roles upholding group cohesion, mock battle rituals also serve important social functions to their individual participants. For young men (the overwhelming majority of participants), they often serve as a venue to gain martial prestige. In the Venetian *Pugni*, "success at the bridges paid off in the coin of status," and *pugni* veterans "were frequent guests at noble palaces" (Davis, 1994, pp. 79–80), where they gained the patronage of powerful patricians (Davis, 1994, p. 143). In a calcified society where privilege was tied to birthright, the *Pugni* presented a liberating parallel system of prestige where working-class young men could gain upwards mobility:

> The world of the *pugni* represented a popular answer to the centralising encroachment of the patrician state. At the same time (indeed, often at the same moment) that the rulers of the Republic [...] gathered in San Marco in the ritual expression of the hierarchical rigidity, civic harmony, and political unity that made the continuation of Venice possible, thousands of ordinary citizens (and a good many patricians as well) were deserting the city centre for outlying bridges, where they demonstrated precisely the opposite spirit. (Davis, 1994, p. 45)

Similarly in Korea, while state officials imposed an immovable social order under the banner of Confucian harmony, peasants could still win prestige above their station by proving themselves at *sŏkchŏn* as "an indicator of manliness" (Siegmund, 2018, p. 136). *Sŏkchŏn* champions were rewarded with "handsome gifts" (Siegmund, 2018, pp. 150–153) from individual nobles, and exalted in poetry for their martial spirit (Siegmund, 2018, pp. 148–149). In both the *Pugni* and *sŏkchŏn*, we see how mock battles can provide participants with opportunities for advancement outside the context of ordinary social hierarchy.

In addition to prestige, mock battles also provide spaces to fulfil gendered expectations of masculine violence that are ordinarily criminal or taboo. In New Guinea, for example, manhood is culturally tied to warrior status. When the colonial state brought an end to tribal warfare, men were left without a venue to fulfil this role. The Pig-Kill ritual provides Papuan men with a rare space where they can embody a traditional warrior masculinity, "displaying his strength, his power, his wealth, and his position in the group" (Schecher, 2003, p. 69), without violating the law. Wild West shows in the US were similarly touted as "an antidote to the constraining and feminising aspects of modern life [which] celebrated the virtues associated with an earlier time: rugged individualism and the martial spirit" (Haddad, 2008, p. 11). In societies with long-running mock battle traditions, the association between the ritual and gendered expectations can even make participation an obligate component of manhood. Young women at *sŏkchŏn* battles often humiliated "those who attempted to flee [...] by calling them cowards" (Siegmund, 2018, p. 136), and in the Andes "young males returning to their community without signs of having participated [in the *Tinku*] are not welcomed" (Martin, 2002, p. 396). Mock battles, thus, serve as a test of courage through which men validate their masculine identities.

Liberation as an Incentive for Scripted Losers

Mock battles are intuitively empowering for those who stand a chance of winning the fight, but in conquest reenactment type battles, half of the combatants are scripted to lose. Why knowingly volunteer their own ritual defeat? In fact, in Harris's study (2000) of *Moros* performers, he found that overwhelmingly participants prefer the losing role: "to be a Moor is more desirable than to be a Christian" (p. 221). For those performing as Moors, the festival is license to provoke, mock, and cavort in costume with all the liberation of a Carnival devil—the Moors are *fun* in contrast to the reactionary policing Christians putting an end to the party. Harris's interviewees often expressed kinship with the Moors in recognition of their own dark-skinned heritage; "the Moors are not just symbols. They are something in us. Look at our faces. Many are Moorish. [...] The Moors are not bad" (Harris, 2000, pp. 221–222). Rather than a trial of humiliation for the losers, *Moros y Cristianos* is a special time of year when they can celebrate normally subordinated aspects of their identities and rule for a day. The liberating character of the ritual is even more pronounced in South America, where indigenous performers have transformed the ritual into a coded mockery of their conquerors. Harris (2000) notes the use of exaggerated clownish masks used in Mexican performances to portray the Christians (p. 22), as well as the mock beheading of John the Baptist standing in for pre-Columbian human sacrifice rituals (Harris, 2000, pp. 11–12). He argues that this syncretism has contributed to the enduring popularity of the festival among the people whose conquest it nominally celebrates: "the capacity of the tradition to embrace such dissent is one reason it has survived so long in so many different settings" (Harris, 2000, p. 19). *Moros* festivals are sanctioned opportunities to subvert hegemony.

For indigenous performers in Wild West shows, the liberating aspect of the ritual was material as well as symbolic. Government reservations confined formerly nomadic tribes and brutally suppressed their culture, while travelling shows offered freedom of movement as far afield as Paris or London (Harris, 2000, p. 22). Much like *pugni* and *sŏkchŏn* fighters, Native American performers also gained access to ruling elites who sponsored their mock battles. The ceremony of official meetings with presidents, monarchs, and diplomats reaffirmed the authority of indigenous leaders, and, at least symbolically, placed them on a temporary equal footing with their conquerors. Performers were also given a rare platform to express themselves to a mass

audience through newspaper interviews (McNenly, 2014, pp. 156–157), in which they lobbied public sympathy for their struggles.

Conclusion

Whatever the realities of power outside the context of the ritual, mock battles create a space for imagining an alternative world in which ordinary hierarchies are temporarily subverted. In civil war-type rituals, this is a world where grievances against neighbors can be safely acted on without rupturing social harmony. In conquest reenactments, it is a world where the conquered can once again confront their conquerors, before being reincorporated as subjects under the olive branch of friendship. Both serve important functions addressing cleavages within communities, and as a venue for prestige and gender performance for individuals.

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Impacts of Climate Change on Vertical Migration of Zooplankton Species

Florida Atlantic University Harbor Branch Oceanographic Institute
Camila Rimoldi Ibañez, Dr. James Masterson (Faculty Advisor)

Abstract

Vertical migration in marine and freshwater environments stands as one of Earth's largest daily animal movements concerning biomass. It serves as a crucial life pump, shaping phytoplankton grazing patterns, facilitating nutrient transport through the water column, and providing vital sustenance for deep-water organisms lacking access to surface phytoplankton. Despite enduring millennia of climatic shifts, contemporary anthropogenic climate change surpasses historical rates of change, posing unprecedented challenges. Climate-induced phenomena increasingly affect various species, including vertical migrators. This paper delves into Diel Vertical Migration's ecological significance and anticipates detrimental effects of climate change on individual animals' physiology and migration patterns. Foremost, rising temperatures are likely to shrink migratory species and hinder their migration frequency, potentially leading to species decline. As climate change intensifies, understanding and mitigating its impacts on these crucial ecological processes becomes imperative.

Research Focus and Question

This literature review focuses on the ecological significance of Diel Vertical Migration of phytoplankton species and the anticipated effects of climate change on their physiology and migratory behaviors. Several topics will be explored to answer the following questions:

- How do rising temperatures and changing environmental conditions influence the migratory patterns and physiological health of zooplankton?
- What implications do these changes have for ecosystem dynamics?

Given the rapidity of climate change, understanding these relationships is crucial for predicting declines in migratory species and addressing broader ecological consequences.

Introduction

Species across 15 distinct phyla at all latitudes partake in vertical migration (Orcutt & Porter, 1983; Bandara et al., 2021). There are two types of vertical migration: the shorter, one-day migration referred to as Diel Vertical Migration (DVM), and the annual Seasonal Vertical Migration (SVM; Bandara et al., 2021). Both help transport organic nutrients to deeper waters, but DVM plays a more critical role in nutrient transport than SVM due to its higher frequency of occurrence (Bandara et al., 2021). This review will prominently focus on DVM and the expected influence climate change will have on this behavior and the animals that perform it.

Mesopelagic animals that perform diel vertical migration (DVM)—such as copepods, larval shrimp, and zooplankton, typically do so to escape their predators and increase their feeding opportunities, which are directly linked to survival and fecundity (Almén et al., 2014; Frank & Widder, 1997; Bandara et al., 2021). The planktonic animals, organisms below one centimeter in body length, can travel between tens to hundreds of meters in roughly three hours (Brierley, 2014; Bianchi et al., 2013). Nearly all mesopelagic zooplankton and larvae engage in nocturnal migration, making it the most common vertical migration pattern (Bianchi et al., 2013). In this trade-off type of trend, animals spend the day in deeper, darker water to avoid predators and ascend to surface waters at night to feed on phytoplankton, which has spent the day photosynthesizing (Bianchi et al., 2013). However, these organisms face many difficulties during migration, including pathogenic microorganisms in the water column and the high metabolic cost of swimming such long distances (Bandara et al., 2021). This characterizes the behavior as a trade-off between the food source and the difficulties faced during migration. Another type of migration performed by some species is twilight DVM; in this type of movement, the species ascend at dusk and descend at midnight (Bandara et al., 2021). This allows the migrators to avoid diurnal predators while taking advantage of surface production.

DVM is guided by many factors including light, physical and chemical cues, food availability, salinity levels, dissolved gases, water density, and the presence of predators (Almén et al., 2014; Bianchi et al., 2013; Bandara et al., 2021). Some studies have found that a driver of DVM could be the animals trying to avoid exposure to damaging ultraviolet radiation (Brierley, 2014). Still, light and temperature are known to be the most influential factors. Species perceive and respond

accordingly to changing light intensities, which activates migratory behavior by enabling the genetic expression of photoreceptive proteins called cryptochromes (Bandara et al., 2021). Yet, the specific cue most influential to the behavior can depend highly on the species. For instance, *Daphnia* is ultraviolet-sensitive and avoids ultraviolet radiation at the surface during the day, whereas copepods avoid the surface regardless of ultraviolet exposure (Cooke et al., 2008).

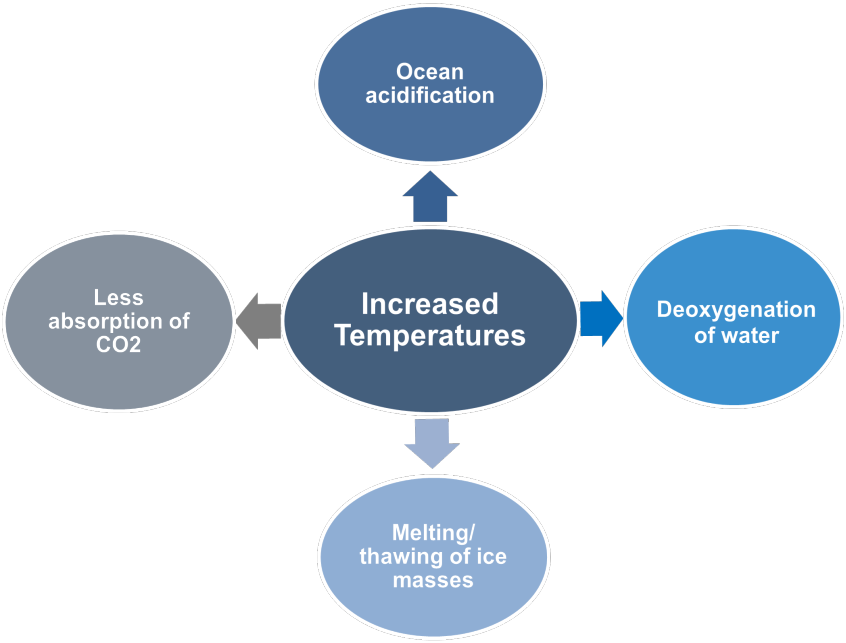
The benefits associated with DVM are of great importance for biological and physical marine systems. Biologically, these dominating herbivore species serve as the primary food source for most deep-water organisms, including but not limited to pelagic larvae, a variety of juvenile commercial fish species, shrimp, squid, and ctenophores (Frank & Widder, 1997). They also serve a major role in cycling nutrients and other materials from the surface to deeper waters (Orcutt and Porter, 1983). Specifically, DVM alters grazing patterns in surface waters and the biogeochemical fluxes of nutrients, such as carbon and oxygen, throughout the ocean. DVM constitutes approximately 10 to 70% of particle export, helping sustain mesopelagic communities' metabolic necessities (Orcutt & Porter, 1983; Bandara et al., 2021). The speed of active particle transport associated with their daily migration outperforms passive transport, such as the sinking of dead phytoplankton, by 40% (Brierley, 2014). Physically, the migrating species' synchronized swimming motion can significantly affect vertical mixing throughout the water column (Bandara et al., 2021). Given the critical importance of these migrating planktonic species in food, oxygen, and nutrient cycling to maintain deep-ocean communities, any change that would negatively affect the species could indicate trouble for much of the deep-sea life (Almén et al., 2014)

The first critical step in understanding the shifts expected to impact DVM patterns due to climate change is to look at how these organisms have dealt with changes in the past. Migrating copepods are one of the most ancient taxa in Earth's aquatic systems, and thus, have survived and adapted to significant temperature and pH changes for millions of years (Almén et al., 2014). Migrating species presently face physicochemical changes in their environment that are equal to or larger than those expected with climate change, such as pH changes higher than 0.5 and temperature changes upwards of 5^{o}C, as they move through the water column (Almén et al., 2014). The short- and long-term adjustments these organisms already experience should be promising in the face of climate change. Yet, the rate at which these

big climatic changes occurred in the past was much slower than the current rate, which provided valuable time for the animals to adjust (Almén et al., 2014). Current CO_2 levels are 40% higher than historical records before the Industrial Revolution (Lønborg et al., 2020). These expedited rates due to climate change are exceptionally fast compared to those seen previously throughout Earth's history. Even the frequency and intensity of massive, long-term heat waves that can extend over a large area of thousands of square kilometers have been increasing, and they are expected to continue increasing over the coming decades (Lønborg et al., 2020). Extreme changes like those of the present will have the greatest effect on zooplankton, especially copepods, different from the slow change in average conditions as previously seen (Almén et al., 2014).

Expected Impacts of Climate Change on Migrators' Environment

Figure 1
The four main threats to diel migrating species caused by increased temperatures as a result of climate change.



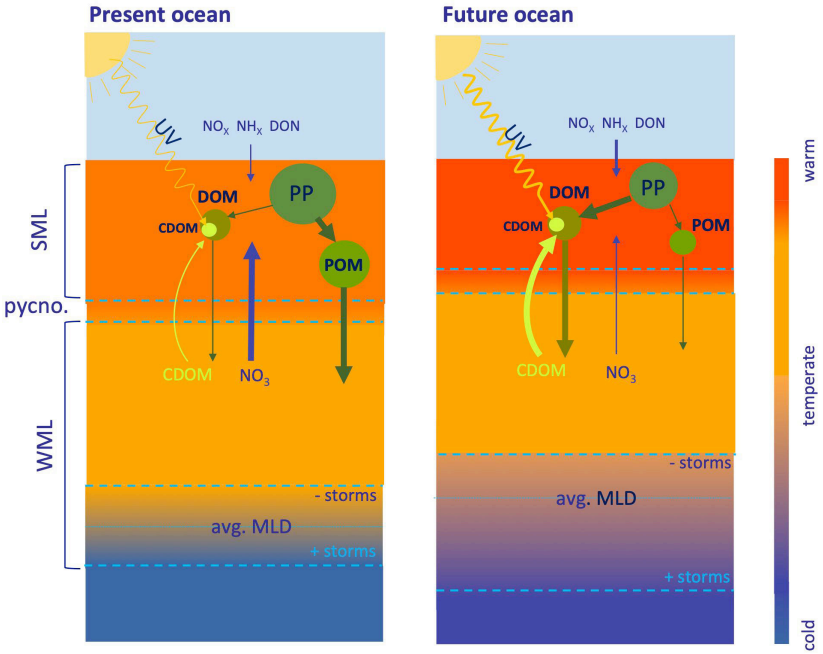
Climate change is a complex web of interconnected issues that affects all life on Earth. When thinking specifically about DVM, evidence shows that increased temperatures resulting in ocean acidification, deoxygenation, and melting and thawing of ice masses will dramatically impact migratory species (Lønborg et al., 2020, Meredith et al., 2022, Almén et al., 2014). Increased temperatures are expected to increase oceanic stratification as warmer air temperatures transfer heat to ocean surface water (Lønborg et al., 2020). The ocean absorbs approximately 90% of the heat generated from greenhouse gas emissions, which are predicted to increase sea surface temperature by up to 4ºC in the next few decades (Lønborg et al., 2020).

As excess CO_2 comes into contact with surface seawater, it forms carbonic acid (Meredith et al., 2022). This reduces pH, in turn decreasing calcium carbonate and oxygen concentrations (Almén et al., 2014). As the temperature of surface water increases, water density will decrease accordingly as the molecules expand and take up more space. This will make the difference between stratified water masses throughout the vertical water column more pronounced, causing the dividing boundaries above and below the pycnocline to disjoin further (Lønborg et al., 2020). The intensified stratification will eventually prevent the vertical exchange of material—like nitrogen or oxygen—across the pycnocline, sometimes referred to as the thermocline (Lønborg et al., 2020).

Ocean deoxygenation is predicted to impact the deep ocean and thermocline the most, the areas where migrating species reside during the day (Lønborg et al., 2020). Eighty-five percent of declining O_2 levels in the open ocean are attributed to ocean stratification (Lønborg et al., 2020). This will heavily impact the Oxygen Minimum Zone (ocean depths with the lowest oxygen saturation) worldwide. Over the past 50 years, Oxygen Minimum Zones (OMZs) have expanded worldwide (Riquelme-Bugueño et al., 2020). Their expansion is predicted to progress as a warmer climate takes over Earth's atmosphere. As OMZs become more pronounced, constraints will be set on migrating zooplankton, as they typically aggregate just above the oxycline boundary (Tutasi & Escribano, 2020). Having to move higher up in the water column to stay above the oxycline boundary will force the animals to reside in better-lit waters, raising their risks of being spotted by a predator.

An increase in primary production would hypothetically accompany light intensity and availability increases. Since this will result in a larger biomass of phytoplankton, it will consequently increase

Figure 2
Depiction of the expected stratification change due to the effects of climate change, such as higher air temperatures



Note. From "Impacts of Global Change on Ocean Dissolved Organic Carbon (DOC) Cycling," by C. Lønborg, C. Carreira, T. Jickells, and X. A. Álvarez-Salgado, 2020, *Frontiers in Marine Science*, 7. https://doi.org/10.3389/fmars.2020.00466. Copyright 2020 by Cristian Lønborg, Cátia Carreira, Tim Jickells, and Xosé Antón Álvarez-Salgado. Reprinted with permission.

dissolved organic matter (DOM) from dead phytoplankton cells and excretions as they sink (Lønborg et al., 2020). Organic matter is crucial in absorbing harmful ultraviolet (UV) rays that would otherwise harm many microscopic organisms living in the ocean (Lønborg et al., 2020). UV-B radiation, or electromagnetic radiation within the 280 to 315nm range, influences carbon, nitrogen, sulfur, and metal cycling (Zepp et al., 2007). This accelerates the transformation and availability of colored dissolved organic matter (CDOM), a UV-absorbing substance (Zepp et al., 2007). More CDOM means more UV radiation will be absorbed, leaving less sunlight available for photosynthesizing organisms and potentially reducing primary productivity. Such consequences would cause a direct negative impact on migrating species (Zepp et al., 2007).

Another issue associated with climate change is the melting and thawing of glaciers and permafrost areas. In general, Arctic air temperatures have surpassed double the global average in the past two decades and are predicted to continue rising (Meredith et al., 2022). Even the abnormally high temperatures observed in winter were double those of previous abnormalities in 2016 and 2018 (Meredith et al., 2022). These temperature changes have already been associated with a decline of sea ice age, as warmer temperatures hinder the formation and continual growth of sea ice. The quantity of sea ice found to be at least five years old comprised 30% of all Arctic sea ice in 1979; by 2018, that sea ice coverage had exponentially decreased to just 2% of the total mass (Meredith et al., 2022). By 2100, 21 to 75% of the total ice volume in glaciers and ice caps is expected to have melted due to climate change (Lønborg et al., 2020). Some predictions of possible emission levels hypothesize a seasonally ice-free Arctic before 2050 (Lønborg et al., 2020). With increased ice and glacial melting, it is harder for these ice bodies to contain meltwater, causing a surge of freshwater runoff and increasing stratification and DOM concentrations from terrestrial ecosystems (Meredith et al., 2022; Zepp et al., 2007). An expected trend shows that the rise in sediment deposits and the influx of nutrients will increase primary production in polar waters (Meredith et al., 2022). The daunting issue of permafrost hosts yet another set of effects. Currently, permafrost acts as a massive storage of carbon dioxide and methane, a powerful greenhouse gas. At the current melting rate, 37 to 174 gigatons of carbon are expected to be released into the atmosphere by 2100, expediting global warming (Meredith et al., 2022). Yet, some predict melting permafrost will not be entirely detrimental, as the post-permafrost soil will be available to host a variety of plant species which can work towards sequestering some of those carbon and methane emissions (Meredith et al., 2022).

Climate Change Effects on Migrators' Physiology and Migratory Patterns

Analyzing the climate-change-driven shifts overall, the described shifts will significantly impact one of the ocean's largest biological carbon sequestration structures (Bandara et al., 2021). The described effects of climate change have been identified to mainly impact vertical migrating species in two ways: through their physiology and migrating patterns. Warmer temperatures have been linked to physiological changes in body size, a wide range of metabolic functions, and alterations

of environmental tolerance in zooplankton. Body sizes in temperature-stressed zooplankton are typically smaller (De Stasio Jr. et al., 1996; Orcutt & Porter, 1983). These migrating organisms make up the basis of most aquatic food webs, especially in deep-water habitats. A decrease in the biomass of this critical food source could induce catastrophic food shortages, simply because they would not provide the same quantity of food that many species depend on, especially for deep-water organisms that do not participate in DVM (De Stasio Jr. et al., 1996).

Climate change-driven temperature rise will have a negative effect on a wide variety of metabolic functions in zooplankton. Manipulative experimentation on the response of the migrating species *Daphnia parvula* to temperature changes found that the reproduction rates, life span, and growth decrease with increasing temperatures (Orcutt & Porter, 1983). Specifically, these factors were insignificantly affected at constant warm temperatures of 20°C but showed significant responses when exposed to fluctuating temperatures between 10 to 20°C, and above 20°C (Orcutt & Porter, 1983). Maintaining constant moderate temperatures or slowly shifting towards higher temperatures seem to have an insignificant effect on the metabolic activities of these organisms. Fluctuating towards warmer temperatures or constant high temperatures, on the contrary, significantly affects metabolic activity in a way that will negatively impact the migrating zooplankton. A study found that temperature changes as small as 1.5°C affect feeding, swimming, and reproduction in *Daphnia magna* (Cooke et al., 2008). It is fluctuating climatic changes, even small changes, that will impact species the most, and this trend continues to emerge as global climate change progresses. What makes this even more dangerous is that young copepods do not migrate as much as adults, leaving them more sensitive to rapid changes in temperature (Almén et al., 2014). Suppose the new generation of a population cannot adjust to a changing environment. In that case, the population's survival is left largely up to chance, leaving species subject to sharp population decline or extinction.

Most vertical migrators are sensitive to environmental cues that help temporally orient migration, including avoidance responses. Copepods specifically aim to avoid low-pH water, so if pH drops too low in their natural deep-water environment, it is very likely that the individuals will restrict themselves to shallower areas (Almén et al., 2014). Deep water is particularly vulnerable to ocean acidification, which is the primary cause of drops in the pH level. Shallower waters do not provide equivalent protection from predators as the deeper, darker waters can.

Another set of effects associated with climate change is changes in migration patterns, notably the frequency at different latitudes. In warmer tropical waters, there is expected to be less primary production from limited nutrients and light, causing a decrease in DVM (Brierley, 2014). Migrating is a costly behavior, and it is presently worth the use of energy sources because of the food availability. In the absence of current phytoplankton abundance, it would not benefit migrating species to use up so much energy with such a poor reward. In the Arctic, however, primary productivity is expected to increase. As sea ice continues to melt, it will give way to higher light penetration through the water column (Brierley, 2014). The increase in light availability predicts that there will be an increase in phytoplankton primary production in this partially inhospitable environment. With the increase in food availability, migrating species could increase their carrying capacity appropriately (Bandara et al., 2021). With better-illuminated waters, zooplankton will most likely reside and migrate from even deeper water to stay safe from predators, sequestrating carbon deeper in the water column as they do so (Brierley, 2014).

Yet, this scenario would only occur under the ideal condition that climate change only affects light exposure, but this is not the case. As mentioned, there are a multitude of issues branching off from climate change, making it a much more complex problem. The expected alteration in rain patterns and large-scale glacial melting would bring more runoff (Meredith et al., 2022). With increased runoff comes more turbid waters, making it harder for light penetration and ultimately leading to lowered levels of primary production accompanied by decreased migration (Bandara et al., 2021). Turbidity is also directly related to lowering migration rates because migrating zooplankton residing in deep water rely on the slightest changes in light intensity to signal the start of the migration cycle. These organisms have evolved to respond to certain degrees of changes in light intensity, and variations to these long-standing intensities would alter their response. Any further changes to migrating zooplankton could be dangerous. It is hypothesized that these organisms are already at their physiological limit in the highly variable areas they live and migrate through (Bandara et al., 2021).

Implication in the Freshwater Environment

Understanding new migratory trends or the physiology of migrating zooplankton in freshwater lakes can better predict what will happen in harder-to-study open ocean areas. De Stasio Jr. et al. (1996)

observed how climate change may affect *Daphnia* and its predators in the freshwater systems of Wisconsin Lakes. Additionally, it is essential to analyze lakes because most are a source of CO_2, rather than carbon sinks, due to oversaturation that could contribute to climate change in the future (De Stasio Jr. et al., 1996). In freshwater lakes, data suggest deeper thermoclines and more drastic stratification due to higher air temperatures and winds, reducing the amplitude of *Daphnia*'s DVM (De Stasio Jr. et al., 1996). This study found two new potential trends in DVM and a change to the physiology of zooplankton due to increased temperature from climate change. The first trend showed that migratory amplitude decreased across all modeled warmer temperatures; the second showcased dependence of migratory behavioral changes and predator-prey interactions (De Stasio Jr. et al., 1996). Physiologically, migratory species are expected to shift towards smaller sizes, and long-term selective pressure may favor smaller species (De Stasio Jr. et al., 1996).

Unknowns

Though DVM has long been observed, there are still a lot of unknown variables about the behavior. Because the poles are such a hard *in situ* field space, DVM patterns of different species are unknown. For instance, *Neogloboquadrina pachyderma*, a dominant planktonic foraminiferan found in northern polar latitudes, is known to perform a vertical migration towards deeper water for reproductive purposes. Still, it is unclear if the species perform Diel Vertical Migration (Greco et al., 2019). Only one study has aimed to research this, and it found no definitive indication of DVM. However, the experiment was conducted during the midnight sun, which has poor changes in light intensity in the poles (Greco et al., 2019). If no significant environmental changes are present to cue DVM, it is impossible to observe this behavior in a migratory species. The bigger-picture point with this information is that foraminifera plankton is affected by ocean acidification in an even more direct manner. Foraminifera produces calcium carbonate shells, a compound that is more easily dissolved by the relatively acidic waters that result from ocean acidification. An uncertain but confirmed migratory species for selective processes, known for its critical importance to the carbon cycle and nutrient cycling, foraminifera may already be on track for population collapse due to drastic pH shifts in deep ocean water (The Ocean Portal Team, 2018).

Conclusion

Diel Vertical Migration (DVM) is one of the most common and widespread migratory behaviors completed by thousands of microscopic organisms daily, making it the largest migration on Earth in biomass. The migrants, a variety of zooplankton species, ascend to the surface to feed on phytoplankton and do so at nighttime to avoid predators. They then descend back towards deeper water to avoid environmental stress from factors like ultraviolet (UV) radiation. These species use an array of environmental cues, including light, temperature, UV intensity, and water density, to signal when their migratory journey should commence. Climate change and its associated destructive impacts continue to change the environment in which migratory zooplankton live and travel. As these changes occur at unprecedented rates, zooplankton will face changes that will negatively impact their physiology and migratory trends. Given the critical importance of DVM and the significant threats posed by climate change, further research is essential to identify potential solutions for phytoplankton adaptation, develop effective regulatory measures, and enhance efforts to limit and reduce CO_2 emissions. Such initiatives are vital for the protection of these organisms and the ecosystems they inhabit.

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Exploring Infant Toy Interactions in Free Play Environments

Charles E. Schmidt College of Science, Florida Atlantic University
Emma Bermudez, Jacqueline Hammack,
Dr. Teresa Wilcox (Faculty Advisor)

Abstract

Infants' interactions with toys play a crucial role in their early cognitive and motor development, yet much of the research on this topic has been conducted in controlled laboratory settings, which may not accurately reflect the diversity of toys and interactions infants experience in their natural environments. This study uniquely examined these interactions in a home setting by utilizing an online video conferencing platform. This study investigated how toy properties influence manual infant-object interactions during dyadic tabletop free play, where mothers selected the toys for the session. Within the confines of this study, results show that infants spend significantly more time manipulating traditional organizational/fine motor toys compared to other toy categories (i.e., responsive, art, etc.). This study highlights the importance of considering the natural context in which toy interactions occur and provides insights into how different types of toys impact early behaviors and exploration.

Introduction

Object manipulation is a type of object interaction that is crucial to infant development, supporting sensory, motor, and cognitive growth (Ruff, 1984). Research shows that infants' preferences for manipulating toys differ depending on the toys' physical and cognitive properties. For example, McCall (1974) found that, in the context of sight-based manipulation, 9.5- to 10-month-old infants significantly preferred flexible and sound-producing toys (e.g., thin tissue paper, flexible springs, and bolted-shut pots containing five items inside) over rigid or noiseless ones (e.g., thin cardboard, non-flexible springs, and bolted-shut pots containing 0 to 4 items inside). Building on the foundational insights of McCall (1974), who highlighted the impact

of toy flexibility and sound on 10-month-old infant toy manipulation behaviors, Bjorklund and Bjorklund (1979) further categorized toys based on their developmental implications, and explored how toy qualities (i.e., organizational, symbolic, and responsive toys) affected infant's preferences for manually playing with different toys. Bjorklund and Bjorklund (1979) observed that older infants (12, 16, and 24 months) significantly preferred spending more time manually contacting toys from the organizational category (e.g., stacking rings, puzzles) compared to responsive toys (e.g., squeak toys) and symbolic toys (e.g., dolls, toy phones). Both studies likely reflect Piaget's Theory of Cognitive Development, which explains that older infants, aged 12 to 18 months, begin experimenting with objects and using trial-and-error methods to solve problems (e.g., playing with shape sorters and puzzles). In contrast, younger infants, aged 4 to 8 months, are more focused on simple behaviors that involve interacting with the external world, such as shaking a rattle (associated with responsive toys), as their attention shifts from self-directed actions to exploration of their environment. Complex, creative symbolic thinking, which supports play with dolls or toy phones (associated with symbolic toys), typically begins to develop around 18 months and continues through early childhood (2 to 7 years; Sanghvi, 2020).

Many studies on infant object manipulation focus primarily on physical properties like color, shape, and texture, rather than considering the functional, cognitive, and social aspects of the toys (Ruff, 1984; Rochat, 1989). Additionally, most research emphasizes how factors such as posture, age, and social touch influence infant-object interactions, rather than how toy properties themselves might influence an infant's interaction (Soska & Adolph, 2014; Mlincek et al., 2022; Needham & Nelson, 2023; Tanaka et al., 2021).

While toy properties influence how infants interact with objects, the variety and availability of toys play a crucial role in shaping these interactions and supporting infant development (Swirbul et al., 2022; Healey et al., 2019; Wachs, 1985). Research shows that infants spend significant time interacting with diverse objects found in their home environments, including books, toy vehicles, stuffed animals, electronic toys, and everyday household items like boxes and blankets (Swirbul et al., 2022). However, lab-based studies often fail to reflect this diversity, as they typically use a restricted set of toys that represent only a few toy categories (Bjorklund & Bjorklund, 1979; Koşkulu et al., 2021; Miller et al., 2017). This broader variety is usually reserved

for studies conducted on preschool children (Rubin & Howe, 1985; Pereira et al., 2021). Furthermore, although Swirbul et al. (2022) and Herzberg et al. (2022) documented the variety of toys infants were exposed to at home, they did not document how much time was spent with each toy variety. Instead, they categorized the manual object interactions as toy, non-toy, or mixed.

To address these limitations, this study examines manual object interactions with the toys infants have at home during unmoderated dyadic free play. By allowing mothers to choose the toys to bring to the play session, this naturalistic approach allows infants to access a greater variety of toys, reflecting their typical environments. This study also expands the categorization of toys, organizing them based on shared cognitive and physical properties, allowing for a more comprehensive understanding of the underlying influences that may be dictating infant preferences for manipulating certain objects more than others (Bjorklund & Bjorklund, 1979; Healey et al., 2019; Cates et al., 2023). Furthermore, this study explores interactions within the broader context of caregiver-infant play at home, where crucial moments of learning and development occur (Schneider et al., 2023).

To our knowledge, no studies to date have investigated or observed the influence of toy types on infant-object manipulation preferences during unmoderated, dyadic mother-infant tabletop play. Our exploratory analysis aimed to fill these gaps by:

1. Analyzing the composition of infant toy environments
2. Exploring the overall time infants manually engage with toys
3. Examining the duration of interaction with each toy type
4. Assessing how toy properties influence infant preferences for manipulation
5. Investigating whether infants exhibit different preferences for toy manipulation when exposed to a greater variety of toys in home environments compared to the limited variety in lab studies

These findings offer insights into how infants' typical toy environments influence engagement and preferences, contributing to a deeper understanding of how early experiences shape object exploration. Examining these patterns is crucial for understanding infancy, as the types of objects infants interact with can affect their cognitive, motor, and social development. Understanding these dynamics can help researchers and caregivers identify factors that support healthy development during this crucial time.

Methods

Forty-seven infant-mother dyads (N_{male} = 21) aged 12 to 24 months (M = 17.21, SD = 3.22), participated in a four-minute naturalistic free play session conducted via Zoom, an online video conferencing platform. The sample was predominantly non-Hispanic Caucasian (59.57%), with remaining participants identifying as Hispanic (29.78% infants, 31.91% mothers), multiple races (8.51% infants, 6.38% mothers), and African American (2.12% of infants and mothers). Mothers were instructed via email to bring about five non-electronic toys to the play session. Toy selection was at the mothers' discretion, allowing flexibility to create a more naturalistic setting. They were instructed to sit at a tabletop position with their infant for the duration of the session and to "play with your infant just as you normally would." This instruction was intended to simulate naturalistic free play and minimize the controlled conditions typical of laboratory experiments. An undergraduate or graduate student researcher moderated the session, assisting with video setup and ensuring optimal video quality. Trained coders analyzed the recordings to document the time infants spent manipulating objects, the types of toys manipulated, and all toys present in the infant's immediate environment (whether the infant interacted with them or not).

Recruitment and Inclusion Criteria

Participants were recruited through Facebook ads and local events in West Palm Beach, Boynton Beach, Delray Beach, and Boca Raton, Florida. Infants aged 6 to 24 months were initially recruited, but only those aged 12 to 24 months were included in the study. Only data from the first play session were used to minimize familiarity with the experiment's procedures, excluding any second sessions (if a dyad returned to participate again). Infants were screened using the *Ages & Stages Questionnaire®*, *Third Edition* (*ASQ®-3*) and excluded if they fell below the cutoff in any developmental domain (communication, gross motor, fine motor, problem-solving, personal social). The initial sample size was 52 dyads; four participants were excluded for not meeting the criteria, and one was dropped due to technical issues.

Coding Object Interactions

Object interactions were recorded each time an infant displaced an object. Object displacement refers to the act of an infant moving or manipulating an object (Swirbul et al., 2022; Herzberg et al., 2022).

Coders tracked the onset and offset of object displacements, also called displacement bouts. A displacement bout is a continuous period of manipulation separated by pauses of more than three seconds (Swirbul et al., 2022; Herzberg et al., 2022). Onsets were coded when the infant first moved the object with any part of the hand, through actions like lifting, holding, pressing, grasping, banging, or shaking. A displacement event was counted even if the infant was not looking at the object (e.g., carrying it). Resting hands on or lightly touching an object without moving it did not count. Offsets occurred when the infant stopped touching the object or paused moving it for more than three seconds. If part of the object were visible but the hands were not, the coder could deduce manipulation. If the hands were out of view for less than three seconds and returned with no change, it was coded as an object interaction; if the hands were out of view longer and the manipulation was unclear, it was coded as missing (e.g., hands in a toy chest).

Object displacement bouts were coded using Datavyu (https://datavyu.org), a digital coding program for frame-by-frame behavioral analysis synchronized with video footage. The coding scheme for this study was adapted from Swirbul et al. (2022), whose coding manuals, Ruby scripts, analyses, example video clips, and data are publicly available at https://nyu.databrary.org/volume/1417. Fundamental coding rules, created by infant play experts from universities across the United States, are outlined on the PLAY project website (https://play-project.org/coding.html#Object_Interaction_coding; Herzberg et al., 2022). To ensure reliability, 20% of the object displacement interactions were coded by a secondary coder, achieving an average agreement of 94.7% (range: 70.59% to 100%).

Coding Toy Types

Toy types were categorized as responsive, symbolic, organizational/fine motor, art, language/concept, non-toy/household, and gross motor (Bjorklund & Bjorklund, 1979; Healey et al., 2019; Cates et al., 2023). Refer to Table 1 for the characteristics and properties of each toy category.

Some toys could fit into more than one category. In these cases, toys were categorized based on their predominant properties. For example, Mr. Potato Head could be placed in both the symbolic and organizational categories but was categorized as organizational due to its primary purpose (assembling the pieces).

Looking at Table 1, some toy names appear in multiple categories (e.g., 'animal' is listed in both the symbolic and organizational/fine motor categories). This indicates one animal toy served a predominant symbolic function, while another animal toy functioned as puzzle pieces and was categorized as organizational/fine motor. The simplicity in the naming of toys facilitated the coding process. When analyzing the data, toys were evaluated and separated by their categories, not their names, ensuring repeating names did not interfere with the analysis of toy types. These categories were further subdivided into traditional (non-electronic) and electronic versions, as some parents brought electronic toys to the play session. The categorization is based on the Availability of Learning Materials (ALM) subscale of the StimQ, an interviewer-administered evaluation of the cognitive enrichment children experience at home (Cates et al., 2023). Toy types were coded by modifying the coding scripts from Swirbul et al. (2022), so coders were able to code the types of toys infants displaced within object displacement bouts. For toy types, coders averaged 99.98% agreement (range: 98.9% to 100.0%).

Results

Types of Toys Present

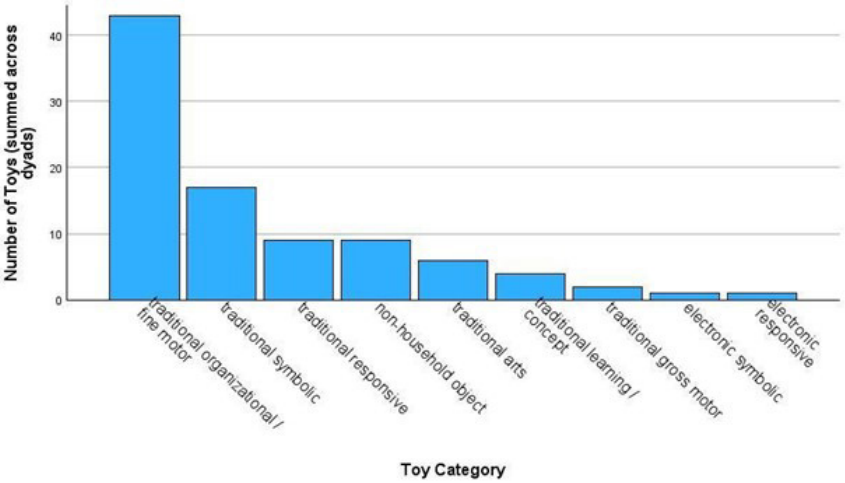
Across all dyads, infants had an average of 5.13 toys present in their environment (SD = 3.42, range = 1.0 to 14.0). The toy types most frequently present in the infants' environments were those in the traditional organizational/fine motor and traditional symbolic categories. The traditional organizational/fine motor toy category was the most prevalent, which accounted for 49.26% (SD = .35) of all toys (Figure 1). This was followed by traditional symbolic toys, making up 23.45% (SD = .31) of the total. Other toy categories included traditional art (9.32%, SD = .25), traditional non-toy/household items (6.44%, SD = .12), traditional responsive (5.32%, SD = .12), traditional learning/concept (2.95%, SD = .09), and traditional gross motor toys (2.55%, SD = .11; Figure 1). Electronic toys were the least present, with both electronic responsive and electronic symbolic categories each comprising 0.35% (SD = .02) of the toys.

Table 1
Toy Categories and Definitions with Examples of Object Names

Toy Type	Object Names
Traditional responsive *Objects created to produce sounds and/or visual effects when a child interacts with them (Bjorklund & Bjorklund, 1979).*	ball_shaker instrument jack_in_the_box maraca pop_up toy_bar
Traditional symbolic *Objects intended for use in pretend play or to be imagined as something different from what they are (Bjorklund & Bjorklund, 1979).*	animal character doll kitchen_toy stuffed_animal tea_set toy_chair toy_cup toy_food toy_pan toy_telephone vehicle
Traditional organizational/fine motor *Objects that are created for the systematic organization of related components, allowing a child's interaction to establish connections between these parts, also includes objects that supporting fine motor skills (Bjorklund & Bjorklund, 1979; Healey et al., 2019)*	activity_cube animal ball ball_run blocks car_tower character container fidget_toy fidget_tube linked_toy magnet_tiles nesting_dolls peg_sorter pop_it puzzle shape_sorter stacking_cups stacking_flowers stacking_rings tissue_box toy_bar uno_moo
Traditional art	crayons paper playdoh playdoh_utensil stickers
Traditional non-toy/household	bracelet container floss water_bottle yarn
Traditional gross motor	ball
Electronic responsive	wand
Electronic symbolic	character

Note. A list of object names sorted by toy category, representing the entire sample and only the toys infants displaced.

Figure 1
Total Number of Toys Present (by Category) Across All Dyads



Note. This illustrates the total number of toys present (by category) across all infant-mother dyads.

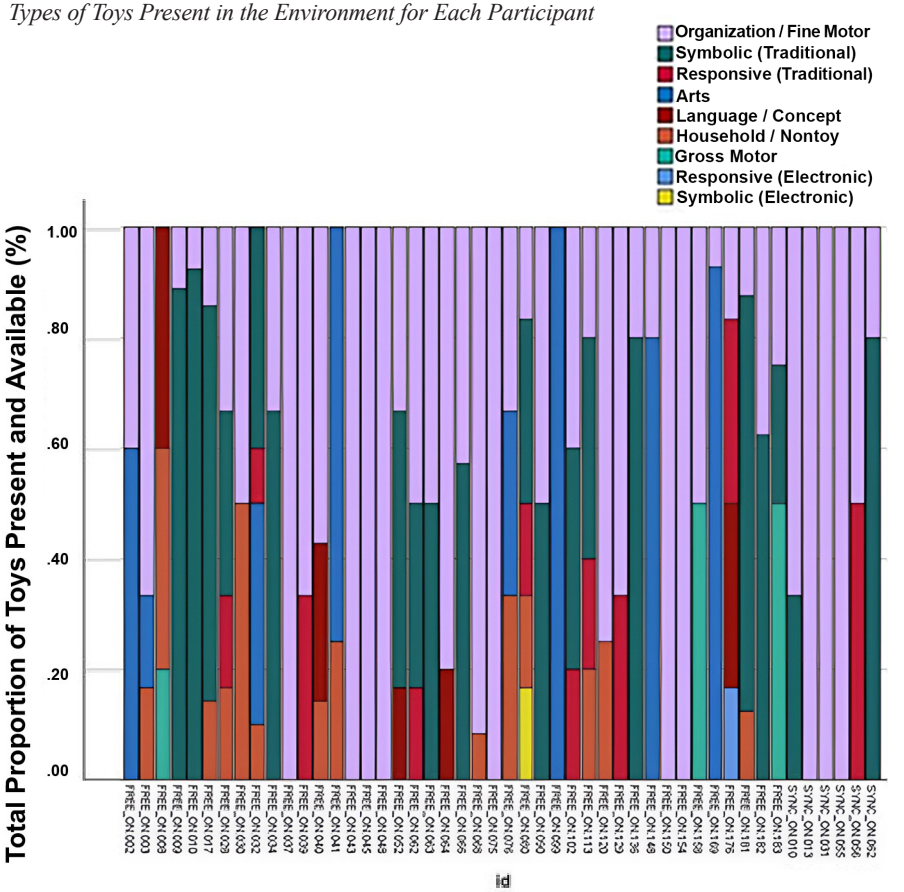
Time Spent Manipulating Objects

We assessed the amount of time infants spent manipulating objects and the amount of time spent without manipulating any objects during the free play session. A paired-samples t-test revealed that on average, infants spent significantly more of their time manipulating objects (t [46] = 11.108, p = <.001, m = 76.6%, SD = .16) compared with time spent without manipulating any objects (M = 23.4%, SD = .16; see Figure 3).

Time Spent Manipulating Objects by Toy Categories

We also examined the percentage of time infants spent manipulating each type of toy. A repeated measures analysis of variance (ANOVA) was conducted with the percentage of time spent manipulating each of the nine types of toys as within-subjects factors. The ANOVA was significant ($F[8,36] = 255.671, p < .001$). Follow up paired comparisons revealed that infants spent most of their time manipulating traditional organizational/fine motor toys (50.52%, SD = .33) compared to all other toy types (p < .001; Figure 4). Traditional symbolic toys followed behind, with infants spending 9.84% (SD = 9.84) of their time with these types of toys, and traditional art toys accounting for 8.61% (SD = .26) of their playtime (Figure 4). Less time was spent manipulating other toy types: traditional responsive toys (3.17%, SD = .11), traditional

Figure 2
Types of Toys Present in the Environment for Each Participant



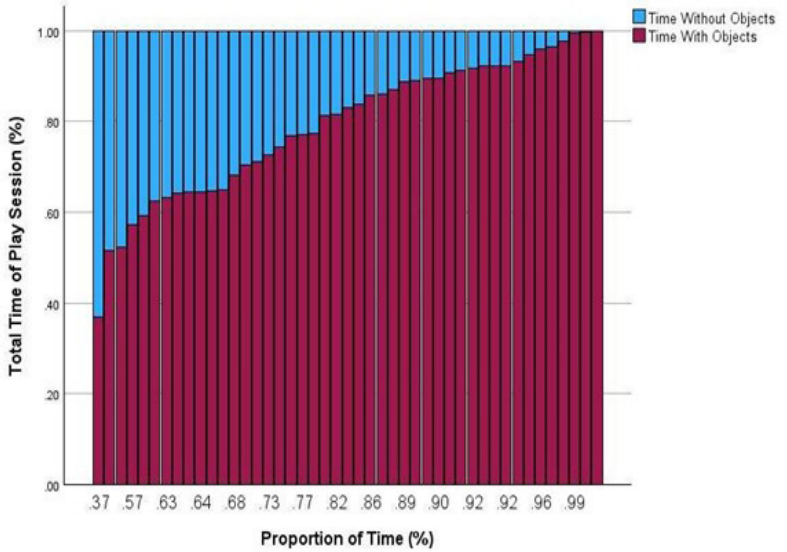
Note. This graph displays the proportion of toy types in each infant's environment, with each bar representing an infant and each color corresponding to a toy category.

learning/concept toys (1.40%, SD = .09), traditional nontoy/household items (2.61%, SD = .10), and traditional gross motor toys (0.33%, SD = .02; Figure 4). Interaction with electronic toys was minimal, with infants spending 0.07% (SD = .005) of their time on electronic responsive toys and 0.06% (SD = .004) on electronic symbolic toys (Figure 4).

A repeated measures ANOVA was also conducted to examine the average bout duration for each toy type across all dyads. The average duration of independent bouts of time that infants spent manipulating various toy categories varied significantly ($F [8,46] = 14.634, p < .001$; Table 2). Follow-up paired comparisons

Figure 3

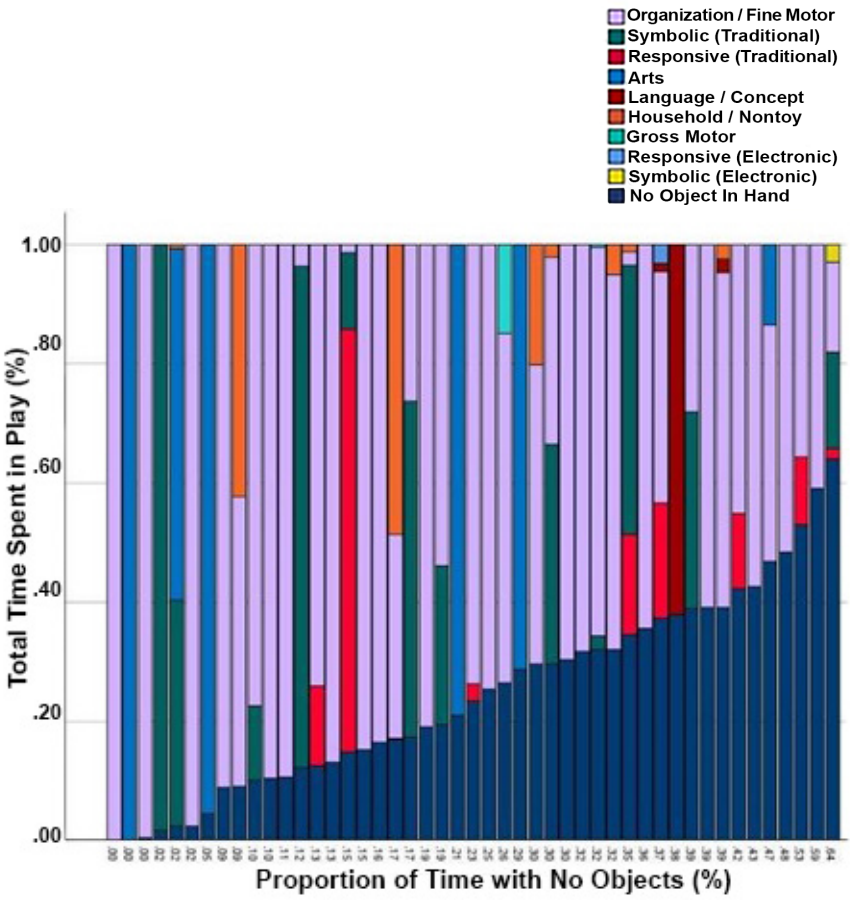
Proportion of Time (by Percentage) Infants Spent Manipulating Objects and without Manipulating Objects



Note. This figure shows the proportion of time infants spent manipulating objects and not manipulating objects during the four-minute free play session. Bars, arranged from least (left) to most (right) time spent manipulating objects, represent individual infants.

revealed that infants' bouts with traditional organizational/fine motor toys were longer, on average, compared to all other toy categories (M = 30.634 seconds, SD = 7.31, range = 0 to 240.32; $p < .030$). Traditional symbolic toys had an average interaction time of 3.55 seconds (SD = 6.50, range = 0 to 27.07), which was significantly longer than average bouts of traditional gross motor (M = .1231 seconds, SD = .677, range = 0 to 4.49; $p = .031$), electronic responsive (M = .163 seconds, SD = 1.12, range = 0 to 7.67; $p = .040$), and electronic symbolic (M = 0.153 seconds, SD = 1.05, range = 0 to 7.21; $p = .023$). Traditional art toys had an average interaction time of 3.04 seconds (SD = 11.44, range = 0 to 62.36). Traditional responsive toys displayed an average of 1.70 seconds (SD = 10.72, range = 0.34 to 33.65). Interaction with traditional non-toy/household items averaged 8.56 seconds (SD = 8.24, range = 0.54 to 24.58). Traditional learning/concept toys exhibited an average of .319 seconds (SD = 1.61, range = 0 to 10.66).

Figure 4
Proportion of Time Spent in Object Manipulations by Toy Categories



Note. The bar graph shows the proportion of time each infant spent manipulating each toy category and without an object in hand (represented in dark blue at the bottom of each bar). Each bar represents one infant, with colors indicating specific toy types or the absence of manual interactions.

Table 2
The Average Bout Duration for Each Toy Category Across All Dyads

Toy Type[a]	Mean (seconds)	Standard Deviation	Min. (seconds)	Max. (seconds)
Electronic Responsive	0.163	1.12	0.00	7.67
Electronic Symbolic	0.153	1.05	0.00	7.21
Traditional Art	3.04	11.44	0.00	62.36
Traditional Gross Motor	0.123	0.68	0.00	4.49
Traditional Learning/ Concept	0.319	1.61	0.00	10.66
Traditional Nontoy/ Household	8.56	8.24	0.54	24.58
Traditional Organizational/Fine Motor	30.63	7.31	0.00	240.32
Traditional Responsive	1.70	10.72	0.34	33.65
Traditional Symbolic	3.55	6.50	0.00	27.07

Note. The table shows the average bout duration (in seconds) for each toy category across all infant-mother dyads, along with the standard deviation and the minimum and maximum durations.
[a]Limited to first 100 cases

Discussion

Types of Toys Present

Our findings show that, within the scope of our study, traditional organizational/fine motor toys dominated infants' home environments during dyadic tabletop play, comprising 49.26% of the total toys, followed by symbolic toys at 23.45%. This may reflect mothers' preference for toys that support joint participation, as these types of toys encourage shared play and mutual involvement, unlike toys that may be for individual use and participation, such as rattles (from the responsive category) or art supplies.

Caregiver beliefs and decisions, such as purchasing toys, strongly influence the toys present in an infant's environment (Michael Cohen Group, 2019). Allowing mothers to select toys for the play session created a naturalistic approach but introduced potential bias. Mothers might have, consciously or subconsciously, prioritized certain toys based on perceived developmental benefits or to avoid judgment, which may have influenced

observed object manipulation preferences. Future studies could address this by surveying home toy inventories and randomly selecting toys for play sessions to reduce bias and better represent the infants' overall toy environment during dyadic tabletop play.

Time Spent Manipulating Objects

During the four-minute play session, infants spent significantly more time, on average, manipulating objects (76.6%) than not manipulating objects (23.4%), with mothers present throughout. This strong preference for manual object play aligns with research showing that, over a two-hour period, infants engage in manual object play for most of their time (60%) at home (Swirbul et al., 2022). The presence of a caregiver may also influence this high percentage of manual object engagement by keeping infants on-task and redirecting them if they become distracted. This is supported by a study showing that infants interacted with objects longer when their mothers were actively engaged with them compared to when they played independently (Schatz et al., 2022). These findings suggest that social context, particularly caregiver presence, plays an essential role in infants' object interactions, guiding early cognitive and motor skill development with objects through shared engagement.

Time Spent Manipulating Objects by Toy Categories

During dyadic tabletop play at home, with the toys available to them, infants aged 12 to 24 months displayed a significant preference for traditional organizational/fine motor toys, spending over half of their manual object interaction time with these toys. This likely reflects their developmental stage, as problem-solving activities like fitting blocks into a shape sorter are more accessible than symbolic, artistic, or conceptual play, which involves advanced cognitive processes emerging between 18 to 24 months and developing fully at ages 2 to 7 years (Sanghvi, 2020). Responsive play, typical for younger infants aged 4 to 8 months, was less engaging for this age group, explaining their limited interaction with toys from that category (Sanghvi, 2020). Similar preferences were observed in a laboratory study by Bjorklund and Bjorklund (1979), where infants aged 12, 16, and 20 months preferred manually engaging with organizational toys during non-dyadic play, a preference also observed in our study when infants were in the presence of caregivers. The consistency between these findings and our study suggests that, even with greater toy variety within and across toy categories, infants'

preference for organizational/fine motor toys persists across settings, offering insights into early cognitive and motor skill development.

Average Bout Duration by Toy Categories

Traditional organizational/fine motor toys had the highest average bout duration (30.63 seconds), significantly longer than all other toy categories, suggesting they promote longer, more focused play. This type of focused engagement is developmentally beneficial, as sustained attention is linked to improved cognitive and self-regulatory functions. A longitudinal study found that sustained attention at age one correlates with better executive functioning and effortful control at age two (Johansson et al., 2015). These findings highlight the potential developmental benefits of activities like playing with organizational/fine motor toys, which may foster focused attention and early executive functioning.

The minimal interaction with and presence of electronic toys was expected, as mothers were instructed to bring non-electronic toys to the session, with only two infants having electronic toys in their environment. However, future studies should compare interactions with electronic versus traditional toys, especially given concerns about the potential negative developmental effects of electronic toys (Miller et al., 2017; Wooldridge & Shapka, 2012). With the increasing prevalence of electronic toys in today's society, further research is needed to explore their potential detrimental effects on infant development. To our knowledge, no studies have indicated any developmental benefits of electronic play.

Conclusions, Limitations, and Future Directions

Our study shows that infants, with the toys available to them, prefer manipulating traditional organizational/fine motor toys during dyadic free play in a tabletop setting at home. However, limitations in this study suggest areas for future research. One limitation is that object manipulation alone may not fully capture engagement with symbolic and artistic toys, which may require less hands-on interaction than organizational/fine motor toys. Future research could address this by observing additional behaviors during manual object interactions, such as gaze direction, joint attention, and positive affect, to explore the relationship between these behaviors and gain a more comprehensive understanding of infant engagement with different toy types.

Another limitation of this study is the task constraint requiring mothers to remain seated and play at a table, which may have influenced

their toy selection, favoring tabletop-appropriate toys like puzzles over gross motor toys. This likely contributed to the high prevalence of organizational/fine motor toys in the toy distribution. Future studies could capture more natural dyadic interactions by allowing infants and mothers to play in a less structured setting, potentially observing a broader range of toy types and behaviors.

Infants may have spent more time with organizational/fine motor toys due to their greater availability, suggesting that toy accessibility influences infant-object interactions. Understanding this distinction is important for determining whether infants engage with certain toys because of their properties or their availability. Future studies could use self-reported inventories or video tours to assess toy availability, providing insights into whether infants' preferences are shaped more by the toys' accessibility or their specific characteristics.

Finally, this study's participant sample was predominantly white and non-Hispanic. To better understand infant behaviors across cultural contexts, future studies should prioritize sample diversity, enhancing the generalizability of findings. Conducting research in infants' home environments, rather than unfamiliar laboratory settings, may also improve participant comfort, yield more natural behavior, and increase accessibility for a broader range of caregivers, potentially promoting greater population diversity. These insights could deepen our understanding of how toy types may impact early cognitive and motor development.

Acknowledgements

Thank you to the Office of Undergraduate Research and Inquiry (OURI) for funding this research. I am especially grateful to my graduate advisor, Jaqueline Hammack, and faculty advisor, Dr. Teresa Wilcox, for their invaluable assistance and support throughout this entire process.

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From Company to Crown: The 1858 Government of India Act in the British Press

Dorothy F. Schmidt College of Arts and Letters,
Florida Atlantic University
Benjamin Cohen, Dr. Douglas Kanter (Faculty Advisor)

Abstract

In the aftermath of the Indian Mutiny and the perceived failures of the British East India Company, the British Parliament passed the 1858 Government of India Act, which liquidated the Company and placed India under direct rule by the Crown. The historiography on the Company's end and the Raj's beginning has not given adequate attention to the Act itself, portraying it as an uncontroversial measure used to change India's governing body. This article addresses the controversial nature of the Act. By examining geographically and politically dispersed newspaper articles from when the Act was being considered in Parliament, this research shows that the 1858 Government of India Act was contested, and there was no uniform reaction to the Act's introduction or passage through Parliament.

Introduction

India was long one of the most important colonies in the British Empire. From its origins as a pathway into a new area for exploration and trade to a land filled with opportunities for cultural and economic expansion, India was the "jewel in the crown" of the British Empire, according to historian James Lawrence (1997). Historians have explored how India developed from an uncharted land to a bustling center for trade in the Empire and how India's colonial history had a lasting impact on the rest of the Empire, even in the modern era. But they have not paid adequate attention to the political transition in India from rule by the East India Company to direct government by Britain, which occurred in 1858. Historians have treated the switch from the Company to the Crown as a uniformly accepted decision. However, there is insufficient scholarship on how the press reacted to the end of a major imperial institution.

This article argues that the 1858 Government of India Act, which facilitated the end of the British East India Company and instituted direct Crown rule over India, was extremely controversial. Through an analysis of nine British and Irish newspapers, it demonstrates that there was no uniform press perception of the bill while Parliament considered it. This research will examine press commentary discussing the merits and drawbacks of the bill and Company rule over India in general. Newspaper coverage both reflected and informed public opinion. In an effort to provide a representative sample of attitudes to the Government of India Act, the journals examined in this essay are geographically dispersed and politically diverse. Previous scholarship does not discuss the bill, leading historians to believe that it was noncontroversial; however, the different opinions seen across the United Kingdom show that this was not the case and that there was no universal opinion of the bill.

Most of the historiography on British governance over India does not address the 1858 Government of India Act; instead, it frames the legislation as the instrument that switched Indian governance from the British East India Company to the Raj. In their major works about the British Empire, Antoinette Burton, William Dalrymple, and Thomas Metcalf all use it in this manner, failing to discuss the Act's public perception (Burton, 2015; Dalrymple, 2006; Metcalf, 1960). While this approach is valid, it does not give enough credit to the circumstances and opinions that created the Act in the form it was enacted.

Historians have neglected to consider how the Act itself was highly controversial and there have been no other studies specifically on the Act's public perception. These controversies could have been foreseeably carried into the early years of the Raj. Thus, historians' ability to analyze the Raj effectively in its early years is limited. While there is no directly relevant literature, the relevant historiography addresses the results of the Indian Mutiny that pushed British officials to make this change in governance. Literature on British opinions of why the Act was necessary will add to this and help to illustrate why scholars found the British East India Company to be an ineffective governance structure, necessitating the Raj.

The British East India Company came from the rapid expansion of British exploration in the late sixteenth and seventeenth centuries (Andrews, 1985, p. 256). By the middle of the eighteenth century, the Company had established a durable presence in India and had become indispensable to the Imperial economy. After defeating

a vassal within the Mughal Empire, the Mughal Emperor gave the Company a monopoly on trade within the area and the right to manage the finances of the Mughal Empire, including collecting taxes (Parsons, 2019, p. 45). The Company became India's dominant political and governmental power, forcing administrators to balance their profitability and governance responsibilities.

Several aspects defined the Company's administrative structure in India. While Company administrators retained control over most Company affairs, many aspects of Indian governance relied on the colonial populations. Colonial subjects formed the basis for the Company armies. While officers and some soldiers were European, Indians comprised most of the common army. They also formed much of the civil and administrative service, with Indians being a crucial part of the Company police force in India (Parsons, 2019, p. 49). As the Indian people began to take more prominent positions in roles traditionally seen as "European," more Western education institutions were established. These aimed to bring "evangelism, Western law, and English education" to the people of a land considered to be barbaric (Parsons, 2019, pp. 52–53). The graduates of these programs were seen to be more "Western" than the other colonial subjects and were put into the civil service, albeit at lower levels (Parsons, 2019, pp. 52–53; Mookerjee, 1944, p. 30).

While Company rule had, in many ways, helped bring economic improvement to the region, their actions caused resentment among the Indian people. Heavy land taxes on the Indian people fueled discontent with the Company administration. The anglicization of the Indian people also made many colonial subjects feel as if their culture was being alienated. When the British introduced the Enfield Rifle as the primary weapon of the primarily Hindu and Muslim *sepoy* army, there was an outcry amongst soldiers that resulted in a series of violent outbursts (Yadav, 1994, pp. 140–142; Burton, 2015, p. 58).

The reports of violence caused by the rebels during the Indian Mutiny/Rebellion of 1857–1858 helped to change many perspectives about how India should be governed. Before, many British administrators saw India as a land that could "be as progressive as England itself" by bringing British customs and culture to the land. The Mutiny forced the British government to view their governance of India through a more authoritarian lens (Metcalf, 1960, p. 25). The Mutiny highlighted the lack of benefits of Company administration in India. Many realized that the Company did not bring much to Indian governance (Spear, 1965, p.

229). After realizing the benefits of direct rule over India, Parliament began discussing the future of the British East India Company.

Lord Palmerston noted several reasons for the Government of India Bill when it was introduced to Parliament on February 12, 1858. He explained the positive aspects of Company rule in India and that the Indian people benefited under the Company. However, Palmerston argued that imperial administration should be accompanied by accountability to Parliament. By keeping the Company, still an independent organization, as the administrative body of British India, Palmerston argued that India had a "double government" that created a cumbersome bureaucracy. This, he argued, in part contributed to the Indian Mutiny. Although the government had no control over the situation, they were blamed for these failures (*Hansard*, 12 Feb. 1858 col 1277). Lord Stanley and Benjamin Disraeli continued to advocate for the bill when the Conservatives took control of Parliament in late February of that same year. While some particulars of the bill were changed, its focus was the same as when Palmerston initially introduced it.

The basis of the bill was to create a system whereby Parliament would more directly control the administration of India, allowing them to have more control over how India was governed (*Hansard*, 12 Feb. 1858 col 1277). Palmerston proposed that the Crown control British possessions in India to achieve this goal, with a Secretary of State for India established to oversee the colony in conjunction with a fifteen-member council. The Act also established the Indian Civil Service and put all Company contracts onto the Crown. These changes were guided by the goal of merely being a change in governance rather than a change of the government system. While many members of Parliament saw this change as necessary based on the events in India and the perceived issues with the Company governance system, others saw flaws in the bill. An early opponent was Thomas Baring, the Conservative MP for Huntingdon. His main objection was to the bill's timing, arguing that the unrest in India would have to settle before this bill could be passed. This, he argued, would put the government in a better position to enforce the change in the system (*Hansard*, 12 Feb. 1858 col 1296).

As the proceedings carried on, members of Parliament raised other objections. Parliament amended the bill several times, which many MPs saw as weakening the structures that could have checked the Council set up to assist the Secretary of State for India (*Hansard*, 25 Apr. 1858 col 1692). One of the chief criticisms was that the government did not state its exact grievances with Company rule; rather, ministers alluded

to several factors culminating with the Mutiny. Most MPs overcame these objections through Lord Stanley and Disraeli's work, and the bill received royal assent on August 2, 1858.

English Press Reactions

The English press in London was at the center of the debates surrounding this bill. As a significant piece of legislation that occupied Parliament's attention, many newspapers in London paid close attention to the India bill. As one of the major liberal newspapers in London, the *Daily News* regularly published articles about the bill's progress (Brake & Demoor, 2009, p. 158). The *Daily News* editors immediately began to attack the bill after it was introduced. The liberal paper believed that changing the governing structure for India was too radical of a move, warning that "[the government of India] is not a thing to be trifled with" (*Daily News*, 17 Feb. 1858). As Parliament amended the bill and the government sponsoring it changed, the *Daily News* attacked both the way that Parliament considered the bill and some of the new aspects of the bill. It argued that the House of Commons did not fully comprehend how Indian affairs would be affected by this system (*Daily News*, 18 Jun. 1858). The *Daily News* continued with this trope as the bill approached passage, indicating this paper's strong opposition to the bill.

Compared to the policies of the *Daily News*, London's major conservative newspaper, the *Standard,* firmly supported the India bill. The *Standard* was circulated well beyond London and used in other conservative publications (Brake & Demoor, 2009, pp. 596–597). When the bill was introduced in February, the *Standard* writers pointed out how Company governance was flawed from the beginning, eventually leading public opinion to turn against them (*The Standard*, 12 Feb. 1858). During the revisions to the bill, the *Standard* continued to publish accounts of the Indian Mutiny, something that was not seen as much with the *Daily News*. The *Standard* continued to voice its support for the bill, arguing that the measure's opponents based their opposition on the timing of the legislation rather than an actual need for the bill. The paper used intelligence reports from India to counter these claims. Towards the passage of the bill, its correspondents in India argued vehemently for a change in government (*The Standard*, 6 Apr. 1858, 20 Jul. 1858).

These two newspapers provide ample information as representatives of the larger English press. The *Daily News*, one of the

leading liberal newspapers, opposed the bill until the end. This pattern contrasts with the *Standard*, which followed what the Conservative party leadership advocated by supporting the bill. Its coverage of the bill's passage makes it clear that many members of the English press believed that the Mutiny demonstrated the failures of the Company. This evidence from the press demonstrates how English reactions to the bill were split based on political leaning.

Irish Press Reactions

The English liberal press primarily opposed the India bill because of its timing. The Irish liberal press, on the other hand, had vastly different reasons for opposing the bill. The *Northern Whig*, a prominent Irish liberal newspaper based in Belfast that circulated throughout Northern Ireland, followed the bill's passage in Parliament (Brake & Demoor, 2009, p. 461). Its editors described it as "a masterpiece of human stupidity" (*Northern Whig*, 12 Apr. 1858). They argued that the politicians who introduced the bill neither understood nor cared about the Indian people who would, upon passage, be subject to their direct rule. The editors believe that the Company had a major impact on the negative actions in India; however, the *Northern Whig* argued that the Indian people would be no better served by a group of people who had never been exposed to the area and could not be expected to care. While the bill's specifics changed over the six months it was being considered, the *Northern Whig* never substantially changed its views. One of its main criticisms was how the House of Commons considered the bill. They argued that the increasing specificity of the bill did not address the key concerns and opposed it until it was passed (*Northern Whig*, 11 Jun. 1858).

Irish conservatives initially followed much of the same trends as Irish liberals. The *Belfast News-Letter*, a major conservative paper, opposed the bill (Brake & Demoor, 2009, pp. 44–45). When it was introduced, editors for the paper explained how putting control over India in the hands of the new Council, which the Prime Minister's government would ultimately choose, would adversely affect Indian governance (*Belfast News-Letter*, 19 Feb. 1858). However, the *Belfast News-Letter* writers changed their views on the bill once it was reintroduced by Benjamin Disraeli, requiring some members of the Council to have experience in India. The editors fully supported these changes and believed they would strengthen the new Council. They also felt that it would improve the caliber of Indian civil servants

(*Belfast News-Letter*, 30 Mar. 1858). The paper continued its support until the bill was adopted, writing that it was in its best form yet and that it was necessary to enact it immediately to prevent future issues under the Company (*Belfast News-Letter*, 9 Jun. 1858).

While the liberal and conservative perspectives are essential to understanding Irish opinions of the bill, the Irish nationalist perspective holds equal weight and must be considered. This is seen through examining the *Freeman's Journal*, one of the leading Irish nationalist newspapers that "reflected mainstream moderate nationalist opinion" (Brake & Demoor, 2009, pp. 230–231). This paper expressed concern over the bill and took issue with how Parliament considered the bill, citing some major issues as symptomatic of a broken state. One of the paper's major apprehensions was the new Council members' high salaries (*Freeman's Journal*, 19 Jul. 1858). The *Freeman's Journal* saw the bill as evidence of Parliament's indecisiveness and division in creating policy, explaining how the bill was "intolerable" and "required only to be exposed in order to cover its proposers and abettors ignominy and disgrace" (*Freeman's Journal*, 28 Jul. 1858). These opinions reflected their general concern with English governance seen in the Irish nationalist movement.

The Irish press's reactions to the bill were as diverse as those in England. The *Northern Whig* authors reported that their views were consistent for Irish liberals, indicating that they generally opposed the legislation. While Irish conservatives initially opposed the bill, their concerns were addressed in due time, and they eventually supported the measure, recognizing that it was the best way to fix the Company's broken governance structure. The Irish nationalist press did not take a firm stance on the bill but saw some specific aspects and the process in which it was considered as symptomatic of the English government's struggles. The Irish press was divided on the bill, with each side of the debate having different opinions and motives.

Scottish Press Reactions

While the liberal press from England and Ireland opposed the India bill, the Scottish press reflected a different attitude. The *Scotsman* was one of the major liberal newspapers in Scotland and heavily commented on the bill while it was being considered in Parliament (Brake & Demoor, 2009, p. 562). Reporting on the bill's initial introduction by Palmerston, editors from the *Scotsman* brought up how there was little evidence that the Company government was

"a model of perfection" as other liberal commentators had suggested (*Scotsman*, 17 Feb. 1858). Their treatment of the bill was extremely balanced. They discussed the bill's benefits and drawbacks, especially the new mechanism of having men of experience as a part of the Council supervising Indian affairs. The *Scotsman* continued to fight for its passage, but the bill faced some opposition because it gave the government too much power. "Imperial matters," the *Scotsman* commentators wrote, "cannot be given over to local management; and if the management is to be imperial, it must go to the Imperial Government" (*Scotsman*, 3 Apr. 1858). While Scottish liberals saw inherent issues with the bill, they believed this system was better than the Company governance system. While they may not have fully favored the bill, they were not opposed.

Similarly to the conservative journals from England and Ireland, the *Edinburgh Evening Courant*, a major Scottish conservative journal with a broad readership, supported the bill (Brake & Demoor, 2009, pp. 189–190). Like the *Scotsman*, the *Courant* treated both sides equally in describing the bill's introduction. However, it pushed back against arguments for delaying reorganization until the Mutiny finished. The *Courant* editors insisted that "...at no time could it be more desirable than now" (*Edinburgh Evening Courant*, 16 Feb. 1858). One of the key ways that the *Courant* advocated for the bill was by attacking the Company governance system. The editors characterized it as a system that made the Company a ruling "caste" in India. One of the arguments against the bill was that the Indian people would not accept the change; however, the *Courant* used evidence from their Indian correspondents to disprove this claim, stating that "the future increase of British influence in India accords no more ground for apprehension" (*Edinburgh Evening Courant*, 24 Apr. 1858). As the bill took its various forms and was eventually passed, the *Courant* demonstrated optimism for its passage and effectiveness (*Edinburgh Evening Courant*, 19 Jun. 1858).

The Scottish reaction to the India bill differed vastly from England's or Ireland's. The latter two expressed more vocal opinions about the legislation, either for or against. The same cannot be said for Scottish conservatives or liberals, who were less vocal about the issue than the English or Irish papers and tended to be more informational than argumentative. Unlike the English and Irish liberal papers, which took a firm stance against the bill, the Scottish liberal press supported it. The Scottish press uniquely endorsed the bill across party lines.

Welsh Press Reactions

The liberal press in Wales opposed the bill. The *North Wales Chronicle*, one of the oldest weekly papers in Wales, indicates how many Welsh liberals reacted to the bill (Brake & Demoor, 2009, p. XI). The paper listed many grievances with the bill. Like other liberal papers, the *North Wales Chronicle* believed the timing was improper because of the ongoing violence (*North Wales Chronicle*, 20 Feb. 1858). It also argued that if one of the two governments in the double governance system had to be abolished, the East India Company should remain since it is already present in the country. This point of contention was not common in other liberal newspapers (*North Wales Chronicle*, 20 Feb. 1858). The newspaper was also opposed to the bill because of the perceived flaws in the system. The editors maintained that the Secretary of State for India should be a permanent official to avoid political influence rather than a Member of Parliament with political motives. They also believed that the new governance structure would create a "stronghold of families" that would control India (*North Wales Chronicle*, 17 Apr. 1858, 3 Jul. 1858). While they opposed the bill, their criticism was more targeted, focusing on specific aspects of the measure.

The general conservative consensus surrounding the bill supported its adoption and extended to Wales's conservative press. The *Cardiff and Merthyr Guardian,* a conservative newspaper based in southern Wales with circulation across the broader region, supported the bill from its inception. The paper endorsed the perspective of one of its India correspondents, who believed that Company policies were the main cause of the Indian Mutiny and that eliminating the Company was the only way for "the people of England…to be proud of a magnificent empire" (*Cardiff and Merthyr Guardian*, 13 Mar. 1858). As the bill neared passage, the paper's editors summarized their support for the bill: "There may be no radical change in the government of the great dependency, but power will be more concentrated, and a corresponding authority will be enacted from all who wield that power" (*Cardiff and Merthyr Guardian*, 17 Jul. 1858).

As in England and Ireland, the Welsh press was divided on the bill. The Welsh liberal press opposed the legislation, whereas the Welsh conservative press favored the measure. However, one distinction is the specificity with which the Welsh press discussed the bill. With the press from the other kingdoms, the commentary on the bill was broad; however, much of the discussion surrounding the bill in Wales, both for and against its adoption, was specific. Liberal papers stated their

opposition through clear arguments about major parts of the bill. This was uncommon with newspapers in other kingdoms, where much of the opposition targeted the bill as a whole rather than a particular part. The conservative press was the same, defending certain parts of the bill as being vital to addressing the failures of the Company. Apart from this distinction, much of the Welsh press's views, rhetoric, and opinions aligned with those elsewhere.

In the final analysis, the United Kingdom's press never reached a consensus on the Government of India bill. The general trend was that liberal newspapers opposed the bill, and conservative newspapers supported it, but there were also differences in editorial perspectives based on geography. The English liberal press was vehemently against the bill, and its rhetoric reflected this strong opinion. The Irish liberal press had many of the same strong opinions. These, however, contrasted with liberal newspapers in Scotland and Wales, which were much more subdued. The conservative press mimicked this pattern. Conservative newspapers in England and Ireland were much stauncher in their support for the legislation, while the conservative press in Scotland and Wales offered a more muted endorsement. This evidence supports the conclusion that there was no uniform response to this contentious bill and that these differences were based on geography and political leaning.

As previously explained, the historiography relating to the Company's dissolution and the Raj's establishment treats the 1858 Government of India Act as merely an instrument to change the government structure. Scholars have not given much attention to the Act itself, which may lead some to believe it was a relatively uncontentious issue. This research demonstrates that this was not the case. While the bill was passed with overwhelming support in Parliament, the press did not reflect this attitude throughout the United Kingdom. Therefore, the bill was more than just a simple means to transfer control from the Company to the Crown, but was a highly contentious political measure that shaped the nature of British colonialism. Divergent press responses serve as a reminder that there was nothing "natural" or "inevitable" about the form of imperial governance imposed by Britain on India in 1858; rather, the development of the Raj was contested from the very beginning.

It is easy to gloss over the Act: it came after the Mutiny, a time of extreme political and military tensions in the Empire, and before the Raj, which started a new era of British imperial history. The attitudes

and arguments discussed in this essay show how the press, and by extension, the broader British public, was divided over the bill. By failing to discuss how the public reacted to this novel institution, historians have failed to recognize the controversy surrounding the bill and, by extension, the nature of imperial governance in Britain's most important colony. This research fills this critical gap in the historiography and provides a deeper understanding of the complex political atmosphere.

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Emotional Development in Infancy

[1]Harriet L. Wilkes Honors College, Florida Atlantic University
[2]Charles E. Schmidt College of Science, Department of Psychology and Behavioral Neuroscience, Florida Atlantic University
Claire Sanford[1], Samantha Gott[2] (Doctoral Candidate in Behavioral Neuroscience and Lead Research Assistant), Dr. Nancy Aaron Jones[1,2] (Faculty Advisor)

Abstract

Developmental science has documented that infants' emotional reactions are shaped by individual dispositions and their environment. This study investigated fear responses along with patterns of brain development in infants 6 to 10 months. Nine infant-mother dyads participated, with each infant observed twice (18 visits). An electroencephalogram (EEG) was used to analyze brain activity during resting-state and fear-eliciting conditions. Infant fear vocalizations were evaluated and coded during two conditions, i.e., an unfamiliar object (an electronic toy-spider), and an unfamiliar adult approaching. Results indicated infants' fear vocalizations were stimuli-specific, with more fear expressed towards approaching strangers than the novel object. Findings also showed a relationship between heightened fearful vocalizations towards strangers in certain infants and right frontal-region EEG asymmetry. These findings support that infant fear responses vary based on the type of fear-eliciting stimuli as well as the role of individual dispositions, with patterns of infant right frontal EEG asymmetry linked to temperamental fear.

Introduction

Infants' behaviors vary in the emotional situations they experienced throughout their first year as well as their individual/temperamental responses to emotion-eliciting events. Early-life experiences, especially those involving novel and potentially fear-provoking stimuli, are theorized to influence brain development (Filippi et al., 2022). Developmental psychologists have investigated the emotion of fear and examined infant temperament alongside physiological reactions to fear-eliciting conditions. These early responses are

foundational, as situational and temperamental fear reactions are considered precursors to a trajectory of anxious behaviors in childhood (Filippi et al., 2022). Evidence from several studies found that early signals of anxiety occurred in infancy, with more fearful responses and behavioral inhibition indicating future risk for anxiety-related disorders (Gartstein et al., 2010). Along with the plethora of novel and potentially fear-promoting experiences an infant encounters in their environment in the first year of life, studies also link a pattern of right frontal EEG asymmetry to both temperamental and experiential fear responses during the events (Gartstein et al., 2010; Jones et al., 2020). Given the role of early emotional experiences affecting development outcomes, there is growing interest in examining these patterns longitudinally as well as identifying markers, both behavioral and physiological, that could indicate a heightened risk for exaggerated fearful and anxious responses in infancy and early childhood.

Fear

Fear is a startling or distressed response to sudden changes in stimuli that includes novel physical objects or social stimuli (Gartstein & Rothbart, 2003). Fearfulness in infancy can vary depending on the type and intensity of the environmental event and the infant's understanding of the situation (Bittner et al., 2007). Within the first year of infant development, the most studied fears include fear of heights, fear of spiders and snakes, fear of separation from familiar adults and fear of strangers or unfamiliar adults who approach the infant (LoBue & Adolph, 2019). Researchers typically examine infants' behavioral and biological reactions to these novel events to determine if they are fearful because infants cannot verbalize their fear (LoBue & Adolph, 2019).

Fear responses that are observed as distinct reactions to distressing or alarming events have been found to emerge early in infancy, with findings of wariness towards strangers and startling reactions to fear-provoking stimuli like snakes and spiders developing around six months old (LoBue & Adolph, 2019; Gartstein et al., 2010). Due to the infant's lack of verbal skills, their responses often involve crying, clinging to caregivers, facial distress, widening eyes, and freezing or avoidance behaviors such as turning or mobilizing away from the fearful stimulus or event (Gartstein et al., 2010). However, not all infants and not all conditions produce behaviors that are equally demonstrated when infants are presented with these potentially fear-eliciting experiences (LoBue & Adolph, 2019).

Theories suggest that infant fear of the unfamiliar, such as strangers and spiders/snakes, stems from evolution: it is a natural unfolding of an underlying genetic predisposition as humans adaptively needed this innate fear response as a survival mechanism (LoBue & Adolph, 2019). Some research indicates that infants exhibit increased fear of strangers, heights, and snakes/spiders around eight to ten months due to their increased self-generated mobilization at this age, whereas other studies propose that fear of these stimuli could be learned through observing/modeling parents' reactions across age (LoBue & Adolph, 2019). One question the LoBue and Adolph (2019) study addresses is whether the infant is actually experiencing fear. They argued that for an infant to be exhibiting fear, there must be evidence of both behavioral and physiological responses to these environmental conditions.

Temperament

The goal of understanding normative versus heightened risk (non-normative) development has been to design longitudinal investigations of patterns of emotional development as well as the influence of individual variation in temperamental dispositions across infancy. The emotional and biological tendencies that arise in early development mold an infant's temperamental disposition and, ultimately, their personality (Howarth et al., 2017). Temperament, although shaped across infancy via experiences and interactions with social others, is thought to be relatively stable across one's life (Bornstein et al., 2015), so infant temperamental traits formed in infancy are thought to be foundational across the lifespan. In infants, individual differences in emotional responses and overall temperament can be observed in behaviors even a few months after birth (Gartstein & Rothbart, 2003). In addition, temperamental traits are believed to encompass biologically based individual differences in emotional reactivity, arousal, activity level, and self-regulation, all of which emerge in infancy and likely form the basis of personality development (Bornstein & Cote, 2009).

Individuals vary in how they respond to typical and novel situations due to the interplay of innate and environmental factors. Therefore, fearful temperament, characterized by nervousness and avoidance of new situations, is linked to heightened physiological reactivity and is identified as a risk factor for childhood anxiety, suggesting a relationship between temperament and potential risks for psychopathology (Trent et al., 2021). Filippi et al. (2022) argued that there is a gap in understanding the links between the development of

negative emotions and the neural correlates of behavioral inhibition, which is involved in childhood fearful temperament.

EEG and Right Frontal Asymmetry (RFA)

Understanding how the brain is involved in temperament and emotional development is critical in addressing developmental questions surrounding normative versus the potential for risk in patterns of socio-emotional development. One way to study important processes in the brain related to emotions and temperament is by using electroencephalography (EEG) which measures the brain's electrical activity across the scalp (Bell & Cuevas, 2012). During the first year of life, the brain rapidly changes and grows. In developmental research, the EEG is typically used to study cognitive and affective development and their relations to changes in brain patterns across hemispheres and regions due to its low impact on typical behavior (Bell & Cuevas, 2012). EEG measures allow researchers to noninvasively document changes in brain and behavioral development, as prior research indicates that the entire cortex, particularly the frontal region of the brain, continues to mature during infancy (Bell & Wolfe, 2007). Infancy research relies on EEG as it enables the investigation and evaluation of early emotional and cognitive processes. This method bypasses the requirement for infants to demonstrate verbal skills they have not yet acquired, offering a window into their earlier developmental processes (Jones et al., 2020). However, there are challenges when it comes to collecting an EEG on infants, the greatest being undesired movement (Jones et al., 2020). To combat this limitation, in this study, we recorded both resting-state and stimulus-linked EEG responses to precisely locate when the stimuli started and ended. This helped us to document both tonic, non-stimulus responses as well as stimulus-linked patterns of the EEG to be used as a reliable measure of plasticity of the brain and changes across development with experiences (Jones et al., 2020).

Similarly, it is known that the EEG changes in frequency parameters across development and studies have documented that the alpha frequency band is commonly used in research with infants. Bell and Cuevas (2012) have established that alpha band activity in the 6 to 9 Hz band in infant is similar to the adult alpha band (8 to 12Hz) and akin to the state of relaxed wakefulness, which decreases with concentration, stimulation, or visual fixation (Allen & Reznik, 2015). One measure that is utilized to measure brain activity from the alpha band is asymmetry, as not only are there changes in the frequency

parameters but also changes in the left and right hemisphere activation patterns (across the corpus collosum) amongst regions of the brain. Asymmetry is defined as the difference in activity over the right versus left hemisphere of the brain (Allen & Reznik, 2015). Asymmetry values are relatively stable across short and long periods in infancy, making it a reliable way to study cognitive, socio-emotional and brain development (Brooker et al., 2017; Jones et al., 2020). Finally, studies by Fox and colleagues (1994) have linked changes in frontal lobe development with increasing development of emotions and emotion regulation. Notably, studies of RFA in the alpha band have been associated with negative emotions and withdrawing-type behaviors whereas left frontal asymmetry has been associated with positive and approach-type emotions during development (Fox, 1994).

In research with adult populations RFA has been shown to be a biomarker for negative affect and disorders associated with negative mood (Shanok & Jones, 2023). Thus, RFA has been suggested as a potential physiological index across development as it has also been found in children with anxiety and panic disorders (Shanok & Jones, 2023). Further, infants with an increased resting-state RFA showed more fearful responses than infants with relative left frontal EEG asymmetry (Hane et al., 2008) and other studies have found that infants with extreme RFA had high levels of cortisol, which is a stress hormone that can be elevated when a person has heightened anxiety or is under a lot of stress (Jones et al., 2020).

Current Study

This study investigated emotional development in early infancy by examining behavioral fear responses to a standardized set of emotion-eliciting stimuli and events in 6- to 10-month-olds. The fear reactions of infants, starting at 6 months, were examined across two conditions. This was done because developmental changes in an infant's response to fearful stimuli could be related to their level of reaction across age as well as to their brain activity (Jones et al., 2020). Behavioral fear responses are also examined to see if younger infants respond differently to fearful stimuli compared to older infants, or if there are certain infants that are more or less responsive, as suggested in early theories of emotion/fear reactions and temperament across development (Lobue & Adolph, 2019). For brain activity, this research examines whether behavioral fear responses are associated

with frontal region asymmetry and if the responses are during the stimulus event or evident during resting-EEG condition.

It was hypothesized that younger infants would exhibit less fearful responses than older infants in response to different fear-eliciting stimuli, as mentioned in the evolutionary theory presented in Lobue and Adoplh's (2019) paper. It was also hypothesized that there would be a greater fear response to the unfamiliar, stranger-approach than to a novel, "scary" toy-spider stimulus. In addition, we expected infants to be fearful across both stimuli/situations across age. For the measure of brain activity, it was hypothesized that infants with a greater fear response to the stranger-approach stimuli would have an RFA score versus infants with more regulated responses, as mentioned in Jones et al. (2020). Learning more about early fear development through investigating the simultaneous association with the development of brain activity can help identify the variation in responses across infant development.

Specific Aims

The aim of this study is to investigate the development of fear responses in infants and the neurophysiological mechanisms associated with them. Specifically, this study seeks to examine how fear-eliciting stimuli, such as a stranger or a novel object (e.g., a toy spider), influences infant behavior and brain activity, using an EEG. By tracking both age-related and individual differences in fear responses, the study aims to identify early markers of fearful temperament which could be used to identify potential risk factors for later emotional disorders.

The purpose of this research is to better understand the neural pathways of fearful temperament in infancy and how these early patterns of brain activity may predict emotional development outcomes. This knowledge may help identify infants at heightened risk for anxiety-related disorders and inform interventions aimed at supporting their emotional development.

Method

Participants

The data used was part of the Baby BEAR study, currently conducted at Florida Atlantic University (FAU), Washington State University, and Virginia Tech. Nine participants qualified for inclusion

in this study, with 18 visits recorded. All participant visits were conducted at the FAU WAVES Emotion Lab in Jupiter and Boca Raton, Florida. There were two groups of participants: the younger (6- and 8-month-old group) and the older (8- and 10-month-old) group. Participants were recruited via flyers sent to their families' homes, with families identified via birth records from the local region or from recruitment events in West Palm Beach geared towards families. This study is part of a larger project in which infants will be observed bimonthly from 6 to 24 months, with over 100 infants already seen across sites.

Procedure

Before each visit started, the infants' mothers signed consent forms allowing their children to participate in the study and completed a questionnaire asking for personal information. During the visit, each mother sat next to their infant. Different stimuli were shown throughout the visit to elicit a response in the infant; this study looks at the baseline, the Goldsmith and Rothbart (1996) LabTAB, spider task, and the stranger approach task.

To measure frontal asymmetry scores, the infants wore an EEG cap with 32 electrodes, which recorded brain wave activity throughout their visit. At the start and stop of each condition, a research assistant hit the biomarker button on the EEG machine to record the start and end of the condition. In the baseline condition, the infants were shown a short video of *Sesame Street*. The baseline was used to examine the infant's brain activity in relaxed conditions compared to fearful conditions. In the standardized stimuli condition, a remote-controlled spider was placed on the table before the infant and moved around for 60 seconds. Finally, in the stranger approach condition, a research assistant in a lab coat approached the infant for approximately 40 seconds, motioning as though they were going to pick up the infant but not actually doing so. While all these conditions were in action, mothers were instructed to refrain from intervening unless necessary.

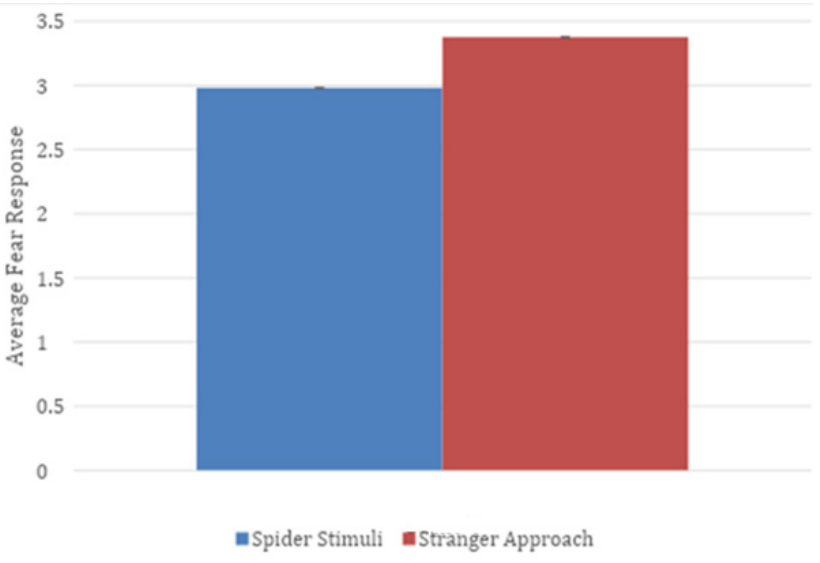
Results

Behavior and Age

Using the Statistical Package for the Social Sciences (SPSS), an omnibus 2 (younger versus older ages) by 2 (standardized by stranger condition) by 4 (10 second intervals within each condition) repeated-

measures MANOVA was computed to analyze the difference in fear vocalization scores during internals, for the conditions and between the age groups. No effects were found between the intervals nor for age groups ($p > .05$). However, there was a significant multivariate effect when comparing the two conditions in which the infants were exposed to fear stimuli, with infants showing more fear vocalizations during the task with the stranger approach and less during the standardized stimulus ($F [1, 16] = 5.95$, $p = .03$; Figure 1).

Figure 1
Fear Vocalization Responses During the Spider Stimuli and Stranger Approach



Note. Comparison between the standardized stimuli and the stranger approach.

In a separate analysis in which we compared 6-, 8-, and 10-month-old infants as a between subject variable (rather than older or younger groupings), there were also no significant age effects nor interactions with age, ($p > .05$); however, there was a trend for the 6-month-old infants (compared to 8- and 10-month old) to show increased fear vocalizations during the stranger condition ($F [1,16] = 2.90$, $p = .09$).

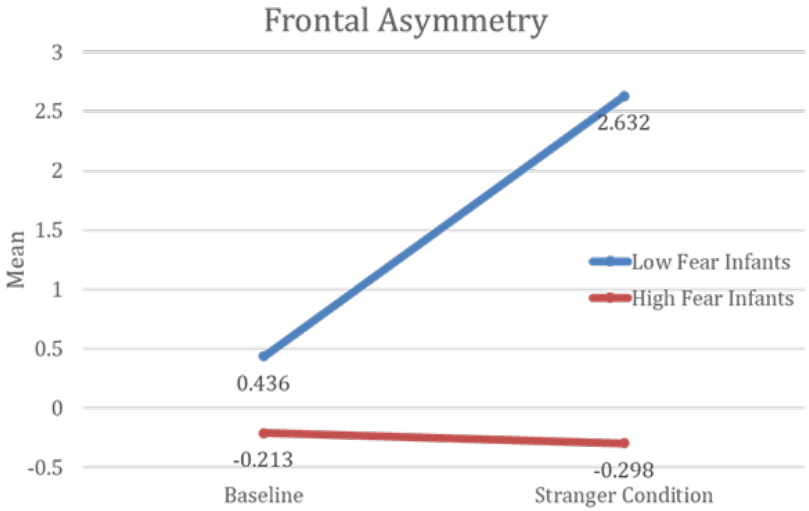
EEG and Behavior During Resting-State Versus Stranger Approach Condition

To examine those with greater fear responses, the participants were separated into two groups based on fear vocalization scores: a score of 4 or above put them in the high temperamental fear group (versus low fear/more regulated responses that scored 3 or below in fear vocalizations). A repeated measure MANOVA was then computed to examine frontal EEG asymmetry in the alpha band (6 to 9 Hz) in the prefrontal (Fp2 to Fp1), mid-frontal (F4 to F3), and lateral frontal (F8 to F7) regions of the brain to examine behavior and neural activity during the resting-state (baseline) and the stranger approach conditions.

During the resting-state EEG condition, no significant differences were found in asymmetry between those infants with increased responses in fear vocalizations versus those with low fear vocalization ($p > .05$). A repeated measures MANOVA was also conducted to test the hypothesis that infants with increases in fear response during the stimuli will have more RFA than infants with more regulated responses. In particular, this analysis looked at the infant's frontal EEG asymmetry during the stranger approach condition. There was a significant effect, with infants with higher fear vocalizations having more right than left frontal asymmetry (compared to those with lower fear/more regulated responses; $F [2,11] = 7.23$, $p = .02$; Figure 2).

Figure 2

Average Frontal Asymmetry Scores Between Low and High Fear Infants



Note. The averages were calculated using SPSS.

Discussion

This study investigated emotional development across ages, specifically the development of infant fear as a potential precursor for emotional risks for an anxiety disorder. During a standardized stimuli task (an electronic spider) and a stranger approaching, fear vocalizations were examined along with EEG data during resting-state and fear-promoting stimulus conditions.

The first part of this study examined how infants responded differently to different fear-eliciting stimuli. This is important because when examining infant fear development if the stimuli used do not elicit a fearful response, it does not show how infants exhibit fear (LoBue & Adolph, 2019). Our study showed that the unfamiliar spider and stranger elicited different fear vocalization scores, with the stranger approaching eliciting increased fear vocalizations.

How infants react to novel emotional situations and how their brain responds to these conditions is important because this may reveal risk factors for avoidance behaviors and potentially later emotional/anxious disorders. Further, infants experience novelty on a daily basis, and those who react with increases in negative affect during new situations may avoid them in the future. Our study showed that those high in fear vocalizations during the stranger approach had greater right frontal brain activation patterns than infants who showed lower fear and more regulated vocalization. This is an important distinction between the two groups because it reveals how infants react to fear may be linked to both the level of emotional responses and associated patterns of frontal region brain development. Also, this study could allow future research to uncover reliable biomarkers in infants who are likely to develop anxiety as children.

Fear Behavior in Infants

Classical evolutionary developmental psychology suggests that an individual's fear of spiders/snakes and strangers is innate, emerging at predictable stages of development reflecting biologically programmed responses to specific stimuli (Lobue & Adolph, 2019). However, in this study, infants displayed a higher fear response to the stranger approaching them than to the novel spider stimulus. This could mean that the fear of spiders may not be innate, as the infants in this study were not scared of the novel stimuli in the shape of a spider. The findings of this study are more indicative of a dynamic system approach because it seems that fear emotion emerges as a

consequence of contextual, dispositional, and experiential factors. Another consideration is that the stranger approach exhibited more fearful behaviors, as infants are likelier to interact with strangers than spiders prior to 6 or 8 months of age (Lobue & Adolph, 2019).

Another possible factor in the fear vocalization response is age, with other studies indicating that older infants typically express more fearful behaviors with a stranger than younger infants (LoBue & Adolph, 2019). However, in our experiment, there was only a trend for younger infants to exhibit more fear vocalizations during the stranger condition. The difference in our findings compared to previous research could be due to the focus on fear vocalizations rather than other fear-linked responses or it could be that in our study, the infant's mother was present and sitting next to the infant, which could act as a buffer for the infant's fear responses (Jones et al., 2020).

EEG, Temperament, and Behavior Implications

Previous studies have indicated that infants with a resting RFA showed more fearful responses than infants with relative left frontal EEG asymmetry (Hane et al., 2008). In this study, we tested the hypothesis that infants with a greater fear response will have a RFA score than infants with more regulated responses. We found this when it came to the stranger approach: infants with higher fear vocalizations also had more RFA and only during the EEG recorded during the stranger approach condition than infants with low fear vocalizations, indicating a potential link between elevated fear and neural activation patterns. However, the infants with more fear vocalization during the stranger stimuli task did not have significantly higher RFA compared to the low-vocalization group during the baseline conditions, but they did have significantly higher RFA during the stranger-approach condition. Our findings support the capability model advocated by Coan et al. (2006) that individual differences in EEG patterns will be more pronounced during the emotion-based stimuli task. These findings suggest that further research is warranted to uncover the underlying mechanism for greater fear responses, possibly with more measures of fear (arousal, motor and facial activity) as well as with other measures of brain functioning (e.g., EEG coherence or during other fear-eliciting stimuli like maternal separation and fear of heights and falling) which can predict greater RFA in EEG patterns in infants prior to one year of age.

Limitations and Future Directions

This study does have some limitations. The study is ongoing, so the data evaluated here is limited and may not fully represent infants fear across development in the first year. Another limitation of this study is that only fear vocalizations and EEG behaviors were coded and analyzed, omitting other fear responses like withdrawal responses, eye gaze and facial expressions. The EEG collection had some artifacts due to movement during the fear conditions, as well as only focusing on the frontal leads due to their primarily being associated with fear. Future research should examine whole brain patterns, as well as other biological and physiological measures that could be related to the development of fear.

Despite the limitations, the findings suggest that fear responses vary among individuals with a fear temperament, and not exclusively by age groups. Fears of spiders/snakes, heights, maternal separation and strangers approaching appear to be both a temperamental variable and likely learned behaviors and not present in all infants as an evolutionary adaptation, as suggested by past theories presented in LoBue and Adolph (2019). This study also highlights the importance of examining fear along with heightened patterns of temperamental fear responses over time to detect possible patterns as precursors for anxiety behaviors and anxiety risk, as programs related to infant mental health may help to provide interventions for these risks.

For future studies, increasing participant numbers, increasing the number of fear-promoting conditions and including other fear measures like facial expressions and motor behaviors, along with measures of temperamental fear using standardized questionnaires, could increase reliability and generalizability. In addition, integrating other biological measures like cortisol levels with fear responses and EEG, and other computations besides frontal region alpha power and asymmetry, could provide a better predictor for anxiety because increases in biological and physiological measures may also be linked with anxiety (Jones et al., 2020).

The findings suggest complex interactions between behavioral fear responses and brain activity across infant development. Our findings show that different stimuli used to elicit fear responses will produce different fear vocalizations in infants, and certain infants, i.e., those with heightened temperamental fear, demonstrate patterns of right frontal EEG asymmetry during fear-reactions. This study highlights the importance of integrating multiple methodologies

across development, such as behavioral observations and neurological processes. This is needed to fully understand the dynamics of emotional development as well as infant mental health risks, especially in investigations during the infant's first year, as infant fear responses can be a precursor for risk for anxiety.

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A Comparative Analysis of the Voicing Systems in Tagalog and Hiligaynon

Dorothy F. Schmidt College of Arts and Letters,
Florida Atlantic University
Ty Craaybeek, Dr. Michael Hamilton (Faculty Advisor)

Abstract

Tagalog and Hiligaynon are both languages from the Philippines that use the unique Philippine-style topic marking system. This system illustrates a special relationship between the verb and the subject or object through an affix marking focus. Tagalog is well-studied, but there is much debate on its voicing system. Hiligaynon and its voicing system are understudied. This paper explores the voicing system in both languages to better understand their similarities. Tagalog and Hiligaynon have related voicing systems, however, Tagalog uses five voices, separating the beneficiary and locative voices, while Hiligaynon has four, combining these two voices into the referential voice. Even though the systems in the two languages are comparable, more research should be undertaken to better address how affixation used in each language can carry multiple pieces of information, and semantic ambiguities in the verbs and noun phrases can lead to problems when counting the voices present.

Introduction

Tagalog is probably the most well-studied language in the Philippines as it is the basis for the official language. Most of the population has knowledge of how to use it as it is spoken in 10.5 million households (Philippine Statistics Authority, 2023). Tagalog is a member of the Central-Philippine branch of the Austronesian language family and closely related with languages like Cebuano or Ilokano (Zorc, 2006). Another closely related language to Tagalog is Hiligaynon. According to the Philippine Statistics Authority (2023), Hiligaynon is a language in the Philippines that is spoken in around 1.9 million households, or 7.3% of the population, and it is also a member of the Central-Philippine branch (Zorc, 2006). Similar

to many other languages in the Philippines, Hiligaynon exhibits the unique *Philippine-style topic marking*, which demonstrates a special relationship between the verb, and the most grammatically prominent noun phrase (typically the subject or object) in the sentence (Pereltsvaig, 2020). This system has been referred to by many names, such as voicing, Austronesian alignment, pivot, and focus, but each describes the process of placing emphasis on a specific noun phrase within a sentence and expressing the relationship with a topic marker as the specifier and special verbal inflection (Chen, 2021; Pereltsvaig, 2020; Schachter, 2009). In Tagalog, similar sentences in English may look slightly different depending on how one wants to place emphasis. Example 1a shows the sentence "*AJ brought cake from Lia for Joy"* in actor voice (AV in the examples throughout), which emphasizes the actor, *AJ*. 1b shows the same sentence in patient voice (PV in the examples throughout), with emphasis on the direct object, *the cake*:

(1) B**<um>**ili **si** **AJ** ng keyk mula kay Lia para kay Joy
Buy$_{\underline{<AV>}}$ $_{\underline{PN.Pivot}}$ **AJ** $_{DET}$ cake $_{P}$ $_{PN}$ Lia $_{P}$ $_{PN}$ Joy
"**AJ** bought cake from Lia for Joy"

Bi-bilih-**in** ni AJ **ang** **keyk** mula kay Lia para kay Joy
$_{CONT}$-buy-$_{\underline{PV}}$ $_{PN}$ AJ $_{\underline{D.Pivot}}$ **cake** $_{P}$ $_{PN}$ Lia $_{P}$ $_{PN}$ Joy
"AJ will buy **cake** from Lia for Joy"
(Chen, 2021, p. 94).

While Tagalog is one of the most well-studied languages with the Philippine-style topic marking, Hiligaynon has been understudied. Although both languages exhibit Philippine-style topic marking, the similarities between their systems are less clear. Even with the extensive research on Tagalog, there is still a debate on the number of voices it has, as well as the structures used to express this information. The purpose of this examination is to see how the Hiligaynon voicing system compares to Tagalog in the number of voices, their functions, and the syntax within the system. Some sources explain that Tagalog utilizes five voices, while others argue there are more or fewer. Additionally, the broader structural systems have been debated with questions concerning how voicing affects the syntax or even the ergativity of these languages, as discussed in Chen (2017). The hypothesis is that as Hiligaynon is an Austronesian language closely related to Tagalog, the system it uses is similar to Tagalog since this

system is consistent across Austronesian languages. However, based on information from prior research, Hiligaynon clearly only makes a four-way distinction, but Tagalog utilizes up to five voices. While both share actor, patient, and instrumental voice, Tagalog differentiates between locative and beneficiary voice. In contrast, Hiligaynon uses referential voice to express the location or beneficiary of an action.

Overview of Tagalog's Voicing System

Many researchers, including Chen (2021), consider Tagalog to have a four-way voicing system: actor voice (AV), patient voice (PV), locative voice (LV), and circumstantial voice (CV), which are all marked with verbal morphology. Others, like Schachter (2009), establish five voices with the beneficiary voice (BV) being a separate voice from the locative voice. Each of these voices has been referred to by various names in other sources: circumstantial voice has sometimes been referred to as instrumental voice (IV), locative and beneficiary voice have both been labelled as referential voice (RV) in some sources, and patient voice has been called the objective voice or goal focus. These are the five most agreed upon voices for the language, and each of them follows the same processes of marking the verb and noun phrase complement.

In Philippine-style topic marking, the noun phrase complement should be marked as the topic, and the verb must carry the affix that highlights what voice is being used. First, Tagalog marks focus on the noun phrase that is the topic of the sentence. For most noun phrases, the word *ang* will mark the topic, but *si* can be used before proper names (Schachter, 2009). The marker *ng* serves to mark non-topic actors and is sometimes referred to as an oblique noun phrase construction, while *ni* is used prior to personal names that are not in focus (Adams, 1988; Schachter, 2009). Personal pronouns change forms based on their role within the sentence or the information that they carry (Adams, 1988). Second, Tagalog uses verbal affixation to indicate the semantic role of the grammatical subject, while the semantic roles of other arguments are expressed in the noun phrases themselves (Schachter, 2009). Affixes must be added to illustrate what voice is being used in the sentence, which is essential because the affixes carry meaning about tense, aspect, and mood. This process can be observed in examples 1a and 1b. The University of Hawai'i at Mānoa (University of Hawai'I, [UH], n.d.) has created several charts to provide examples and clarification on some of the possible affixes that can inflect verbs based on the voice, tense, aspect, and mood.

Actor voice in Tagalog describes topic and subject agreement (Chen, 2021). It signals the subject of the sentence, which is usually the performer or doer of an action, as the topic. In the (2) below, the speaker is placing focus on the actor, *AJ*, with the topic marker *si,* which is used for names. Additionally, the infix *–um-* is added on the verb to help indicate that it is the performer who is in focus.

(2) B**<um>**ili **si** **AJ** ng keyk mula kay Lia para kay Joy
Buy$_{\text{<AV>}}$ $_{\text{PN.Pivot}}$ **AJ** $_{\text{DET}}$ cake $_{\text{P}}$ $_{\text{PN}}$ Lia $_{\text{P}}$ $_{\text{PN}}$ Joy
"**AJ** bought cake from Lia for Joy"
(Chen, 2021, p. 94).

Passive voice in Tagalog observes agreement with the topic and the direct object (Chen, 2021). In (3) below, the direct object of a sentence, in this case *ang keyk*, is in focus as *ang* marks the topic for common nouns, and its grammatical role is further clarified with the suffix *–in* on the verb, which is used to show it is the direct object that is being highlighted.

(3) Bi-bilih-**in** ni AJ **ang** **keyk** mula kay Lia para kay Joy $_{\text{CONT}}$-
buy-$_{\text{PV}}$ $_{\text{PN}}$ AJ $_{\text{D.Pivot}}$ **cake** $_{\text{P}}$ $_{\text{PN}}$ Lia $_{\text{P}}$ $_{\text{PN}}$ Joy
"AJ will buy **cake** from Lia for Joy"
(Chen, 2021, p. 94).

The locative voice emphasizes the topic and locative agreement (Chen, 2021). Focus is placed on exactly where the action is occurring. In (4) below, *si Lia* is the source of the cake, and as a result, *Lia* is considered the location in which the action occurs, with *si* being used to show that the name of a person is the topic. Furthermore, the suffix *-an* is added on the verb to show that *si Lia* is acting as the location or source of the interaction.

(4) Bi-bilih-**an** ni AJ ng keyk **si** **Lia** para kay Joy
$_{\text{CONT}}$-buy-$_{\text{LV}}$ $_{\text{PN}}$ AJ $_{\text{D}}$ cake $_{\text{PN.Pivot}}$**Lia** $_{\text{P}}$ $_{\text{PN}}$ Joy
"AJ will buy cake **from Lia** for Joy"
(Chen, 2021, p. 94).

In (5) below, *-an* is again being used to mark the location of the action. This time, *sako* is described as the topic and follows the common noun topic marker *ang*.

(5) Aalis-**an** ng tindero ng bigas **ang** **sako**
Take.out-FUT.**LV**D storekeeper D rice **D.Pivot** **sack**
"A storekeeper will take some rice **out of the sack**"
(Schachter, 2009, p. 838).

Notably, Schachter (2009) and UH (n.d.), among others, have described the beneficiary voice to be distinct from the locative voice. While researchers like Chen (2021) do not distinguish between these two voices morphologically, Schachter and UH have found examples, as seen in (6) below, that the two categories use different morphological markings. Furthermore, UH (n.d.) has created a chart illustrating affixation for each voice with the morphemes applied onto the beneficiary voice not used in other voices. In the following example, *ipag-* is a prefix indicating the focus of the sentence is the beneficiary. As a result, *ang babae* is clearly marked as the topic of the sentence with the common noun topic marker *ang* and identified as the beneficiary of the action.

(6) **Ipag**-aalis ng tindero ng bigas **ang** **babae**
BV.FUT-take.out DET storekeeper DET rice **DET.Pivot** **woman**
"A storekeeper will take out some rice **for the woman**"
(Schachter, 2009, p. 838).

The circumstantial voice simply expresses a spelled-out topic (Chen, 2021; Schachter, 2009). Although it can be mapped to various pivots (Chen, 2017), Schachter only exemplifies instrumental noun phrases, where the verb expresses that an action is done with something, such as *ipan(s)ulat* meaning 'write with s.t.,' as the prefix *i-* expresses the instrumental voice (Schachter, 2009). Schachter (2009) also only uses the term instrumental voice, and he never discusses circumstantial voice as its own concept or a distinct voice. In either case, the prefix *ipang-* is added to the verb to mark the circumstantial voice. *Sandok* is marked with *ang* as the topic of the sentence as the tool used to accomplish the goal of the action. This can be seen in (7a):

(7a) **Ipang**-aalis ng tindero ng bigas **ang** **sandok**
IV.will-take out DET storekeeper DET rice **DET.Pivot** **ladle**
'The storekeeper will take out some rice **with the ladle**'
(Schachter, 2009, p. 838).

Despite this, Chen (2021) has only noted one instance using circumstantial voice to indicate the beneficiary of the action. In (7b), *I-* is added on the verb to mark the voice as being circumstantial, while *Joy* is marked with the personal name topic marker *si* to be the focus, but this time as the recipient of the action.

(7b) **I**-bi~bili ni AJ ng keyk mula kay Lia **si** **Joy**
CV-CONT~buy PN AJ DET cake P PN Lia **PN.Pivot** **Joy**
'AJ will buy cake from Lia **for Joy**'
(Chen, 2021, p. 94).

In summary, depending on the source, Tagalog uses between four or five voices when emphasizing noun phrase complements. Actor and patient voice in Tagalog place emphasis on arguments in a sentence, while the circumstantial, beneficiary, and locative include an adjunct topic (Chen, 2021). These distinctions may be even more complex. For example, Pereltsvaig (2020) describes eight types of topics in Tagalog (actor, theme, location, reason, direction, etc.) that all have their own verbal morphology. These have not been discussed in this section because reliable information on the validity of these voices is scarce. Of the well-documented voices, they can be expressed by marking the verb to agree with the focused noun phrase complement.

Overview of Hiligaynon's Voicing System

According to Wolfenden (1971), Hiligaynon has four voices: actor voice, patient voice, referential voice, and instrumental voice. The grammatical relations that are marked highlight "l) the actor, 2) the goal or receiver of the action, 3) that which is used to accomplish the action, or which is implicated as the conveyed object in the action, or 4) the beneficiary or the place of the action" (Wolfenden, 1971, p. 77). Each of these voices has other names as well, such as actor voice being referred to as active or subjective voice, accessory voice being known as instrumental voice, patient voice being called goal focus, and referent voice being called benefactive or locative voice.

There are two indicators for focus in Hiligaynon: the first is applied on the noun phrase that will be in focus, and the second will be added on the verb (Wolfenden, 1971). The most common marker in Hiligaynon for the topic of the sentence is the word *ang*, however, *si* is required if a proper name is the focus (Zorc, 2006). Additionally, pronouns and deictics can take special forms if they are the topic of the sentence, and they have forms for when they are used in oblique and locative contexts (Zorc, 2006). Noun phrases and names have other forms for when they are not the focus of the sentence. According to Motus (1971), when a phrase is not the focus, "then the marker *sang* precedes a common noun, or a *ni* precedes a personal name, or the source pronoun set is used. Another particle, *sa*, marks direction of an action, be it to a place, person, or thing" (p. 114). The verb and the proper noun phrase complement need to reflect agreement for the voice. Inflecting the verb to mark focus accounts for the factors of aspect, tense, and mood, along with the voice, resulting in many different affixes that can be added onto the verb, as shown by a chart created by Zorc (2006). The affix being added on the verb must agree with what the speaker wishes to place in focus, or the sentence will appear unnatural or carry a different meaning or interpretation from what the speaker intends.

Actor focus has the topic of the sentence being the actor, or the performer of the action (Motus, 1971). In (8), the infix *–um-* is added into the verb to show the topic will be the actor, while the actor itself is marked as *sia*, which is the topic form of the third person singular pronoun.

(8) **L<um>uto** **sia** sang linapuhan
<FUT.**AV**>cook **3.SG.Pivot** D vegetables
"**He** will cook some vegetables."
(Wolfenden, 1971, p. 81).

Patient voice marks the object that directly receives the action as the focus of the sentence (Wolfenden, 1971). The direct object is being emphasized in the patient voice. In the following example, the prefix *gin-* is added on the verb to mark the direct object as the topic, while *ang* marks *mangga,* or *mango*, as the focus of (9).

(9) **Gin**-kaon mo **ang** **mangga** sa kusina.
PST.**PV**-eat 2.SG **D.Pivot** **mango** in.D kitchen
"You ate **the mango** in the kitchen."
(Wolfenden, 1971, p. 79).

Referent voice places focus on the location or beneficiary of the action; hence it is sometimes referred to as beneficiary and locative voice (Wolfenden, 1971). Even though the focus on locations and beneficiaries of an action are semantic categories, the locations and beneficiary of an action are grouped together because the verbal affixation and function markers are the same (Motus, 1971). In (10) below, *-an* is the suffix marking referential voice, and in this case, is used to show a location is the topic as *ang* marks *lamesa* as the focus.

(10) Hampang-**an** ko sang madyong **ang** **lamesa**
Play.on-FUT.**RV** 1.SG D madjong **on.D.pivot** **table**
"I will play madjong **on the table**."
(Wolfenden, 1971, p. 79).

In (11), instead of topicalizing the location, *-an* is being used to mark a recipient. In this case, *si* is the personal name topic marker highlighting *tatay*.

(11) Lutu-**an** ko sang pagkaon **si** **Tatay**
Cook.for-FUT.**RV** 1.SG D food **PN.pivot** **Father**
"I will cook some food **for Father**."
(Wolfenden, 1971, p. 79).

The instrumental voice puts the focus of the sentence on what was used to do the action (Wolfenden, 1971). Wolfenden clarifies that it can be confused with the goal, but the instrumental voice is frequently used to highlight the instrument used to accomplish the goal, such as a pencil used to write a letter, or it is something implicated in an action, which could be a banana tree that is planted. Also, beneficiaries of actions can appear as the topic within this focus. It is also the most rarely used focus type in Hiligaynon (Motus, 1971). In (12), *I-* is a prefix that describes the voice of the sentence is instrumental. Additionally, *ang* is the common noun topic marker identifying *chalk* as the topic. This combination of the affix and topic marker shows that *chalk* is described as the instrument used to accomplish the action.

(12) **I**-sulat mo sa pisara **ang** **chalk**
IV.FUT-write 2.SG on.D blackboard **D.Pivot** **chalk**
"You will write on the blackboard **with the chalk**."
(Wolfenden, 1971, p. 80).

The four voices in Hiligaynon have their own morphological marking applied to the verb that separates them from one another. Any further distinction between anything would only be based on semantic differences, such as the locative and beneficiary voice using the same morphology but semantically expressing different concepts. Moreover, the actor voice and patient voice focus on the arguments of the sentence, while locative and instrumental emphasize an adjunct. Based on these observations, Hiligaynon most likely contains four voices only.

Discussion

The hypothesis was supported as both Tagalog and Hiligaynon have identical systems. The major difference between the two was Tagalog appears to make a five-way distinction between its voices (Schachter, 2009), while Hiligaynon uses a four-way distinction (Wolfenden, 1971). Whereas Tagalog marks the locative and beneficiary voices with separate morphology, Hiligaynon does not by using the same affixes for both noun phrases. Furthermore, the charts provided by UH (n.d.) illustrate how affixation can change based on the voices and differentiate the beneficiary and locative voices. There do not seem to be any more voices reflected in the morphology beyond these five. Topic marking systems in both languages can become extremely complicated and one of two semantically ambiguous noun phrase complements could be focused under a single voice within the same sentence.

Although Tagalog and Hiligaynon are mostly similar and only differ by one voice, determining whether Tagalog and Hiligaynon share the underlying structure in the system is difficult. Researchers disagree about the morphosyntax in the topic marking system in Tagalog, even though it is one of the most studied languages in the Philippines. Hiligaynon has had relatively little research conducted on it. There are grammars of Hiligaynon and descriptions of how the syntax and morphology work (e.g. Wolfenden, 1971), but there remains a lack of resources. While the work of Wolfenden and Zorc is important, they are the only linguists who have published on the language. However, it is clear that Hiligaynon and Tagalog do not have the exact same voicing system. Many of the systems at play are shared, and the core idea of their voicing system is the same, even though they differ by one voice.

Each verbal affix in Tagalog and Hiligaynon carries meaning about the voice, tense, aspect, and conditionality; therefore, extra consideration and attention must be placed when studying Philippine-style topic marking. Full charts explaining all of the possible affixes added to verbs and using example sentences under various conditions help clear the ambiguity. An effective analysis can be done by testing when subjects and objects can be topicalized, and which affixes are used. By analyzing the grammatical and semantic roles of nouns in the sentence as well as observing the affix attached to the verb, linguists can draw conclusions about what voice is being used. Speakers should also be consulted as they can provide insight on what combinations are also grammatical and what voice is being used in the sentence. However, there is another

problem of ambiguous noun phrase complements, which may create problems in identifying the voice being used.

Some noun phrases in both Hiligaynon and Tagalog may appear semantically ambiguous. In a case where an instrument, location, or beneficiary described in an action would normally be in focus under their respective voices, the direct object can be topicalized instead as it is the goal. This results in speakers being able to choose a noun phrase complement to highlight under a single voice. Wolfenden (1971) notes that roots that are properly inflected do not reflect a focus that is expected, which is because some roots carry an inherent focus and topics are not described in general enough terms. Wolfenden (1975) explains that some roots with the same verb voice can equate ambiguously with different non-topic phrases, which he exemplifies with (13):

(13)	**I**-asal	ko	sang	isda	**ang**	**lipak**
	IV.FUT-pierce	1.SG	D	fish	**with.D.Pivot**	**skewer**
	"I will pierce the fish **with the skewer**"					
	(Wolfenden, 1975, p. 98).					

The noun phrase within the preposition phrase *ang lipak*, which means 'with the skewer,' is the instrument of the sentence, and, as expected, is emphasized in the instrumental voice. Although 'with the skewer' is the focus of this sentence, a speaker could, within the same sentence, use the instrumental voice to mark 'a fish' instead, as in (14):

(14)	**I**-asal	ko	**ang**	**isda**	sa	lipak
	IV.FUT-pierce	1.SG	**D.Pivot**	**fish**	with.D	skewer
	"I will pierce **the fish** with the skewer"					
	(Wolfenden, 1975, p. 98).					

Speakers can highlight either 'the fish' or 'with the skewer' as the focus within the same sentence. Only one noun phrase complement can be in focus, but speakers can choose to mark either the instrument or the direct object as the topic. In the case of (14), although instrumental voice is used, 'the fish' acts as the goal, so it can be topicalized. This concept illustrates the importance of the relationship between the verbal affixation and the focused noun phrase, and it shows that it is not always possible to simply follow the topic marker within the sentence to understand what voice is being used within the sentence. Because of these circumstances, it is extremely important for future work to

be precise to ensure the right voice is being used, to understand what verbs are affected by this, and whether this can be replicated in other languages that use the same system to mark focus.

Conclusion

Hiligaynon and Tagalog have very similar systems for marking the topic, but Tagalog marks the beneficiary and locative voices with their own morphology, while Hiligaynon uses the same affixes for both. This topic is still debated, and more research should be conducted because understanding the underlying structures in these languages may show similarities that explain all languages that use Philippine-style topic marking. The verbal morphology in both languages carries meaning about many factors, and the ambiguity present in some sentences allows for the same voice to focus on different noun phrases within the same sentence depending on the context and the verb root. Additional research could consider the specifics in Hiligaynon's voicing system, such as the underlying syntax. Analyzing the syntax and comparing it to Tagalog could be useful to understand Hiligaynon's system, and Philippine-style topic marking. So, although it appears clear they have differences in their systems, more can and should be done in understanding the voicing systems present in each language to get a better understanding of their systems and for language in general.

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Use of a Novel Plant Essential Oil-based Insecticide for Insect Control in Household Gardens and Lawns

[1]Charles E. Schmidt College of Science,
Department of Biological Sciences, Florida Atlantic University
[2]Penta 5, USA, Sarasota, FL 34234, USA
Mia Michnik[1], Alysa Suissa[1], Liah Brussolo Cremona[1], Annalise Wellman[1], Nicholas Nifakos[1], Alexander Spanoudis[1], Dr. John M. Harlin[2], Dr. Xing-Hai Zhang[1] (Faculty Advisor)

Abstract

Insecticide use has increased as population and agricultural demand for crops grows. Commercial insecticides contain active ingredients that are often harmful to humans and the environment. A viable alternative to this concern is developing and using insecticides with low toxicity and minimum risk to the environment. In this study, we sought to empirically assess the effectiveness of an insecticide based entirely on plant essential oil active ingredients, which contain complex secondary metabolites. Our results show that a concoction of several plant essential oils was effective in killing a wide range of household garden insect species and bedbugs, encompassing five orders of Insecta (Coleoptera, Diptera, Hemiptera, Lepidoptera, and Neuroptera) and at least 22 families. This insecticide is also fatal to four tick species (class Arachnida, order Ixodida, family *Ixodidae*). Due to the likely major economic and environmental benefits of using significantly less toxic, plant-based pesticides on a large scale for agriculture and residential housing, our study highlights the potential of plant essential oil-based insecticides as an effective and environmentally safe means of pest control.

Introduction

Insecticides are reagents composed of active ingredients for the purpose of killing insects. The use of insecticides ranges from backyard gardens and lawns to crop protection in agricultural crops.

The active ingredients used in commercial insecticides vary greatly, including chlorinated hydrocarbons, organophosphates, carbamates, and pyrethroids (Zlotkin, 1999; Ndakidemi et al., 2016). A commonly used and effective mechanism of the active biochemical ingredients acts by attacking the insects' nervous system, inhibiting the neural pathways resulting in their immobility and death. These neurotoxic insecticides typically cause the exposed insects to display symptoms of severe neural damage. For example, acetylcholinesterase inhibitors, such as carbamate and organophosphates, are common insecticide components. They work by binding to the acetylcholine receptor inhibiting acetylcholine, an essential neurotransmitter, resulting in the insect experiencing whole-body tremors, convulsion, paralysis and death (Fukuto, 1990; Rajashekar et al. 2014). Pyrethroids, while generally harmless to humans, are axonic excitotoxins to insects and act by disrupting the action of the voltage-gated sodium channels in the axonal membranes, thus paralyzing the insect (Ndakidemi et al., 2016).

When used extensively, all insecticides exert a negative impact on the environment. Because of the frequent use of neurotoxin-containing insecticides, their environmental impacts are becoming a major concern. Insecticides can be identified as environmental pollutants, which have adverse environmental impacts and cause biochemical changes in other non-insect organisms (Van Dyk & Pletschke 2010; Mulla et al., 2020). They may also result in insecticide resistance, via mutations in the receptor of the insect targeted by the insecticide, thus decreasing the insecticide's effectiveness over time (Hemingway et al., 2002). This is an important consideration, especially when insecticides are used regularly and widely, as in agriculture. In a global pesticide pollution study by Tang et al. (2021), it was found that more agricultural activity and agricultural land use leads to more insecticide use. In Europe, the main three countries—Russia, Ukraine, and Spain—with high pesticide pollution risk also had the highest agricultural production. In addition, China had a pesticide pollution from over 20 active ingredients and ranks as the highest-risk (Tang et al., 2021). Furthermore, these pesticides may affect the nervous system in mammals. The toxicity poses a major threat to humans and pets who may be exposed to chemicals in household applications of insecticide. An extensive review of human and animal studies done by Aloizou et al. (2020) suggested mechanisms of pesticides like permethrin may act neurotoxically on humans. The Canadian Study of Health and Aging found that the risk of vascular dementia is doubled with exposure to occupational pesticides (Yan et

al., 2016). Chronic pesticide exposure may increase the risk of cognitive impairment and dementia.

During hundreds of millions of years of evolution, plants have developed intricate defense mechanisms against environmental stresses such as insect attacks. Plants can defend against insects in two main ways: direct defenses via physical or structural characteristics such as thorns or fortified thick leaves, and indirect defenses with release of volatile compounds that ward off herbivorous insects or attract enemies of the insects (War et al., 2012). Depending on the chemical variability, such compounds may repel herbivores at varying degrees. This was seen in leaf extracts from a shrub plant, *Lantana camara*, which showed insecticidal activity by inhibiting acetylcholinesterase in insect pests (Rajashekar et al., 2014).

Essential oils are produced by many plants and are often volatile with a distinct scent (Camele et al., 2021; Soliman 2022). They are more commonly well-known for their applications in essential oil therapy, food safety, medicine, and their antimicrobial and antioxidant properties (Deans et al., 1987; Seow et al., 2013; Bilenler et al., 2015; Rafie et al., 2016; Arena et al., 2021). While plant essential oils are enjoyed by many people and are generally safe to mammals, various essential oils have also long been used against insects. Essential oils contain complex compositions of various fatty acids (Siegler 1924; as summarized from literature in Table 1).

These fatty acids may harm insects and other invertebrates through various means, such as disrupting cell membranes, blocking breathing pathways in the trachea, damaging the skin cuticle, and interfering with hormones and neurons (Murray, 2000). Therefore, plant essential oils have the capability of being active ingredients of safe and environmentally benign insecticides. However, individual essential oils are typically compounds employed as insect repellents. Individually, they rarely kill insects, and repellency is short-lived because of their rapid evaporation. Plant essential oils also exhibit attractant activity due to various chemical compounds including monoterpenes, sesquiterpenes, and phenylpropanoid compounds (Mossa, 2016).

To develop a product that is both effective and safe for use mainly in household gardens and lawns, we explored the insect-toxic yet safe-to-mammal properties of plant essential oils and formulated a concoction with complex compounds and lower evaporation rates that in synergy are expected to have more lasting effect. In this study, we aimed to empirically survey what types of insects this plant essential

Table 1
Major Compositions of Plant Essential Oils used for the Insecticide

	Composition (%)	Reference
Cedarwood Oil		Kamatou et al. (2010)
Thujopsene (widdrene)	47.1	
α-Cedrol	10.7	
Widdrol	8.5	
Cuparene	4.0	
Thujopsenal	3.2	
α-Bisabolol	3.0	
Mayurone	2.6	
Thujopsadiene	1.8	
α-Cuprenene	1.3	
3-Thujopsanone	1.1	
β-Cuprenene	0.9	
Peppermint Oil		Schmidt et al. (2009)
Menthol	40.7	
Menthone	23.4	
1,8 Cineole	5.3	
Menthofurane	3.7	
Limonene	2.6	
Pulegone	1.9	
α-, β-Pinene	1.8	
β-Caryophyllene	1.7	
Germacrene D	0.9	
Thyme Oil		Borugă et al. (2014), Gruľová et al. (2020)
Thymol	47.6	
γ-terpinene	30.9	
p-Cymene	8.4	
Carene	3.8	
Caryophyllene	2.7	
β-Myrcene	1.5	
α-, β-Pinene	1.4	
α-Thujene	1.1	
Cyclohexene	0.8	
Rosemary Oil		Özcan and Chalchat (2008)
p-Cymene	44.0	
Linalool	20.5	
γ-Terpinene	16.6	
α-, β-Pinene	6.4	
Eucalyptol	2.6	
Thymol	1.8	
Myrcene	1.8	
Camphre	1.7	
Camphene	1.4	
Borneol	1.0	
Terpinene-4-ol	0.8	

oil-based insecticide could kill in the household garden/lawn setting, as well as toxicity to ticks and bedbugs. We also discuss the potential benefits and limitations of using plant essential oils as an alternative method to control pest damage and simultaneously minimize negative environmental impacts.

Material and Methods

Plant Essential Oil-based Insecticide

Developing environmentally friendly products was achieved via a concoction of plant oils formulated to serve as active ingredients of an insecticide. Following the guidelines by the US Environmental Protection Agency (EPA), we aimed to design an insecticide that would pose the least toxic impact on people and the environment. Insect traps were employed over nights from August to October in South Florida. We selected cedarwood Texas essential oil (New Directions Aromatics Inc.), peppermint oil Piperita (Bulk Apothecary), thyme oil White (Phoenix Aromas & Essential Oils, LLC) and rosemary Spanish type oil (Phoenix Aromas & Essential Oils, LLC) as the main active ingredients. They can be labeled as a botanical pesticide and are considered to have lower toxicity than commercial pesticides (EPA, 2021a). Significantly, all these active ingredients in these essential oils composing the insecticide, shown in Table 2, are exempt from the U.S. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), with peppermint oil, thyme oil, and rosemary oil permitted for food use (EPA, 2021b). This implies they have been determined by the EPA to have little to no adverse impact on the environment or human health, and products exclusively using these ingredients are not required to follow the guidelines outlined in FIFRA (2021). To enhance their insecticidal potency, the four oils were supplemented with two coconut oil-derived fatty acids: decanoic acid (also known as capric acid, C10) and lauric acid (C12), both of which have been shown to be safe and potent pesticide ingredients (EPA, 2009; Zhu et al. 2018). These active ingredients, each at a percentage range of 2% to 5%, were mixed with a sunflower carrier oil. The final water-based formula was made with triethyl citrate as a plasticizer and lecithin and xanthan gum as emulsifiers.

Table 2
*Composition Analysis of the Plant Essential Oils used for this Insecticide**

	Major Composition (%)
Cedarwood Texas Essential Oil	
Thujopsene (widdrene)	36.86
α-Cedrol	26.37
Peppermint Oil Pipertia	
Menthol	50.59
Total esters	5.12
Thyme Oil White	
Thymol	47.0
γ-terpinene	30.1
Rosemary Spanish Type Oil	
1,8-Cineole	44.5
α-Pinene	11.1
β-Pinene	7.8
Camphene	4.5
β-Caryophyllene	3.5
Borneol	3.0

*Adapted from manufacturers' certificates of analysis.

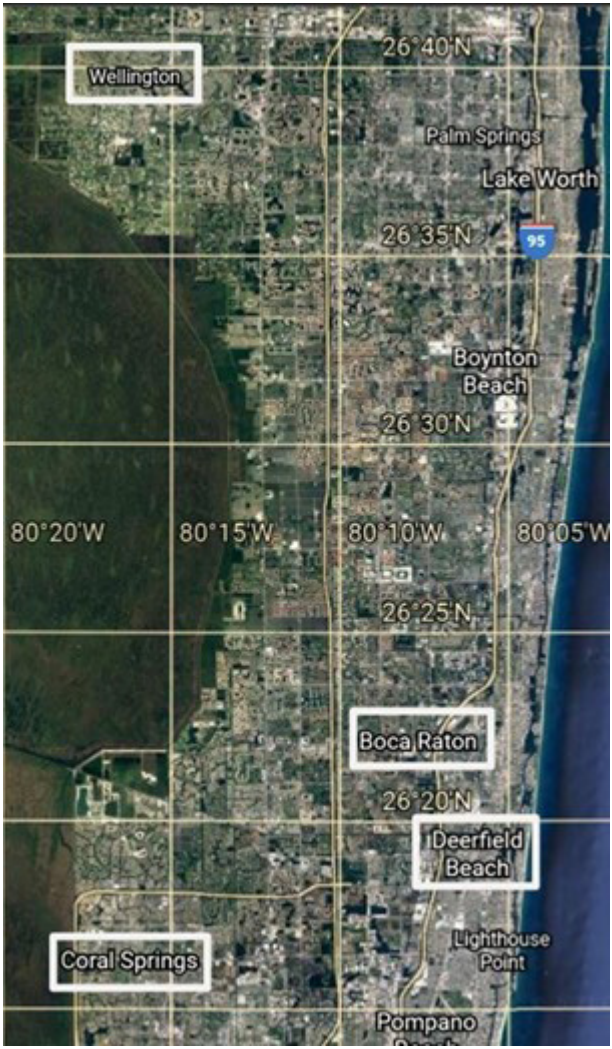
Sampling and Treatments

Capture and treatment of insects were done using a DynaTrap™ DT 2000XL device, with a blue light and a vacuum fan, as shown in "Supplemental Materials, Fig. S1". The blue light aims to attract insects to the trap, where the fan creates a vacuum trapping the insects in the chamber and prevents them from escaping. There was no physical contact between the motor and insects, ensuring they would not be harmed by the device. A filter paper (28 cm in diameter) was placed on the base of the chamber and sprayed with 2 ml of the plant-based insecticide so that the filter was evenly soak-wet. The trap was run overnight for 12 hours from 7 pm to 7 am. The following morning, all trapped insects, dead or alive, were counted. The dead insects were collected into plastic containers and kept at -20°C until retrieved for examination.

This same procedure was performed using a water-soaked filter (mock) as a control, which only collected live insects. A total of four traps were set, one in each city survey site, which included Wellington, Boca Raton, Deerfield Beach, and Coral Springs. Four traps were set up in household gardens with lawns located in Palm Beach and

Broward counties in South Florida, United States, as shown in Figure 1. At each location, trapping was performed repeatedly over three clear nights from August to October.

Figure 1
Trap Locations for Insect Collection, in South Florida, US



To test the effect on ticks, parasitic arachnids that can be health hazardous to mammals including humans, four *Ixodidae* species, *Amblyomma americanum, Dermacentor vaiabillis, Ixodes scapularis* and *Rhipicephalus sanguineus* were purchased from Oklahoma State University, United States. Males and females of each species were tested. In the lab, ticks were placed into small, transparent condiment containers with lids and were sprayed with approximately 0.5 mL of the insecticide. Death was defined as when the ticks no longer showed signs of self-controlled movement when the containers were shaken. Once ticks were confirmed dead, the containers were placed in the -20°C freezer until analysis.

To test the effect on bedbugs, live bedbugs (*Cimex lectularius*) were purchased from cheapbedbugs.com. Death of the bed bugs was defined as being without leg movement upon vigorous agitation of the container over a period of 48 hours. A GF/C glass fiber paper circle (diameter 2.4 cm; Whatman) was placed in the center of 10-cm plastic cups and was soaked with 0.3 ml of the plant-based insecticide. Five bed bugs were placed on the paper circle. The reactions of the bugs to these insecticides were recorded for a period of 48 hours. Each respective insecticide trial was repeated three times. For a duration test, the paper circles soaked with 0.3 ml of the plant-based insecticide were left open lidded on the bench at 24°C for 24 hours, to simulate evaporation. Then, five bed bugs were placed on each of the paper circles. The reactions of the bugs were recorded for a period of 48 hours. Each respective sample trial was repeated three times.

For scanning electron microscopic (SEM) imaging, the treated bedbugs were fixed with 2% glutaraldehyde at 24°C overnight, followed by dehydration with a series of ethanol solutions from 30% to 100%, and submerged in 100% ethanol (Zhang et al., 2020). For SEM imaging, samples underwent a critical-point drying process using Leica EM CPD300 and were mounted and sputter-coated in gold using a thickness setting of 19 to 23 nm on a Leica EM ACE200. The bug samples were scanned with a JEOL NCM-6000Plus system according to the manufacturer's instructions. Images of treated bed bugs were taken post-plant essential oil insecticide and Hot Shot brand pesticide.

Identification of Insect Species

Insect identification was done by photographing and analyzing them under a Leica Zoom 2000 compound microscope. Observed characteristics were compared with insect identification descriptions,

images, and websites to categorize the species taxonomically (see "Supplemental Materials"), with the intention to identify down, when it was possible, to species level. When there was a lack of suitable images or information for comparison, insects were recorded with less specificity. For example, many in Lepidoptera (family of moths and butterflies) were only listed in their order due to uncertainty.

Results

The US Federal Environmental Protection Agency (EPA) has classified bio-pesticides based on their active ingredients originating from plants, fungi, bacteria, or viruses, and has designated three categories: biochemical, microbial, and plant-incorporated protectants (EPA, 2021c). All the active ingredients in the essential oils composing this insecticide (Table 2) are deemed to be safe by the EPA (EPA, 2021b). By contrast, active ingredients of most commercial and non-bio insecticides are not on the Minimum Risk Pesticides List (EPA, 2021a). In addition, due to insect resistance creating challenges with synthetic chemical pesticides, botanical pesticides do not pose a threat to insect resistance to non-target, cross- and multi-resistant insects (El-Shafie, 2021, pp. 109–110).

Plant essential oils have been found to contain complex metabolite compounds (Tables 1 and 2), including eugenol, β-caryophyllene, menthol, menthone, 1,8-cineole, camphor, α-pinene, thymol, p-cymene, linalool and many other metabolites. For example, the essential oil from bark, leaf and fruit of red cedar (*Juniperus virginiana*) was detected to contain compounds such as αpinene, safrole, methyl eugenol, elemol, and limonene (Stewart et al., 2014). Our insecticide is a mixture of essential oils from four different plants, the chemical complexity of which should produce a synergistic effect on pests of plants and reduce, although likely not avoid, the risk of the insect's developing resistance to the insecticide.

Comparing the water-only (mock) traps to those immersed with the oil-based insecticide, we did not observe vast difference in the number or species of insects trapped, contrary to the expectation of more insects attracted to oils. This may indicate that the blue light emitted from the trap was attracting insects in our experimental setting. It may also be due to the small sampling size. When contacted with the plant essential oil-based insecticide, the insects suffered a mortality rate of over 98% overnight, as examples of representative images shown in Figure 2A and 2C. To the best of our identification

ability, the killed insects belong to five orders: Coleoptera, Diptera, Hemiptera, Lepidoptera, and Neuroptera; and at least 22 families, as summarized in Table 3. Among the most abundant were those in order of Lepidoptera, Diptera and Coleoptera. Our results clearly demonstrated that the plant essential oil concoction was effective in killing a wide range of insects within household gardens/lawns. However, this list is likely to be an underestimation because, in some cases, it was not possible to ensure an identification due to partially disfigured insects, faded or altered coloration in death, or unavailability and/or inconsistency of images from identification resources.

Figure 2

Representative Images of Insects Affected by the Plant Essential Oil-based Insecticide. A, green lacewing (genus Crysoperla*); B, ticks (*Ixodes scapularis*); C, metallic fly (genus* Condylostylus*).*

Table 3
Identified Specimens from Class Insecta Killed by Plant Essential Oil-based Insecticide

CLASS INSECTA			
Order	**Family**	**Genus**	**Species**
Coleoptera	Scarabaeidae	*Phyllophaga*	*latifrons*
Coleoptera		*Odontotaenius*	*disjunctus*
Coleoptera	Curculionidae	*Myllocerus*	*undatus*
Coleoptera	Phengodidae	*Phengodes*	*laticollis*
Diptera	Calliphoridae	*Condylostylus*	*occidentalis*
Diptera	Ceratopogonidae	*Culicoides*	*sonorensis*
Diptera	Muscidae	*Musca*	*domestica*
Diptera	Sciaridae	*Bradysia*	*difformis*
Diptera	Tipulidae	*Nephrotoma*	
Diptera	Tephritidae	*Trupanea*	
Hemiptera	Cercopidae	*Prosapia*	*bicincta*
Hemiptera	Cicadellida	*Gyponana*	*angulata*
Hemiptera	Membracidae	*Smilia*	*fasciata*
Hymenoptera	Ichneumonidae		
Lepidoptera	Crambidae	*Herpetogramma*	
Lepidoptera	Crambidae	*Syngamia*	*florella*
Lepidoptera	Geometridae	*Operophtera*	*brusceata*
Lepidoptera	Erebidae	*Seirarctia*	*echo*
Lepidoptera	Erebidae	*Macrochilo*	*orciferalis*
Lepidoptera	Noctuidae		
Lepidoptera	Paralidae or Geometridae	*Epimecis or Eusarca*	*detexta or confusaria*
Lepidoptera	Tineidae		
Lepidoptera	Tineidae	*Tineola*	*bisselliella*
Neuroptera (net winged)	Chrysopidae	*Chrysoperla*	*argentina*
Neuroptera	Chrysopidae		

Table 4
Identified Specimens from Class Arachnida (Ticks) Killed by Plant Essential Oil-based Insecticide

CLASS ARACHNIDA			
Order	**Family**	**Genus**	**Species**
Ixodida	Ixodidae	*Rhipicephalus*	*sanguineus*
Ixodida	Ixodidae	*Ixodes*	*scapularis*
Ixodida	Ixodidae	*Dermacentor*	*variabillis*
Ixodida	Ixodidae	*Amblyomma*	*americanum*

The plant essential oil-based insecticide killed four tick species (class Arachnida, order Ixodidae, family *Ixodidae*), as shown in Table 4 and Figure 2B. All ticks were immediately paralyzed upon contact with the insecticide and confirmed dead within 30 minutes. However, the ticks placed on filter paper coated with insecticide were able to survive by crawling away from contact with the filter paper.

Discussion

It should be pointed out that plant-based insecticides cannot differentiate between insect pests of crops and potentially harmless insects. Some of the insects affected by this insecticide are beneficial entomophagous insects and natural pollinators such as some bees and wasps (Hymenoptera) and moths (Lepidoptera). It is known that use of any types of insecticides carries various degrees of collateral damage and unintended ecological consequences (Hahn et al., 2016; Piechowicz et al., 2017; Pamminger et al., 2018). A study by Smith et al. (2020) suggested that exposure to insecticides during the brood growth stage impacts bumblebee-worker response and learning ability. However, it can be reasonably assumed that the plant-based insecticide used in the study is much less toxic to these groups of pollinators than the commercial insecticides, as suggested by the placement of plant essential oils on the United States EPA's Minimum Risk Pesticides List (EPA, 2021a). Essential oils used in the insecticide relay the lowest acute toxicity to worker bees with no effects on their locomotion and orientation ability (Giunti et al., 2022). Additionally, there is evidence that active ingredients in commercial insecticides can also affect aquatic organisms, showing a wide range of ecological impacts. Seen in a study on Chinook salmon, it was found that the active ingredient bifenthrin in insecticides, combined with rising temperatures associated with climate change, may affect salmon populations in the long-term (Giroux et al., 2019).

Bifenthrin affects gene expression of hormones such as gonadotropin-releasing hormone receptor 2, dopamine receptor 2A and growth hormone 1 in these salmon at the alevin life stage (Giroux et al., 2019). This implies that reproduction of these fish may be affected and at risk, thus leading to population-wide impacts. Plant essential oil-based insecticides, due to their absence of compounds like bifenthrin, are therefore a safer alternative for the aquatic environment as well.

Our insect traps were employed overnight during August to October in South Florida and would not likely catch many of the seasonal or diurnal migrating insects. Thus, this study can only serve as a showcase of the potential utility of plant essential oil-based reagent in insect pest control. Furthermore, the goal of this investigation was to qualitatively assess what types of insects can be killed by this plant essential oil-based insecticide in a household garden/lawn setting. No broader geographic samplings or statistical analyses were attempted for this study. Future investigations are needed to understand the

molecular mechanisms of how this insecticide causes insect mortality, or its quantitative efficacy in different groups of entomofauna. It can be argued that for small scale uses such as household gardens, plant-based insecticides should be the top choice, considering their overall limited environmental impacts/footprints and non-toxicity to humans and pet animals. However, because the plant essential oils are naturally weaker neurotoxins than the commercial, synthesized pesticides (EPA, 2021b), they may be less effective for application at a large scale such as agricultural farmland. For industry use, there will be yield and economic benefit considerations when deciding on choice of insecticides (Ayalew, 2006). Our current study did not examine how long the oil-based insecticide can remain on the plant surface and maintain its efficacy in the natural environment. Therefore, further testing and assessment will be needed to achieve the best overall outcome, both economically and environmentally, for application of plant-based insecticides to large-scale systems.

The plant essential oil-based insecticide was also effective in killing bedbugs on contact in the lab setting. All 20 bugs were paralyzed within five minutes after contact with the insecticide. By 20 minutes, all bed bugs were dead (Figures 3 and 4; Table 5). This effect was long-lasting, as shown by the duration test where all the bedbugs were dead when placed on the insecticide-soaked paper circles that had been left open to air for 24 hours before in contact with the bugs. All these experiments demonstrated the bedbug-killing effect of the plant essential oil mixture.

Images using an electron scanning of bed bug samples post treatment served the purpose to examine any morphological abnormalities. There were no visible morphological differences noticed post insecticide and pesticide treatment; however, it is known that the samples of bed bugs that had undergone each respective treatment had experienced trauma to their neurotransmitter receptors, leading to death due to toxicity. The exterior morphology of the treated bed bugs remains unchanged upon exposure to treatment.

In summary, the plant essential oil-based insecticide examined in this study can kill some of the common insect pests found in South Florida, several species of ticks and common bedbugs. Use of plant essential oils rather than synthetic active ingredients can be more beneficial for the environment as they are less toxic, indicated by their exemption from FIFRA registration. Furthermore, plant essential oil-based insecticides most likely have lesser effects on pollinators in families such as *Hymenoptera* and *Lepidoptera* than most insecticides on

the market. Nevertheless, due to their inherent indiscriminate property against almost all insects tested, the plant essential oil-based insecticides seem most suitable for use in gardens, lawns and indoor households.

Figure 3
Examples of Bedbugs in Contact with the Plant Essential Oil-based Insecticide

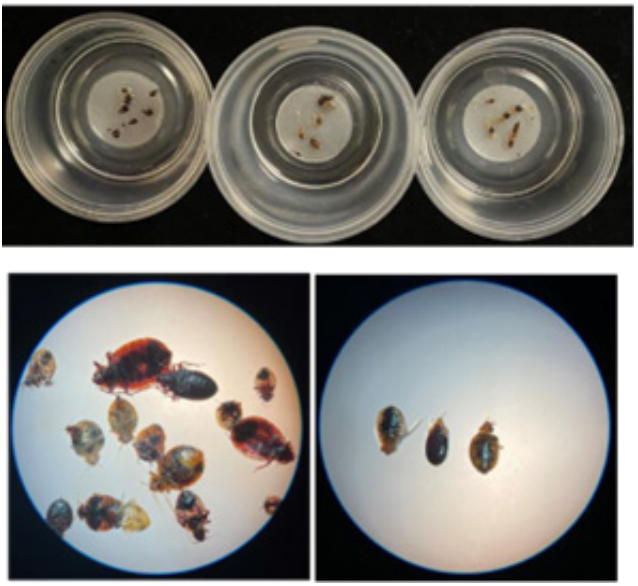


Figure 4
Scanning Electron Microscopic Images of Bedbugs Treated with the Plant Essential Oil-based Insecticide

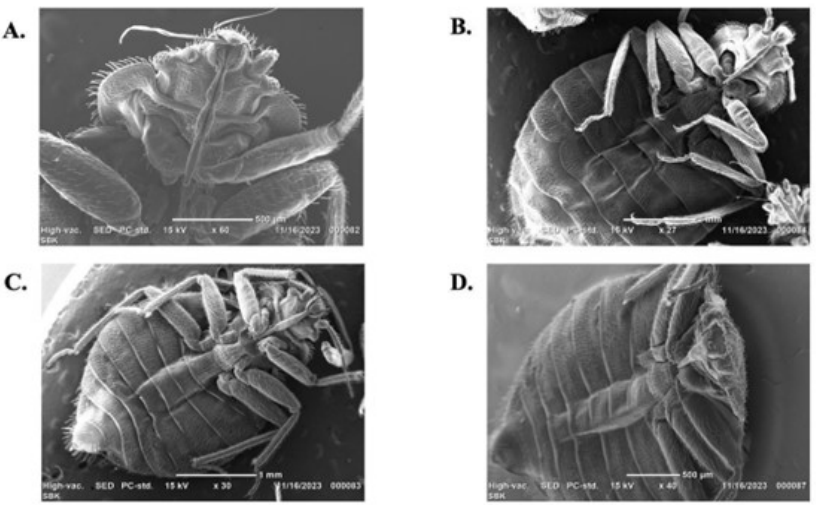
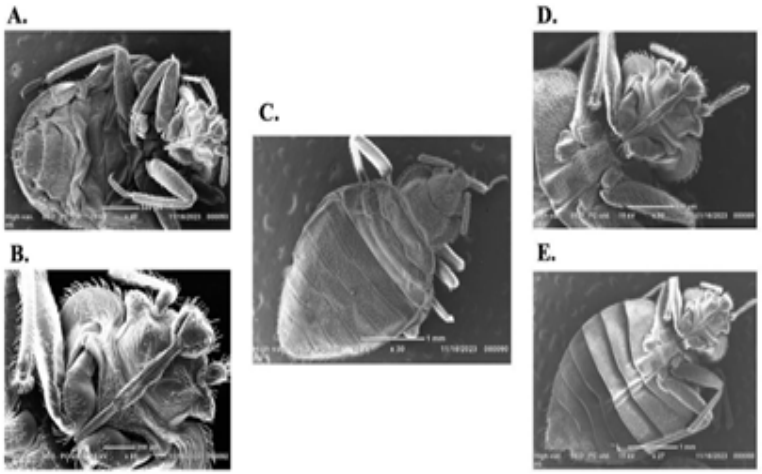


Table 5
Instant Response of Bedbugs to Pesticides upon Contact

Number of bedbugs before contact with pesticide	Number of bedbugs at time after contact with the insecticide		
	5 minutes paralyzed	20 minutes appeared dead	24 hours evidence of life
20	20	20	0

Notes. Table displays no evidence of life 24 hours post contact with insecticide.

Figure 5
Scanning Electron Microscopic Images of Bedbugs Treated with Commercial Hot Shot Pesticide



Conclusions

Our preliminary experiments illustrate that a concoction made from essential oils of cedar wood, peppermint, thyme and rosemary was effective, although indiscriminately, in killing against a wide range of lawn insect species, ticks and bedbugs. Our study highlights the potential of plant essential oil-based insecticides as a beneficial alternative for environmentally safe means of pest control, and the need to further explore the vast resources of still unknown plant metabolite compounds.

Acknowledgements

We thank Dr. Evelyn Frazier and Dr. Diane Owen, Florida Atlantic University (FAU), for their advice on insect identification. The scanning electron microscopy work was funded by and carried out in FAU's A.D. Henderson University High School Owls Imaging Lab with the technical assistance of Dr. Jamie Knaub. This work was supported by FAU Office of Undergraduate Research and Inquiry grants and a fund from Penta 5, USA.

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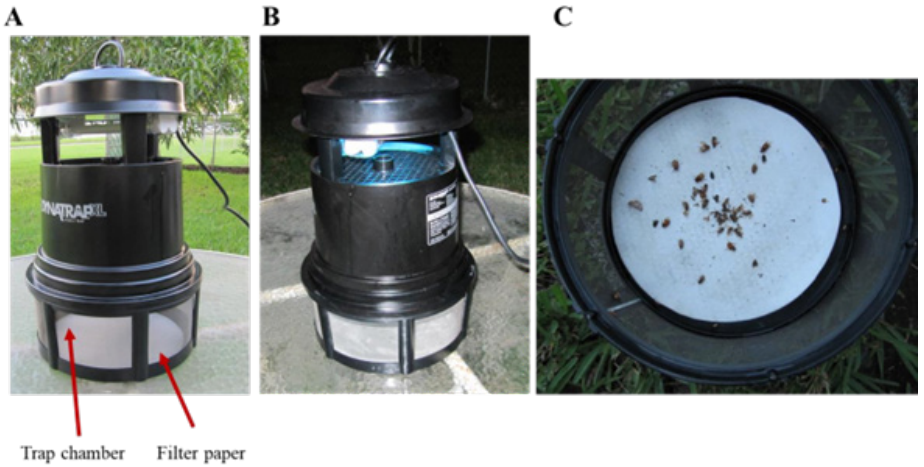
Appendix

Figure S1
Insect Trap Device DynaTrapTM DT 2000XL. **A.** daytime view, with arrows indicating the chamber and the filter paper to be pre-soaked with the insecticide; **B,** nighttime view; **C,** insects trapped and killed.



Bioarchaeological Analysis of Three Individuals Excavated from Palikè di Mineo, Sicily

Dorothy F. Schmidt College of Arts and Letters,
Florida Atlantic University
August M. Stone, Madison Rieth, William Burnett,
Dr. Meredith A. B. Ellis (Faculty Advisor)

Abstract

This study presents the analysis of three individuals excavated from the area of Palikè di Mineo, Sicily in May and June of 2023. The archaeological area contained numerous burials, but this study focuses on two adult male and one subadult (child). Burials are estimated to be from the Imperial Period of the Roman Empire (20 CE - 360 CE). Information collected includes age, sex, stature, and taphonomy of the individuals. Burial style, artifacts, and position of the burials are used to present data on the possible conditions of life in ancient Sicily. This article contributes data to the greater discussion of archaeology in the area.

Introduction

This project presents bioarchaeological analysis of three skeletons excavated in Palikè di Mineo, Sicily, Site 79S-2. Discussion about burial styles, artifacts, and position of the burials is used to suggest possible living conditions in ancient Sicily. Individuals excavated during 2023 were selected for analysis based on completeness and preservation. Individuals 2, 6, and 7, corresponding to Tombs 2, 6, and 7, were chosen. Individuals 6 and 7 are both adult males, and Individual 2 is a child, between the ages of 2 to 3.5 years old. Skeletal analysis and excavation were performed over the same field season in the same area to prevent possible inaccuracies in data.

Palikè di Mineo Archaeological Area

Palikè di Mineo, an archaeological area located in Eastern Sicily, had been inhabited consistently from the Paleolithic period through late antiquity (Maniscalco & McConnell, 2003). Four areas: Hestiaterion,

Complex P, Stoa B, and Stoa FA have been excavated yearly by Laura Maniscalco and Brian McConnell since 1995 (Maniscalco & McConnell, 2003).

Figure 1
Palikè di Mineo Archaeological Area Overlooking Site 79S-2

Site 79S-2 was discovered using ground penetrating radar downhill of the previously excavated areas. Excavation was originally focused on a stone wall in site 79S-2 (McConnell, personal communication, 2023). Burials around the wall changed the focus of the season. Previous excavations in this archaeological area focused exclusively on architectural analyses, yielding no previous in-depth skeletal or material culture context. This project adds dimension and context to historical understanding of the area.

The 2023 field season experienced a lot of heavy rain followed by direct sunlight. Repetitive wetting and drying left skeletal remains friable. Skeletal and material culture analysis was limited due to time constraints and weather. Site 79S-2 was used as farmland for the majority of the year (McConnell, personal communication, 2023). Repeated use of machinery on the land and the area's repetitive field seasons caused severe damage to the archaeological context of the burial area.

Mortuary Context

Tombs 2, 6, and 7 were facing east to west intersecting a stone wall running north to south across Site 79S-2. Each individual had their arms and legs facing straight to the west. Tomb 2 had two stone tiles stacked parallel on top of the individual. Pieces of stone tiles were scattered near Tombs 6 and 7. Similar glass bottles were found oriented north-south at the feet of Individuals 2 and 7, with Tomb 2 containing one glass bottle and Tomb 7 containing two. Individual 6 was buried with a coin positioned in the right hand sitting on the femur. The coin was sent for examination, but no data has been received (McConnell, personal communication, 2024).

Methods

Bone Weathering Stages

All bones from Individuals 2, 6, and 7 aligned with Stage 1 of the Bone Weathering Stages (Behrensmeyer, 1978; Buikstra & Ubaker, 1994).

Age

Individual 2. The age of Individual 2 was estimated using long bone length, epiphyseal union, and tooth growth and development. Elements were selected for measurement based on completeness. The humerus and femur measurements yielded an age range of 1.5 to 3.5 years, and the fibula yielded 2.5 to 3.5 years (Table 1). The clavicle yields 2 to 5 years (Schaefer, 2009).

All deciduous teeth other than one incisor of Individual 2 were recovered. The deciduous canine teeth are root ¾, corresponding to an age of 1.82 years (Moorrees et al., 1963b). The permanent maxillary first incisor is crown ¾, indicating the individual was younger than 5 years (Moorrees et al., 1963a; Table 3).

Taphonomic damage inhibited estimating epiphyseal union for most elements. The cervical, thoracic, and lumbar neural arches were fused, but the centra were unfused, giving an age range of 1 to 3 years (Baker et al., 2005). Fully fused mental symphysis indicates a minimum of 2 years old (Schaefer, 2009; Table 2).

This data suggests Individual 2 died between the ages of 2 to 3.5 years old. Although incorporating all epiphyseal union data would have given a range of 2 to 3 years old, the damage caused by excavation made it difficult to accurately determine whether the elements were fusing

or unfused. This uncertainty was conductive to a more conservative estimate of 2 to 3.5 years old, using the minimum age found in epiphyseal union and the maximum age found in long bone length.

Individual 6. The age of Individual 6 was estimated using the pubic symphysis after Todd (1920) and Brooks and Suchey (1990), and auricular surface after Meindl and Lovejoy (1989). The pubic symphysis is consistent with Phases 7-8, indicating 35 to 45 years old (Todd, 1920), and consistent with Phase 4, corresponding to 23 to 57 years old (Brooks and Suchey, 1990). The auricular surface is consistent with Phases 4-6, indicating 35 to 49 years old (Meindl & Lovejoy, 1989). The final age estimation of Individual 6 is 35 to 49 years old (Table 10).

Individual 7. The age of Individual 7 was estimated using the auricular surface after Meindl and Lovejoy (1989). Both the left and right auricular surfaces are consistent with Phase 7, indicating Individual 7 is between the ages of 50 to 59 years old (Meindl & Lovejoy, 1989; Table 11). Due to damage during excavation, no other elements were complete enough to be analyzed for age estimation.

Sex

Individual 6. The sex of Individual 6 was estimated using cranial elements, os coxa, and femoral and humeral head diameters after Buikstra and Ubelaker (1994). The mastoid process, supraorbital ridge, and mental eminence score as 3, or indeterminate, while the nuchal crest and supraorbital sharpness score at 4, or probable male (Buikstra & Ubelaker, 1994). Measurements of the femoral and humeral head both indicate probable male (Buikstra & Ubelaker 1994). All landmarks on the os coxa are consistent with probable male (Buikstra & Ubelaker 1994). From this analysis it is estimated that Individual 6 is a probable male.

Individual 7. The sex of Individual 7 was estimated using cranial elements, os coxa, curvature of the sacrum, and femoral and humeral head diameters after Buikstra and Ubelaker (1994). Cranial elements on Individual 7 score between 4 and 5, or probable male and male (Buikstra and Ubelaker, 1994). Mastoid process and supra-orbital sharpness scored at 4, and mental eminence at 5 (Buikstra & Ubelaker, 1994). Greater sciatic notch width scored as 3, or indeterminate (Buikstra & Ubelaker 1994), and curvature of the sacrum indicates

probable male (Buikstra and Ubelaker 1994). Both the femoral and humeral head indicate male (Buikstra & Ubelaker 1994; Table 7). With the measurements nearly all falling between probable male and male, it was estimated that the Individual 7 is male.

Stature

Stature for Individuals 6 and 7 was estimated after the formula by Trotter (1970). Individual 6's humerus, ulna, and femur indicate an estimated stature of 5'5 and 5'6 (Trotter 1970) (Table 4). Individual 7's humerus, ulna, and tibia indicate an estimated stature between 5'7 and 5'9 (Trotter 1970; Table 5).

Results

Individual 2 is a subadult between 2 to 3.5 years old. Individual 2 has no visible pathology. Initial observation recognized potential pathological changes to the long bones. This was later estimated to be appositional (normal) growth for individuals under 4 years of age (Lewis, 2018, p.132; Schrenk, 2017). New bone formation can mimic signs of certain pathological responses in infants (Gleser, 1949). After further consideration of the changes to the cortical bone observed on Individual 2 and comparing them to Lewis (2018), due to the bilateral nature and consistency across long bones, these markers are most likely associated with normal growth for an individual under four (Lewis, 2018, p. 132; Figure 2).

Individual 6 is estimated to be an adult male between the ages of 35 and 49 years old. Stature is estimated to be between 5'5 and 5'6. Enthesophytes are found bilaterally on the long bones, as well as along the nuchal crest and mandibular muscle attachment sites (Minozzi et al., 2014; White et al., 2012). There is a large abnormality on the occipital and right parietal of the crania located superior to the lambdoidal suture (White et al., 2012) (Figure 3). The cause of this abnormality is unknown.

Figure 2
Individual 2 Right Tibia with Markings of Appositional Growth

Figure 3
Abnormality on Crania of Individual 6

Individual 7 is estimated to be an adult male between the ages of 50 to 59 years old. Stature is estimated to be between 5'7 and 5'9. Lipping, osteophytes, enthesophytes, and Schmorl's nodes (Figure 5) are prominent on the vertebrae (Aufderheide & Rodriguez-Martin, 1998, p. 96; Minozzi et al., 2014; Mok et al., 2010; Rogers et al., 1985; Wang, 2023; White et al., 2012). Vertebrae C3+C4 (Figure 4), and C5+C6 are completely fused together due to excess bone growth around several suspected healed fractures (Aufderheide & Rodriguez-Martin, 1998; Rogers et al., 1985; White et al., 2012). Extensive osteophyte and enthesophyte growth among muscle attachment sites and joint surfaces can be found on nearly every element (Bolanowski et al., 2005; Menz et al., 2019; Minozzi et al., 2014; White et al., 2012). Third trochanters are present on both femurs (Bolanowski et al., 2005). Osteophyte growth present on the left ulna and left radius appear to articulate, giving evidence of a past fracture or long-term displacement (Aufderheide & Rodriguez-Martin, 1998, p. 25; Pedersen et al., 2019; White et al., 2012, p. 436). The sacrum is curved to the left, and irregularities appear to be due to a possible fusion abnormality (Park et al., 2017; Figure 6).

Figure 4
Fusion of Cervical Vertebrae 3 and 4 of Individual 7

Figure 5
Individual 7 Lumbar Vertebra 3 with Shmorl's Node and Osteophyte Lipping

Figure 6
Suspected Sacral Fusion Abnormality of Individual 7

Metrics

Table 1
Individual 2 Long Bone Length

Element	Length (mm)	Age Range (years)
Right Humerus	131 mm	1.5 to 3.5
Right Femur	180 mm	1.5 to 3.5
Left Fibula	145 mm	2.5 to 3.5
Clavicle	75 mm	2 to 5

Table 2
Individual 2 Epiphyseal Union

Element	Fusion Status	Age Range (years)
Cervical/Thoracic Neural Arches	Fully Fused	1 to 6
Cervical/Lumbar Centra	Not Fused	1 to 3
Thoracic Centra	Not Fused	1 to 3
Lumbar Neural Arches	Fully Fused	1 to 6
Mandibular Symphysis	Fully Fused	2

Table 3
Individual 2 Teeth

Tooth	Development Stage	Age Range
Deciduous Canines	R3/4	Older than 1.82 years
Permanent Maxillary First Incisor	Cr3/4	Younger than 5 years

Table 4
Individual 6 Long Bone Length

Element	Length (mm)
Humerus	315 mm
Ulna	260 mm
Femur	460 mm

Table 5
Individual 7 Long Bone Length

Element	Length (mm)
Left Humerus	355 mm
Right Ulna	290 mm
Right Tibia	405 mm

Table 6
Individual 6 Femoral and Humeral Head Diameters

Element	Diameter (mm)	Sex Estimation
Left Humerus	44 mm	Probable Male
Right Humerus	45 mm	Probable Male
Left Femur	46 mm	Probable Male
Right Femur	46 mm	Probable Male

Table 7
Individual 7 Femoral and Humeral Head Diameters[5]

Element	Diameter (mm)	Sex Estimation
Left Humerus	51 mm	Male
Right Humerus	51 mm	Male
Right Femur	52.5 mm	Male

Table 8
Individual 6 Sex Estimation Markers[6]

Element	Skeletal Sexing Score	Sex Estimation
Mastoid Process	3	Indeterminate
Supraorbital Ridge	3	Indeterminate
Mental Eminence	3	Indeterminate
Nuchal Crest	4	Probable Male
Supraorbital Sharpness	4	Probable Male
Greater Sciatic Notch	4	Probable Male
Ischiopubic Ramus	4	Probable Male

Table 9
Individual 7 Sex Estimation Markers

Element	Skeletal Sexing Score	Sex Estimation
Mastoid Process	4	Probable Male
Supraorbital Ridge	4	Probable Male
Mental Eminence	5	Male
Supraorbital Sharpness	4	Probable Male
Greater Sciatic Notch	3	Indeterminate
Curvature of Sacrum	4	Probable Male

Table 10
Individual 6 Age Estimation Markers

Element	System	Phase	Age Range (years)
Auricular Surface	Meindl & Lovejoy	4-6	35 to 49
Pubic Symphysis	Todd	7-8	35 to 45
Pubic Symphysis	Suchey-Brooks	4	23 to 57

Table 11
Individual 7 Age Estimation Markers

Element	System	Phase	Age Range (years)
Auricular Surface	Meindl & Lovejoy	7	50 to 59

Discussion

Individuals 6 and 7 both present excessive enthesophyte growth around muscle attachment sites. Enthesophyte growth across the entire skeleton makes it difficult to determine possible activities that could be associated with such widespread bone reaction (Menz et al., 2019). Excessive bone growth around attachment sites corresponds with muscle growth in that area (Bolanowski et al., 2005; Menz et al., 2019; Minozzi et al., 2014), and thus it can be suspected that Individuals 6 and 7 performed physically strenuous labor over long periods of time (Bolanowski et al., 2005; Menz et al., 2019; Minozzi et al., 2014). Individual 7 is notably more robust than Individual 6, as well as being taller and overall larger. It is possible that there is correlation between size and labor done in life (Bolanowski et al., 2005; Minozzi et al., 2014), though it cannot be confirmed. In addition to larger enthesophytes, Individual 7 also shows signs of moderate to severe degenerative joint disease (Menz et al., 2019), presenting as Schmorl's Nodes in the lumbar vertebrae (Mok et al., 2010; Wang, 2023) and lipping around joint articulation surfaces (Rogers et al., 1985). This is most likely due to a combination of old age and heavy labor (Menz et al., 2019; Minozzi et al., 2014).

It was initially thought that Individuals 6 and 7 could have been soldiers during life due to their enthesophyte growth and past injuries (McConnell, personal communication, 2023; Minozzi et al., 2014). It has been speculated for some time that the area of Palikè was used as retirement for the soldiers of Rome (McConnell, personal communication, 2023). Though the theory appears feasible from skeletal analysis (Menz et al., 2019), upon further research it seems unlikely due to the specific and consistent burial styles of Roman soldiers (Gardner 1999; Hope 2010; Rogers 2008; Williams, 2009). Individuals 6 and 7 were not consistent with the burial styles of soldiers during the time period, nor was the general layout of the site consistent with the style of necropolis in which soldiers were buried (Gardner 1999; Hope 2010; Rogers 2008; Williams, 2009). However, even with low likelihood of the individuals being soldiers, this does not negate the fact that the individuals lived in a way that required long periods of heavy labor (Menz et al., 2019; Minozzi et al., 2014). While no lifeway can be proven, some possibilities include farmers or slaves (McConnell, personal communication, 2023; Minozzi et al., 2014).

Individual 7 gave the most insight into what life may have been like during this time because of the injuries he sustained. Fractured cervical

vertebrae are difficult to heal without disrupting the spinal cord (Rogers et al., 1985), and the healed osteophytes around the area show that the individual lived for long enough for the injury to heal to completion (Israelowich, 2015; Menz et al., 2019; Minozzi et al., 2014; Rogers et al., 1985). The individual likely would not have been able to work or participate in society for some period of time, and would have required a large amount of care from his community to survive the injury (Israelowich, 2015). This shows some emphasis in the culture on care for others, and that Individual 7 was valued by his community enough to be cared for during the healing of his injury (Israelowich, 2015).

The fusion abnormality of Individual 7's sacrum also gives room for hypotheses on how childhood may have been experienced for individuals in this society. Though fusion abnormality can happen randomly (Park et al., 2017), the presence of extreme enthesophyte growth may be an indication that the fusion abnormality seen could be due to heavy labor beginning in childhood (Menz et al., 2019; Park et al., 2017; Thomas et al., 1976).

This segue into childhood during this period is particularly interesting with the presence of a subadult burial in this study. Individual 2 was estimated to be between the ages of 2 to 3.5 years old. At this time children were normally buried separately from adults, if buried at all (Boehmer, 2023). In addition to being buried in the same area as adults, Individual 2 was also buried in a very similar manner to the other individuals excavated from this site (Boehmer, 2023). While it cannot be determined exactly how children were viewed in this society, Individual 2 was buried as if the individual had achieved a social acceptance similar to the status of an adult. The fusion failure of the sacrum of Individual 7, indicating possible heavy labor at an early age, could be evidence of children being viewed as active community members much earlier than they are today (Boehmer, 2023; Lewis, 2009; Schrenk, 2017).

Conclusion

Excavation and data collection at the site of Palikè di Mineo in Eastern Sicily took place over a six-week period in the summer of 2023. The combined data from Individuals 2, 6, and 7 suggests the individuals living in Palikè di Mineo during this time lived strenuous lives beginning early in childhood. Enthesophyte growth present on Individuals 6 and 7 demonstrates excessive muscle usage over a long period of time, and the widespread presence of these enthesophytes shows a variety of possible activities (e.g., agriculture, digging,

building, carrying, lifting). The burial style of Individual 2 aligning with an adult burial style suggests an early social age of acceptance, and it is possible that this social age aligned with onset of early labor.

The findings from this project contribute data to a greater discussion of ancient life in Palikè di Mineo during the Imperial Period of the Roman Empire, as well as a deeper understanding of the history of the long life of this area. With a lack of previous in-depth skeletal analysis in the area, these findings provide a new dimension for understanding the history of the area its human inhabitants. A larger sample size of skeletal remains in this area with a wider variety of individuals is needed to draw any firm conclusions about what life may have been like for these individuals. Data including individuals of other ages and sexes would be necessary to gain an understanding of both social age in this society and the roles that individuals may have played. By adding a human skeletal component to past research, we hope to inspire future inclusion of skeletal analysis within the Palikè di Mineo archaeological area.

Acknowledgements

This project was supported by the Florida Atlantic University Summer Undergraduate Research Fellowship. We are grateful to Dr. Meredith Ellis for overseeing this project, to Dr. Laura Maniscalco and Dr. Brian McConnell for granting us access to the site and supporting our research efforts, and to our fellow field school students for their contributions throughout the field season.

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Fast Fashion: Discovering American Consumers' Opinions

College of Business, Florida Atlantic University
Ava Vignola, Mr. Eric Levy (Faculty Advisor)

Abstract

The effects of fast fashion range from human rights violations to environmental devastation. This study uncovers the views of Americans on fast fashion. It was expected to find that many Americans prioritize price when shopping, due to the growth of these companies. The study had 193 people complete a questionnaire on Amazon Turk that contained 16 questions. It found that most Americans consider the price to be more important than the ethics when shopping, with there being a statistically significant difference ($p < .001$) in that older consumers are more likely to prioritize price. It also found that males are more likely to disagree than females with the statement that fast fashion is unethical ($p < .001$). This data can be used to create marketing strategies to encourage American consumers to purchase from sustainable companies.

Introduction

Fast fashion is defined as when companies produce products inspired by luxury clothing brands for a cheap price by cutting development costs to meet increasing demand (Joy et al., 2012). Most often, these cuts in cost accompany negative environmental effects and the inhumane treatment of workers (Bick et al., 2018). Thus, the modern consumer is presented with the decision between purchasing items at a low cost or paying a higher price for more sustainable options (Pasqualicchio, 2021). Ethical clothing is defined as clothing that considers the various consequences of production and sales on the people employed as well as the environment (Mintel, 2009). A previous study found that more people would consider purchasing from more ethical fashion brands if their prices were lower (Joergens, 2006). Despite the ethical questions raised by fast fashion, sales continue to grow at a staggering rate (McNeill & Moore, 2015; Bhardwaj & Fairhurst, 2010), and it is undetermined whether this is because Americans are unaware of the effects of fast

fashion or because they simply choose to prioritize the low price of the items over items being ethically made for a higher price.

What is clear is that fast fashion has grown in tandem with consumer culture in the United States, showing rapid growth since the 1990s with the rise of trends through magazines and runway models, and, eventually, social media (Bhardwaj & Fairhurst, 2010). Social media promotes fast fashion, not only by allowing companies to promote their products to willing customers through advertisements and sponsored posts, but also by trend cycles (Diantari, 2021). Social media encourages people to buy more products to constantly stay in-style—a perfect breeding ground for the promotion of fast fashion (Michaela & Lavie orna, 2015). Stores like H&M, for example, have done particularly well in marketing their products on social media (Bonilla, Arriaga, & Andreu, 2019), with 38.3 million Instagram followers (Instagram, 2024), similar to Shein, Temu, and Zara, all of which have emerged as some of the leaders in the fashion industry while all being fast fashion companies (Perri, 2023). Shein, one of the biggest fast fashion retailers, was valued by the *Wall Street Journal* at $100 billion in 2022, just 14 years after the company's founding (Gottfried & Scott, 2022). According to the Ellen McArthur Foundation, clothing production has doubled in the past 15 years (Ellen Macarthur Foundation 2017). The demand for clothes is growing, and fast fashion is the industry's response to this ever-growing demand (Bhardwaj & Fairhurst, 2010). Currently, it is unclear whether Americans consider the effects of fast fashion when purchasing products.

Some of the downsides of fast fashion include serious environmental impacts and unsafe, inhumane working conditions of laborers (Bick et al., 2018). During production, workers are not only facing health hazards from dangerous chemicals, but fast fashion also leads to billions of pounds of waste being produced each year (Bick et al., 2018), directly impacting the environment. From dangerous chemicals being used in production, to clothing dye being found in nearby water supplies from production centers, to textile waste filling up landfills, almost every part of fast fashion includes cutting costs through unsafe and dangerous means in order to market their products at such low prices (Bick et al., 2018). Furthermore, occupational hazards threaten workers, creating unsafe working conditions (Bick et al., 2018). Workers for these companies face life-threatening medical side effects from working in perilous environments, as well as "respiratory hazards due to poor ventilation…and musculoskeletal hazards from repetitive

motion tasks" (Bick et al., 2018). Research on these topics needs to be continued so consumers are educated on which brands they choose to support and the potential repercussions of that choice.

Even though fast fashion brands are growing, environmental consciousness is growing in consumers as well (Neumann & Martinez, 2020). Consumers' attitudes towards brands can be altered by whether the brand is seen as sustainable and can affect how much the consumers trust the brands (Neumann & Martinez, 2020). Because people care about the environmental sustainability of brands, and this can affect their purchasing decisions (Neumann & Martinez, 2020), it is astounding how the rise of fast fashion continues to grow despite the environmental impact. Our research aims to determine whether it is because consumers are not aware of the environmental impact and repercussions of purchasing from certain brands, or whether they simply do not think about these issues when shopping.

Since fast fashion is so popular in the United States, consumers must either be unaware of the effects of fast fashion, or simply choose to prioritize the price instead of considering the ethics of purchasing products from certain brands. This data can be used to create programs to spread awareness of the adverse effects of fast fashion to change the way consumers purchase products.

Methodology

For the conduction of this survey, we utilized Amazon Mechanical Turk. This service allows users to incentivize other users to take their survey using small payments. The survey used was a 16-question survey with various questions about the topic of fast fashion, as well as demographic questions. This was done to make the survey data easy to categorize and for us to be able to investigate the differences between various demographics in their responses. We expected to receive 200 responses and ended up with seven incomplete responses and 193 usable responses. We then analyzed the data using the computer software SPSS, or the Statistical Package for the Social Sciences. This allowed us to weigh the importance of certain responses, to ensure that the data reflects the United States population based on demographics like race, age, sex, and political party. We used the United States Census for demographic information (U.S. Census Bureau, 2020).

Figure 1
Specific Survey Questions

Specific Questions
How often do you purchase from fast fashion brands?
When shopping, how often do you consider the environmental impact from the production of the goods you purchased?
When shopping, how often do you consider how the workers from the company you purchased from are treated?
To what extent do you agree or disagree with the following statement: Fast fashion is unethical.
How often do you do research on the ethics of the companies that produce the fast fashion brands you purchase from? (Ex. Environmental impact, Unsafe/Inhumane working Conditions, Child labor, Slave labor, & more)
To what extent do you agree or disagree with the following statement: Certain products are too cheap to worry about the ethics behind buying from fast fashion brands.
When shopping, which do you consider to be more important: Price or Ethics?
To what extent do you agree or disagree with the following statement: Fast fashion brands offer an unrivaled convenience and cheapness that makes them appealing.
To what extent do you agree or disagree with the following statement: If other products were cheaper, I wouldn't buy from fast fashion brands.
To what extent do you agree or disagree with the following statement: The United States Government should intervene against fast fashion brands.

Notes. The ten fast fashion survey questions distributed on Amazon Mechanical Turk.

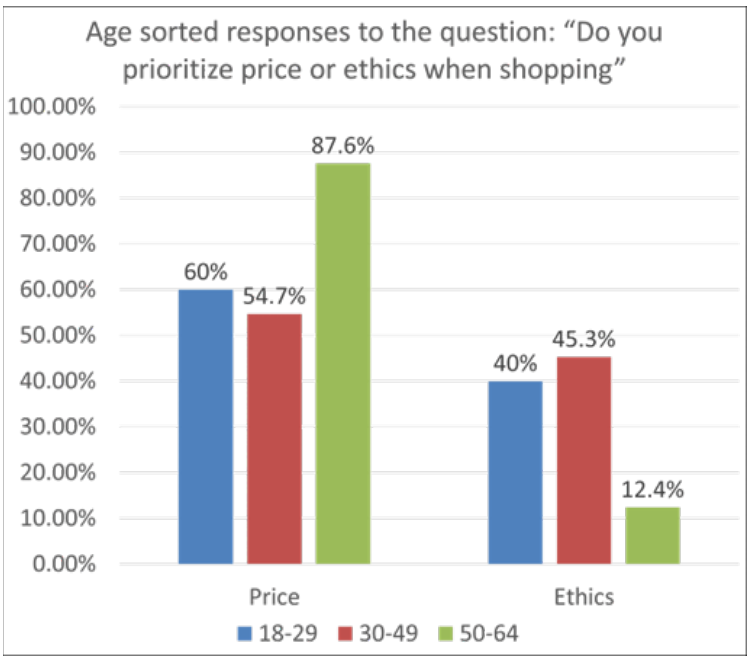
Results

After running multiple chi square tests on SPSS, we were able to find the ways in which different demographics responded to the survey questions. The results of this survey found that most Americans prioritize price over ethics when shopping, with 60% of 18- to 29-year-olds, 54.7% of 30- to 49-year-olds, and 87.6% percent of 50- to 64-year-olds prioritizing price. Participants in the age range of 50 to 64 years old were significantly more likely to prioritize price over ethics when compared to their younger counterparts ($p < .001$).

The results of this survey also found that most Americans at least somewhat agree with the statement that fast fashion is unethical, with 68% of women and 52% of men strongly or somewhat agreeing. It also found a statistically significant difference ($p < .001$) in how different genders view whether fast fashion is unethical. Specifically, males

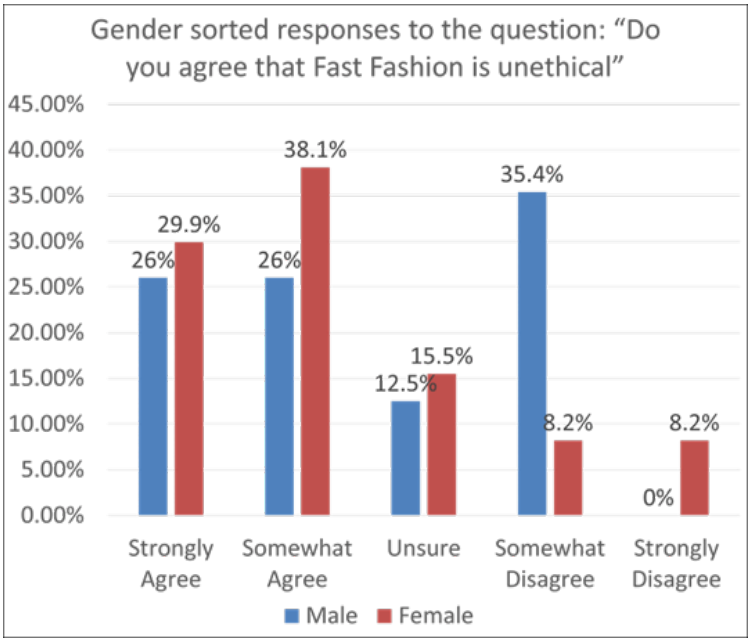
are much more likely to somewhat disagree with the statement that fast fashion is unethical, with 35.4% of males somewhat disagreeing in comparison only 8.2% of females disagreeing. Females are much more likely to agree than males with the statement that fast fashion is unethical, with 29.9% strongly agreeing with this statement, compared to 26% of males; and 38.1% of females somewhat agreeing with this statement, compared to 26% of males.

Figure 2
Results from the Survey when the Question "Do you prioritize price or ethics when shopping?" was Asked



Notes. A statistically significant difference was found in which 50- to 64-year-olds are much more likely to prioritize price than their younger counterparts ($X^2[2, N = 193] = 22.58$; $p < .001$). Of the 50- to 64-year-olds surveyed, 87.6% prioritize price over ethics when shopping.

Figure 3
Results from the Survey when the Question "Do you agree that Fast Fashion is unethical" was Asked



Notes. A statistically significant difference was found in which males are much more likely to disagree with the statement that fast fashion is unethical than females ($X^2[4, N = 193] = 27.04$; $p < .001$). Of males surveyed, 35.4% somewhat disagree that fast fashion is unethical.

Discussion

The overall results from this study revealed valuable insights into how Americans perceive fast fashion and how their opinions affect their purchasing habits. The study found that most Americans prioritize price over ethics when shopping. This means that more sustainable clothing companies may be pressured to compete with fast fashion brands by lowering their prices. This effort to lower prices can lead to the adaptation of more harmful practices like pollution and mistreatment of workers. However, sustainable brands can continue to market less frequent but higher-quality purchases—for example, Patagonia (Freudenreich, 2020; Gossen, 2021). Furthermore, Americans aged 50 and older were much more likely to prioritize price, and currently make up around a third of the United States population. It is important to understand how this demographic makes their purchasing decisions and to ensure they are educated about the effects of their purchases.

The survey also found a difference in the way males and females view fast fashion. Specifically, it was found that males are much more likely to disagree with the statement that fast fashion is unethical in comparison to females. This points to room for more public awareness of the negative impacts of fast fashion. Females also shop online much more than men (Pradhana, 2019), so it is important that advocates are able to continue educating females on the effects of their purchases.

This research is a small step in understanding the complexities of the fast fashion industry and its role in American consumerism. Further research can be conducted on the effects of the fast fashion industry, including the various external benefits and causes, to determine the ramifications of the fashion industry evolving in this way. Research can also be conducted on how to encourage more sustainable consumption habits, and to find ways to lower the impact that fast fashion has on the environment and human rights. This research shows that more awareness can be spread on the effects of one's purchases, and research can be done on how to best effectively communicate this information. Finally, future research can be conducted to determine the best ways to curb the spread of unethical industry practices, in every shopping category beyond fashion.

As technology continues to advance and make every aspect of people's lives easier, the fashion industry acts no differently. It will continue to evolve and aim to become more profitable through increased convenience for consumers. Despite the many benefits this growth in convenience provides to consumers, it is important to consider the side effects and repercussions that many people around the world will face due to increased consumer spending on fast fashion. It is imperative that consumers understand the power they hold in their purchase decisions, and that they become educated in what sort of production practices they are supporting.

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Collaboration against Communism: An Overview of Ukrainian Resistance During the Second World War, UPA

Dorothy F. Schmidt College of Arts and Letters,
Florida Atlantic University
Stefan Tsapenko, Dr. Doug McGetchin (Faculty Advisor)

Abstract

During and following World War II, the Soviet, and later, Russian Federation enforced the narrative that Ukrainians fighting in the war against the Soviets were Nazis. The author utilized his proficiency in the Ukrainian and Russian languages, conducted research regarding Ukrainian participation in WWII, and concluded that this narrative is largely anti-Ukrainian propaganda. The author's research focused on the Ukrainian Insurgent Army (UPA) which was formed in 1942 by the Organization of Ukrainian Nationalists (OUN) as a militant wing. The OUN was the center of Ukrainian nationalism starting in 1929, and strove for an independent Ukrainian state. Russian regimes repressed Ukrainian nationalism, which caused many nationalists to turn to the Reich, who promised Ukrainian statehood. After Ukrainians proclaimed independence in 1941, the Nazis jailed leaders and massacred civilians. This research demonstrates that the UPA fought the Nazis between 1943-1945 and the Soviets between 1939 to 1960, for an independent Ukrainian state during World War II, not for Nazism.

Introduction

With Ukrainian land integrated into the Soviet Union, the German Reich seemed to be a sensible ally to collaborate with in the hope of regaining national sovereignty. This was what many occupied peoples thought during the Second World War, leading to a large number of nationalist army groups to be formed, who collaborated with the German Reich against the Soviet Union. Not all people chose this route; however this was a way that a person could gain weapons and training which put them in a much better position to defend their land. Having gained weapons, many collaborators deserted the Nazis and instead joined their respective nationalist partisan movements,

being now better equipped than ever. The other choices were to join the Soviet Red Army or to join a national resistance force fighting against both Soviets and Germans. These choices existed for many nations during the Second World War, including for Ukrainians who were extremely disillusioned with anything connected to Russia, communism, and the idea of "brotherly nations." This stance emerged due to long-lasting Russian attempts to cruelly and violently subdue Ukrainian nationalism. The most notable of these nationalist resistance groups was the UPA, Ukrainian Insurgent Army (Українська Повстанська Армія). The UPA was the militant wing of the OUN, Organization of Ukrainian Nationalists (Організація Українських Націоналістів), which evolved from the UNR, Ukrainian People's Republic (Українська Народна Республіка). The Ukrainian State formed the UNR after the collapse of the Russian Empire, and lasted officially for four years from 1917 to 1921, existing in exile after 1921. The OUN was formed in 1929 and served as the headquarters of Ukrainian nationalism in Ukraine. With the start of the Second World War, founders of the UPA created the militant group to defend and fight against those looking to harm Ukraine.

Before and during the first half of the war, the UPA collaborated with the German Reich, treating it as a training camp and starting point for the creation of a modern Ukrainian military record. In return, the Nazi army group promised to allow for the formation of a Ukrainian state, however the Nazi high command renounced it quickly (Central Intelligence Agency [CIA], 2007). The Nazis then cracked down on Ukrainian attempts to create a state outside of the Reichskommissariat, the puppet state established in Nazi occupied Ukraine, and sent leaders to concentration camps. Though resistance started in 1942, large-scale UPA resistance began in 1943 and continued long after the end of the Second World War, ending around 1960 (Birchak, 2016).

The Soviet, and later, Russian government put an immense effort into vilifying the organization, just as it did with all previous Ukrainian nationalist movements, through state-indoctrinated propaganda, the effects of which can still be seen today. The OUN-UPA organizations collaborated with the German Reich against the Communists, seeing this as a possible way to establish an independent Ukrainian state, also knowing, however, that they would eventually come to fight the Germans. While Ukrainian nationalist groups may have collaborated with Nazi Germany at the start of the Second World War and later fought against them, a number of these groups were determined to not

have committed war crimes, and their legacy has been manipulated by Soviet and Russian propaganda. This research was conducted by reviewing archival data, historical books, and video accounts, with sources originating from historians around the world.

Historical Background

The choice of joining the Red Army and fighting for the Soviet Union against the German Reich was clearly available to Ukrainians during the Second World War. The thought of fighting on the side of communism was loathsome for many Ukrainian people, given the atrocities that the Soviet Union committed against Ukrainians. Ukraine suffered a mass genocide, known as the Holodomor, only several years prior to the start of the Second World War, that lasted from 1932 to 1933 (Holodomor Museum, 2023). The genocide was artificially induced in the form of mass starvation on a national scale. Four years prior to the Holodomor, the Soviet Union, under Stalin's leadership, introduced a policy known as "collectivization" which confiscated individual farm ownership and transferred it to State ownership (Holodomor Museum, 2023). The Ukrainian people resisted the policy with protests and demonstrations that spanned across the country, numbering around 4,000 demonstrations (Holodomor Museum, 2023).

The official Soviet Union policy for the Holodomor was State "grain procurement" (Holodomor Museum, 2023). According to the official Holodomor Museum (2023), "The leadership of the Soviet Union committed [the Holodomor] in order to suppress Ukrainians and ultimately eliminate Ukrainian resistance to the regime, including efforts to build an independent Ukrainian state". The Soviet Union withheld grain imports, controlled farm ownership, restricted movement for 22.4 million people through a blockade, and banned discussion and the recording of an official death count (Holodomor Museum, 2023). Millions of Ukrainians were killed as a result of the Holodomor, and those who survived held a deep-rooted disdain for the Soviet Union.

Fundamentally, when it came time to make a choice on which side to fight on, Ukrainians remembered all too well what life was like under the Russian "Big Brother." Ukrainians living during the Second World War found themselves in a similar situation to the one that Bogdan Khmelnitsky was in many years ago, stuck between two powerful nations bent on making Ukraine's borders their own. Bogdan Khmelnitsky was the Ukrainian *hetman* (leader) of the *Zaporozhian Sich* (state) and played a pivotal role in Ukrainian history in the 17th

century. Khmelnitsky chose to align himself with the Russian Tsardom against the Polish-Lithuanian Empire, given the immediate threat.

Despite his good intentions, his decision proved to be almost detrimental to the future of Ukrainian independence. Khmelnitsky made this choice in an attempt to preserve the Ukrainian state, the *Zaporozhian Sich*, and to preserve the Orthodox Christian faith, both of which were being threatened by the Polish-Lithuanian *Schlachta* (nobility). This was in vain, as the Ukrainian church was later absorbed by the Moscow Patriarchate in 1686 and the *Sich* was destroyed by Empress Catherine II in 1775 (Religion in Ukraine, Kuksa, 2010). Finally, the most recent example for Ukrainians during the Second World War was the UNR's war for Ukrainian independence, which ultimately ended after a betrayal by Poland. Symon Petliura, the Chairman of the Directorate of the UNR, had come to an agreement with Poland's Marshal Jozef Pilsudski in the Warsaw Pact (Dotsenko, 1923). The agreement restored historical borders, recognized the UNR as legitimate, and created an anti-Bolshevik Polish-Ukrainian alliance (Dotsenko, 1923). Poland, however, later signed the Peace of Riga with the Soviet Union, which marked the end of the UNR in Ukraine (Dotsenko, 1923). Alliance with either Poland or Russia was now unlikely for any members of the UNR, who made up a large portion of the OUN upon its creation. All of these factors likely influenced many Ukrainians in choosing which faction to join and support.

Organization of Ukrainian Nationalists (OUN) Prior to WWII

The history of the Ukrainian Insurgent Army, UPA, during the Second World War begins with the formation of the Organization of Ukrainian Nationalists (OUN). The OUN was formed during the interwar period, in 1929 in Vienna, with the goal of continuing the struggle for Ukrainian independence that was started earlier in the First World War. The group conducted anti-Polish and anti-Bolshevik raids throughout Western and Eastern Ukraine, both of which were occupied by Poland and the Soviet Union, respectively. The organization was led by Yevhen Konovalets, from the time of the organization's formation in 1929 until 1938. Power struggles divided the organization when he was assassinated in 1938 by Pavel Sudoplatov, a Soviet agent. Konovalets was a veteran militant Ukrainian nationalist, having previously founded and commanded a *Sich* riflemen company, a military unit of the UNR.

Following his death, a power vacuum occurred, which by 1940

had sufficiently split the group into supporters of the two most influential men in the group, Andriy Melnyk and Stepan Bandera. Melnyk was second-in-command to Konovalets and was supported by the older veterans of the OUN, while Bandera was supported by younger members (Vedeneev & Bystrukhin, 2006). There was a key disagreement regarding the direction each leader wanted to take the movement. Bandera wanted to create a "Revolutionary Leadership" unit of the OUN in Poland that would plan revolutionary movements and uprisings against the Soviet Union focused on liberating Ukraine from Soviet control (Shurkhalo, 2020). This plan was advantageous in Bandera's perspective because the Revolutionary Leadership would be located close to Ukraine. Melnyk, on the other hand, opposed Bandera's plan because he did not want to risk exposure of the movement and the possible endangerment of the lives of OUN members. Melnyk's plan was to wait until the German Reich invaded the Soviet Union, and to then strike a surprise attack upon a weakened Soviet Union.

The two leaders failed to reach an agreement, which would prove to be detrimental. Bandera decided to go ahead with his plan and create the OUN Revolutionary Leadership, but also to have Melnyk lead it, without his knowledge (Shurkhalo, 2020). This caused fighting within the OUN, and it split into the OUN-B headed by Bandera and OUN-M headed by Melnyk. According to Vedeneev and Bystrukhin (2006), intra-group fighting during the years 1940 to 1941 cost the lives of 400 Melnyk supporters and 200 Bandera supporters.

Collaboration Against Communism

According to a declassified document released by the CIA (2007):

> Realizing that the aspiration for national independence was uppermost in the minds of a majority of Ukrainians, the Third Reich promised at the beginning of World War II that the Ukrainians would be freed from Soviet domination and could found a Ukrainian state. This promise accounts for the welcome the Germans received initially when they arrived on Ukrainian territory. (CIA, 2007)

Prior to the Barbarossa invasion of the Soviet Union, Bandera had been in contact with representatives of the German Reich and was attempting to create all-Ukrainian units. Two such units which were made up of Ukrainian nationalists were Nachtigall and Roland, both

organized in 1941 (Melnyk, 2016). In 1941, the groups were reorganized into the 201st Schutzmannschaft Battalion for one year (Mirchuk, 1953). Roman Shukhevich, the "regional leader of the OUN(b) in the western peripheral Ukraine" was the "commander of the 1st company and deputy battalion commander" (History Institute of Ukraine, n.d.). After the contract expired on December 5, 1942, the battalion members refused to sign a new contract. They were disarmed and taken to Lviv by the Germans (History Institute of Ukraine, n.d.).

In 1942, a mass exodus of Ukrainians from Schutzmannschaft battalions to the UPA occurred. This, along with German losses at the Battle of Stalingrad, prompted the German Reich to form the 14th grenadier division of the Waffen SS Galychyna (Veselova et al., 2008). In 1944, the Galychyna division entered combat for the first time. During the battle of Brody, 4,000 of the 11,000 troops were killed, 1,000 were wounded, and 3,000 were captured (*Military composition and numbers of the Galicia Division 1943-45*; Veselova et al., 2008). These groups carry quite a bit of controversy, as they are believed to have participated in ethnic cleansings. There is, however, insufficient evidence to make a definite argument regarding these claims (Katchanovski, 2015). After the war, the Allies tried these groups at Nuremberg and determined that they were not guilty of war crimes. Similarly, the Canadian Commission on War Criminals tried them and determined them to be not guilty (Commission of Inquiry of War Criminals, 1986). Prior, the security force of the OUN, SB OUN (Служба Безпеки), was created in 1940 by Bandera. This unit was known for its brutal disciplinary methods, as well as its ethnic cleansing of anyone opposing Ukrainian nationalism (Vedeneev, 2002). This was especially true of Communists and Poles, as the SB OUN was engaged in a counterintelligence war against Communist and Polish units (Vedeneev, 2002).

Fighting Against the German Reich

On June 30, 1941, Bandera's OUN made the declaration of the "Act of Restoration of the Ukrainian State After 23 Years of Captivity" (State Archive of the Lviv Region, 2017). This did not sit well with the Reichskommissariat, who sent many OUN-B leaders to the political concentration camp Sachsenhausen. Melnyk was condemned to house arrest in Berlin, and was later similarly sent to Sachsenhausen in 1944 (Foreign Intelligence Service of Ukraine, 2021). According to Komaromi, "From this point on, acknowledging that the Nazis would not support the creation of an independent Ukraine, the OUN-B fought

two authoritarian regimes simultaneously: Nazi Germany and the Soviet Union, murdering anyone who stood in the way of Ukraine's independence" (Komaromi, 2022). The OUN did not, however, immediately rise up against the Germans enmasse. Instead, some members stayed and joined Ukrainian insurgents later.

From the time of the German invasion until 1943, the OUN was under the thumb of the German Reich, engaging with Soviet troops and other nationalist groups such as that of Taras Bulba-Borovets' UPA Polyska Sich group. They participated in the creation of specialized units in the armed forces of the German Reich, of which there are conflicting accounts about their cooperation with the Reich in committing atrocities. These units were secretly loyal to the OUN and were meant to desert when ordered to and enter into the UPA (Katchanovski, 2015).

According to Rossolinski-Liebe, "The OUN joined the Ukrainian [Reichskommissariat] police, in 1941, and helped the Germans murder Jews in western Ukraine," adding he had found no evidence that Bandera supported or condemned "'ethnic cleansing' or killing Jews and other minorities" (Goncharenko, 2022). Scholars are sure that there were Ukrainians who committed atrocities in the Second World War, however they are still divided on who these units belonged to and who permitted these atrocities.

While arrested in the Sachsenhausen concentration camp, OUN leaders urged Ukrainian nationalists to focus on using the German Reich in order to gain proper military training, supplies, and to spread the ideas of the OUN (Foreign Intelligence Service of Ukraine, 2021). The plan was for these "reserves" to join into the ranks of the UPA when the OUN saw that the German Reich and Soviet Union had significantly weakened themselves in their fight with each other, enough for the OUN to begin reclaiming Ukraine (*Military composition and numbers of the Galicia Division 1943–45*). The UPA was broken into sections: North, West, and South, which were further broken down into squads, each of which had a commander (Mirchuk, 1953). Prior to 1943, the OUN had only planned to revolt; however, in 1943, the UPA went on the offensive against the German Reich after the Reich had attempted to liquidate a large portion of the organization (Tys-Krokhmaliuk, 1972). The UPA was able to release a journal titled "To Arms" in eight parts, in an effort to spread the word to allies and solidify the pivotal event in history (Shtender & Potichyi, 1985).

UPA units were able to gain control in the Volyn region, seizing the Kolki village and establishing an administrative structure there for

six months (Lenartovych, 2011). Similar regions existed all over the eastern front, where partisan groups held a strong majority. The UPA structure was made up of primarily high-ranking officers who infiltrated Ukrainian groups collaborating with the German Reich, as well as veterans of the first World War and younger nationalists. The UPA leaders were decreed by the UPA Central Committee as Shukhevich being the commander-in-chief, Klyachkivskyi commanding the north, and Grabets commanding the South (Mirchuk, 1953). Following 1943, the UPA fought against the advancing Soviets and the German Reich which was attempting to crush nationalist activity (Mirchuk, 1953). During the end of the Second World War, the UPA was speculated to be at its greatest size and was able to resupply itself with the arms left by retreating Nazi soldiers (Mirchuk, 1953). The exact number is highly contested by scholars. A CIA declassified document reveals that:

> The messages they [members of the OUN and UPA] and returning German prisoners of war brought, conclusively confirmed that the OUN and the UPA were continuing to fight against the Soviets with the weapons and ammunition which the retreating German armies had left behind. (CIA, 2007)

The Second World War officially ended on May 8 or 9 (contested by Western and Eastern European scholars). However, this was only half the battle for the UPA, who continued their resistance up until 1960 (Birchak, 2016). This marked the end of the Ukrainian nationalists' chapter in the history of the Second World War, and the start of the conflict known as the Partisan war. Shukhevych now led the UPA, having been made the commander-in-chief by the central committee. The Nazis attempted to gain back the support and favor of the UPA, but their efforts proved fruitless, ending with them releasing Bandera and Stetsko in September (Mirchuk, 1953). Bandera continued his fight against communist brutality, now opting to fight politically instead of physically. Bandera worked with the Anti-Bolshevik Bloc of Nations, an organization which advocated against communist regimes, until he was assassinated by a Soviet agent in 1959 (Komaromi, 2022).

Ideology

The political ideology of the UPA was unfettered Ukrainian nationalism. This meant a complete lack of empathy for foes, as members of the UPA understood that a falter would surely mean the

end of the UPA and thus the end of a future for Ukraine in which it was independent from its neighbors (Mirchuk, 1953). The UPA was first and foremost a partisan organization, without a strong national presence. There were no arms manufacturing plants, nor supply factories which would provide sustenance for troops. UPA soldiers gathered necessary supplies, then entered the forest to become partisans. The only way to resupply was to loot enemy positions after successful battles and raids. Help from villagers and supporters was also a method of survival, however this put innocent people who were typically unable to defend themselves at risk from Nazi or Communist soldiers who found out about their actions. According to Volodymyr Kloc, an UPA veteran, "We all knew one thing, (that we need to) infiltrate the division (SS Galychyna), obtain training, obtain weapons, and escape to the woods to be partisans, to join UPA" (Faces Of Independence, 2023, 20:00–20:08). As such, key UPA activity included conducting raids on enemy positions, disturbing supply lines, destroying pathways such as bridges and roads, and setting ambush positions throughout Ukraine. This was the most that the partisan force could do, as entering into a large battle while severely outnumbered would be suicidal. Instead, knowledge of the terrain and having smaller numbers spread across the area assisted them in gaining a tactical advantage in woodland and mountain areas.

Options for Ukrainians in WWII

Joining the UPA was not the only choice for Ukrainians during the Second World War. Groups collaborating with the German Reich were a possible choice that included military training as well as access to weaponry. The UNA, Ukrainian National Army, was formed from the combination of all Ukrainian units fighting for the German Reich (Shandruk, 1959). Another choice was joining the Soviet Union's Red Army, in which training would be similarly received at the expense of one's religious convictions and personal political beliefs of Ukrainian nationalism. Both choices were flawed, with neither prioritizing Ukrainian national priorities. Forced conscription was also quite prevalent, however, after receiving training, the possibility of leaving the Reich or the Red Army and joining the UPA still stayed, a choice which many nationalists made. Another option was to fight for the Ukrainian nation created by Petliura, the UNR in exile. There was a third option: to fight for the UPA Polyska Sich division formed by Taras Bulba-Borovets, which fought against the militant wing of OUN (Bulba-Borovets, 1981). UPA Polyska Sich initially cooperated with

the Nazi Reich, however, in 1942 it began to fight against both the Soviets and Nazis. Bulba-Borovets operated in Volhynia, maintaining a strong anti-communist presence in the area. (Bulba-Borovets, 1981). Joining the UPA meant leaving one's life behind, a complete commitment to the organization, as being caught as part of it was a certain death sentence. It was one of the most difficult choices that Ukranians could make during the Second World War; however, they saw it as a selfless choice that many who valued Ukraine above all made.

Soviet and Russian Propaganda

The Soviet Union was well-known for its usage of propaganda, a tactic which the Russian Federation continued. In order to pacify the Ukrainian population, propagandists carefully combed through Ukrainian history to find desired examples which could be manipulated to fit Bolshevik rhetoric. Cossack leaders and Ukrainian poets were made into proponents of the Russian idea of "brotherly nations". This idea argues that Russia has claim to Ukrainian and Belorussian land. According to this propaganda, Khmelnytsky had the desire to reconnect Malorussia ("small Russia"; used by the Russian Empire to refer to Ukraine) to Russia, thereby restoring the ancient Rus'. In an attempt to gain control over Ukraine and ingrain a hatred for Ukrainian nationalism, the Soviet Union distorted and exaggerated historic events, and made the UPA responsible for ethnic cleansings during the Second World War. The foundation of this propaganda was that the UPA had collaborated with the German Reich, who had committed ethnic cleansings, and that certain Ukrainian units had committed atrocities during the war. This was a generalization of all Ukrainian nationalist movements, spreading distorted history in an effort to suppress Ukrainian nationalism. This logic fails to mention Soviet collaboration with the German Reich during the Molotov-Ribbentrop compact which saw both armies conducting a pincer maneuver around Poland, which Western Ukraine was part of. This was an effort by the Soviet Union to finally crush Ukrainian nationalist resistance that was based in Western Ukraine. A negative image for the UPA was ingrained in Soviet school doctrine, which left students with the impression that Ukrainian nationalism was synonymous with Nazism and fascism. A pro-Russian doctrine was a key factor in the Soviet Union, which held that Russia was superior to all of the other nations present in the Soviet Union (Faces of Independence, 2023). The undoing of this harm by modern Ukrainians is ongoing, however the propaganda can still clearly

be seen in the current war in Ukraine, especially on the easternmost side, which endured the most ethnic cleansing during the Holodomor, followed by repopulation by ethnic Russians. Certain Ukrainian groups' participation in local oppression further supported the Soviet doctrine that Ukrainian nationalists were, are, and will continue to be Nazis. Who exactly pulled the trigger during pogroms and crackdowns remains unclear, and scholars are yet to reach a consensus. Archives have been tampered with by both sides' propaganda: Soviet propaganda which attributes all massacres to Ukrainians, and Ukrainian propaganda which pushes an image of heroic resistance against all foes.

Conclusion

Ultimately, the ultra-nationalist military wing of the OUN, UPA, was anything but perfect. The group collaborated with the Nazis against the Communists, and participated in the creation of specialty national units. These units, which were secretly loyal to the OUN, deserted and joined the UPA, bringing military training and arms with them. Several Ukrainian Nationalist groups were cleared of war crimes at the Nuremberg tribunals and by the Canadian Commission on War Criminals. If the Ukrainian nationalist struggle demonstrates anything, it is as UPA veteran Humenyuk says "the fact goes that there is a nationality that wanted to live freely, in its independent nation like other European nations did" (OUN Archive, 2023, 27:05–27:15). Soviet and Russian propagandists manipulated historical truths to paint Ukrainian nationalists in bad light. The topic lacks scholarly research, which must be done to resolve misconceptions.

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The Current and Potential Applications of CRISPR Technology

Charles E. Schmidt College of Science, Florida Atlantic University
Abhishikta Srigiriraju, Dr. David Binninger (Faculty Advisor)

Abstract

CRISPR-Cas9 is a genetic engineering technology that allows for precise editing of DNA sequences. It utilizes a guide RNA that targets the endonuclease Cas9 to make a double-strand DNA break at a specific site. This technology has proven useful in both clinical and biomedical settings. This review discusses some prominent applications including treatment of Transthyretin Amyloidosis, cell cloning for pharmaceuticals, refractory cancer, sickle cell disease and β-Thalassemia. Furthermore, two methods that utilize CRISPR-Cas9 to prevent hereditary retinoblastoma are proposed.

Introduction

Many human diseases and disorders have their basis in genetics and are inherited, making them treatable but not curable. An example of this is Huntington's Disease, a genetic disorder with symptoms including progressive loss of motor control, changes in mood, and decreased ability to concentrate due to degradation of brain neurons (U.S. Department of Human and Health Services). However, CRISPR-Cas9, a bacterial defense system against viruses (Loureiro & da Silva, 2019), has been exploited to create a revolutionary method to make precise gene-edits, providing the potential to cure genetic disorders like Huntington's Disease and more. CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats" (YourGenome, 2022). CRISPR-Cas9 works by using the Cas9 enzyme, which is directed by a guide RNA (gRNA) to the complementary target DNA sequence; there, the enzyme creates a double-strand break. Specifically, the endonuclease activity associated with Cas9 complex that makes the double-strand DNA cut are HNH and RuvC (Hillary & Caesar, 2022). This break provides an opportunity to make changes to the sequence and then the cell's own DNA repair mechanisms to ligate the DNA back together (YourGenome, 2022). The cell has several options for repairing the damaged DNA, and the chosen

method will depend on the phase of the cell cycle, how condensed the chromatin is at the location of the gene, and the sequence of the gene itself (Xue & Green, 2021).

Two main types of changes, or mutations, can be made: "knock-out" and "knock-in", with knock-in being more complicated (Rasul et al., 2022). Knock-out mutations consist of removing or inactivating a gene by either excising the entire gene, creating a sequence that results in termination of the product before it is finished, or creating a sequence that leads to a nonfunctional product (Ford, 2022). Knock-in mutations consist of adding a novel sequence to a specific spot in the genome; the accuracy required is what makes this more complicated (Koch Institute for Integrative Cancer Research). Despite the variety of methods used to repair DNA (Xue & Green, 2021), double-strand break repair is broadly done in two ways: Non-homologous end joining (NHEJ), which results in the two strands being directly attached to each other by their ends; which is prone to error; and Homology-Directed Repair (HDR), when a complementary sequence is used as a template to attach two strands, which is more accurate (Patrick et al., 2023). Since a knock-in mutation requires more precision, it is usually carried out through HDR with the help of an additional template sequence, which leads to an accurate edit (Stutzner-Gibson et al., n.d). However, knock-out mutations do not have to be so precise, so NHEJ is typically used instead (Stutzner-Gibson et al., n.d.). Overall, the CRISPR-Cas9 system has multiple mechanisms that can be used, and factors that can be adjusted, to create mutations at a specific site in the genome, lending itself the ability to be applied in a myriad of situations.

Methods

In evaluating the current stage of development and application of CRISPR technology, four recent studies that utilized the technology for clinical or biomedical purposes were analyzed. The analysis of each study was divided into a discussion of the purpose of the research, the methodology employed, the results, and their implications.

Review

First, in a study titled *CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis* (Gillmore et al., 2021), a novel gene therapy that utilized CRISPR-Cas9 to make a knock-out mutation was given to six patients to treat their transthyretin amyloidosis, which is due to misfolded transthyretin protein building up in the body (Gillmore et

al., 2021); the liver produces most of the body's transthyretin (Cedars-Sinai). The therapy is named NTLA-2001 and its goal is to reduce levels of transthyretin (TTR) protein in the blood to alleviate symptoms of transthyretin amyloidosis. It uses a lipid nanoparticle (LNP) that contains the gRNA (referred to as sgRNA), which contains a sequence that is complementary to the TTR gene, and a Cas9 mRNA. NTLA-2001 is delivered through systemic intravenous therapy and, following opsonization by apolipoprotein E, the LNP delivers its contents to the hepatocytes. Cas9 is then translated from its mRNA and the Cas9-gRNA complex is formed. The complex creates a double-strand break in the TTR gene, which is then repaired using the error-prone method of NHEJ, leading to a knock-out mutation that produces a nonfunctional TTR protein. Two groups were tested over a 28-day period: one receiving a lower dose, 0.1 mg/kg of NTLA-2001 and the other receiving a higher dose of 0.3 mg/kg. The results showed that the 0.3 mg/kg group demonstrated a greater lowering of average TTR levels in their blood (87%) compared to the 0.1 mg/kg group, which had an average lowering of 52%, suggesting that the treatment is dose-dependent and possibly an effective treatment for transthyretin amyloidosis.

In another study titled *CRISPR-engineered T cells in Patients with Refractory Cancer* (Stadtmauer et al., 2020), CRISPR-Cas9 was used to make a series of knock-out mutations to improve the ability of a genetically engineered T cell to attack cancer cells. Cancer that becomes resistant to treatment is considered a "refractory" cancer (Temple University Health System, 2020). NY-ESO-1 is an antigen commonly present on the cells of certain cancers, and research has shown that genetically edited T cell receptors (TCRs) that target this antigen have the potential to treat these cancers, but that interference with patients' native TCR chains has proven to be an obstacle (Stadtmauer et al. 2020). Previous research suggests that the programmed cell death protein 1 (PD-1) in T cells leads to decreased efficiency in targeting cancer cells. Therefore, the goal of this study was to produce three knock-out mutations in genetically-edited T-cells that contained the NY-ESO-1 T cell receptor (TCR): one each for the genes that code for the α and β chains, referred to as *TRAC* and *TRBC* respectively, of the original TCR present in unedited T cells; and another for *PDCD1*, the gene coding for PD-1. This was done by collecting T cells from patients and using electroporation to deliver a CRISPR-Cas9 system along with three gRNAs, complementary to the sequences for either *TRAC*, *TRBC*, or *PDCD1*. In addition, a viral vector that contained a

plasmid with the gene coding for the NY-ESO-1- TCR was delivered to the original T cells. The fully edited T cells were referred to as NYCE and were infused into three patients who had refractive cancer. The ability of NYCE to stay present in the blood was analyzed in all three patients and the results showed that the levels of NYCE in the blood of all three patients were stable for 3 to 9 months. Furthermore, the patients' new T cell line persisted longer in the blood compared to other studies which also edited the NY-ESO-1 TCR. (Stadtmauer et al., 2020). This suggests that the knock-out of *TRAC*, *TRBC*, and *PDCD1* allowed the NYCE cells to maintain a presence in the blood compared to NY-ESO-1 TCR–engineered T cells that did not receive these mutations. Additionally, during treatment, the cancers of two of the patients stopped intensifying and, although the cancer of the third patient continued to get worse in other areas, his abdominal tumor decreased to about half its original size. Overall, these clinical results suggest that the treatment did have a beneficial impact.

In the study titled *CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia* (Frangoul et al., 2021), a knock-out mutation of the gene *BCL11A* in blood cells was produced to increase fetal hemoglobin levels in a patient who had β-Thalassemia (TDT), a genetic condition that results in lower levels of the protein beta globin (Schultz, 2022). Another patient in the study had Sickle Cell Disease (SCD), a condition characterized by blockages and a lower quantity of erythrocytes in circulation because erythrocytes bend and adopt the shape of a sickle (U.S. Department of Health & Human Services, 2023). Success with patients who have TDT or SCD has been linked to higher quantities of fetal hemoglobin in the blood (Frangoul et al., 2021). The protein BCL11A is a key part of the developmental switch from fetal to adult hemoglobin, and TDT and SCD patients have normal fetal, but mutant adult beta hemoglobin. Using electroporation of stem cells and progenitor cells that develop into blood cells, the researchers introduced a knock-out mutation of *BCL11A* through cleavage of its enhancer sequence. The gRNA (referred to as sgRNA) contained a sequence complementary to the *BCL11A* enhancer, allowing for Cas9 to bind and produce a double-stranded break in the sequence. Although the method of DNA repair used to repair the break was not directly discussed, the attached protocol states that NHEJ was used due to the process being error-prone, favoring the creation of a nonfunctional enhancer sequence. The results showed that during preclinical studies, the blood cells that developed from

the edited stem cells and progenitor cells exhibited greater levels of fetal hemoglobin (29.0±10.8%) compared to control blood cells (10.5±5.2%), suggesting that effective knock-out of *BCL11A* was able to occur due to the interruption of its enhancer sequence. Next, the two patients had their stem cells and progenitor cells collected for genetic editing. The patients received myeloablation of their bone marrow using the chemotherapeutic agent Busulfan, causing destruction of the bone marrow cells (Dana-Farber Cancer Institute) that were not collected and allowing for complete replacement with the edited cells. Fortunately, treatment of both patients was successful; they had higher levels of fetal hemoglobin in the blood and the associated edited gene present in both the bone marrow and blood cells long term. (Frangoul et al., 2021). This treatment, now named "Casgevy", has just recently received approval from the U.S. Food and Drug Administration to be used in patients with sickle cell disease (U.S. Food and Drug Administration, 2023).

Finally, in the study titled *Feasible Development of Stable HEK293 Clones by CRISPR/Cas9-mediated Site-specific Integration for Biopharmaceuticals Production* (Yang et al., 2019), the potential to mass produce certain proteins, by inducing knock-in mutations in standardized cell lines with CRISPR-Cas9, was analyzed. To engineer a cell to express a gene of interest (GOI), the GOI could only be introduced into the genome using homologous recombination. This process would occur multiple times until cell lines that produced a high level of the desired protein were isolated. Nevertheless, this process also requires significant time and effort, making it inefficient. Although multiple nuclease-based technologies are currently available for genetic engineering that are more effective than this process, CRISPR-Cas9 has proven to be the most efficient out of these technologies and is the one used the study (Yang et al., 2019). The cell line of focus in the study was HEK293, a cell line widely used in the biopharmaceutical industry to produce therapeutic proteins and was developed from human embryonic kidney cells (Tan et al., 2021). Furthermore, for gene introduction, the "safe harbor", or region of DNA that is free from position effects, AAVS1 was targeted. To confirm that the AAVS1 locus could provide effective gene expression, the researchers recombined HEK293 cells with *EGFP*, which codes for a green fluorescent protein, at the AAVS1 locus. Interestingly, the researchers used two gRNAs to guide Cas9, referred to as sgRNA A1 and sgRNA A2, which each targeted different sequences adjacent to exon 1 of AAVS1. Along with the CRISPR-Cas9 system, a donor

fragment encoding EGFP was provided to repair the double-strand break with HDR. Using fluorescence microscopy, EGFP expression confirmed that *EGFP* successfully recombined into the genome. The researchers then used the AAVS1 locus to integrate a larger gene that codes for the protein CTLA4Ig, which has shown potential in combating autoimmune conditions (Deppong, 2013). In this case, only one gRNA was used that was complementary to a sequence in the intron located between exons 1 and 2 of AAVS1, and HDR was used to seal the double-strand break. In the control, only the donor plasmid without a gRNA was introduced into the cell. The complete sequence used to insert the CLTA4Ig gene into the plasmid was CMV-CLTA4Ig-IRES-PAC-polyA, with the CMV promoter at the 5' end and the polyA tail at the 3' end of the sequence. Next, PCR and subsequent gel electrophoresis was performed on both the control and gRNA-containing cell lines to determine if the 5' and 3' junctions of CMV-CLTA4Ig-IRES-PAC-polyA were present in the genome. If they were, it would mean the CLTA4Ig gene was properly integrated into the genome. The electrophoresis results showed that for the cell line that received only the donor template but no gRNA, the 5' and 3' junctions were not present. In contrast, the cell line that received both the donor template and gRNA, the 5' and 3' junctions were present and properly integrated into the CTLA4Ig gene. Furthermore, to confirm that the CTLA4Ig gene was properly expressed in the edited cell line, proteins were extracted and a Western blot performed. This technique uses gel electrophoresis to check for the presence of a particular protein by using its molecular weight and identification with a protein-specific antibody (Yang & Mahmood, 2012). The results showed that the HEK293 cells that received the gRNA successfully expressed the CTLA4Ig gene, whereas the control did not express CTLA4Ig gene. Furthermore, to analyze the productivity of the clones in producing CTLA4Ig, an ELISA assay was done on twelve clones. ELISA stands for "enzyme-linked immunosorbent assay" and is used to quantify specific proteins. ELISA analysis showed that 83.3% of the clones efficiently made the CTLA4Ig protein. That the data showed that CRISPR-Cas9 was able to be used to create a knock-in mutation of the CTLA4Ig gene precisely into the AAVS1 locus and that the gene product was consistently expressed in the engineered cells suggests that CRISPR-Cas9 can be successfully applied to other genes to mass-produce therapeutic proteins.

Discussion

Despite the many strides made in the development and uses of CRISPR-Cas9, sufficient research has not been done on the technology's potential to prevent hereditary retinoblastoma. The disease is a type of cancer that begins developing in the retina of the eye (American Cancer Society, 2018). The cancer is caused by a loss of function mutation in the RB1 tumor-suppressor gene. A person who is heterozygous for *RB1* has one wild-type allele that codes for a functional tumor-suppressor gene and one mutant allele (Correa & Berry, 2016). Loss of the wild-type allele through a somatic cell mutation leads to the recessive phenotype, which is a loss of the RB1 tumor suppressor protein. Heterozygotes are predisposed to retinoblastoma through this process of "loss of heterozygosity." CRISPR-Cas9 could be used to prevent this disease through a replacement of the mutant allele with a knock-in mutation of the wild-type allele, with the double-strand break being precisely repaired with HDR. However, before providing the treatment, the first step is to identify who has the disease. The ideal method for this is population-wide genome sequencing, which would allow for easy detection of mutant RB alleles in people who may not have developed the disease yet. Once carriers have been found, the CRISPR-Cas9 system, along with a gRNA complementary to the mutant allele, could be administered in an LNP such as that used in Gillmore et al., 2021. However, systemic intravenous administration may prove difficult because a method to target the eye would need to be found, like how the opsonization by apolipoprotein E in Gillmore et al., 2021 made the LNP specific to the liver. Instead of intravenous therapy, the most feasible local administration is likely an intravitreal injection: a direct injection into the vitreous cavity of the eye that can be used to treat the retina (Lusby, 2022). Another potential method could involve sequencing of a fetal genome early in development, which would allow for early detection of a mutant RB allele. Microinjection of CRISPR-Cas9 into stem cells would be used and this would provide the opportunity for widespread fetal genetic modification before cell differentiation, leading to a homozygous wild-type genotype in each cell post-differentiation.

Altogether, CRISPR-Cas9 technology provides the world with seemingly limitless potential to prevent, cure, or treat a wide variety of human ailments, directly or indirectly. Examples of direct methods include the intravenous administration of CRISPR-Cas9-containing LNPs targeting the liver of patients with transthyretin amyloidosis (Gillmore et al. 2021), the replacement of stem cells and progenitor

cells found in the bone marrow with genetically modified versions to treat SCD and TDT (Frangoul et al., 2021), and the structural optimization of genetically modified T cells to improve the targeting of cancer cells by a patient's immune system (Stadtmauer et al., 2020). Conversely, an example of an indirect method is the creation of standardized cell lines to mass produce therapeutic proteins (Yang et al., 2019). Despite all these applications, CRISPR-Cas9 essentially functions the same way in each: Cas9 is guided to a target sequence by a gRNA to make a double-strand break, which is then repaired using HDR or NHEJ to introduce a knock-in or knock-out mutation respectively. In addition, a potential unexplored application of CRISPR-Cas9 is the prevention of hereditary retinoblastoma by either LNP administration or fetal cell microinjection. Although this would most likely require population-wide and fetal genetic sequencing, the incidence of inherited disorders like hereditary retinoblastoma could be greatly reduced with CRISPR-Cas9. In conclusion, even more effort should be directed towards both improving and discovering additional uses of CRISPR-Cas9 technology to lead humanity to a future of improved health and prosperity.

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AUTHOR BIOGRAPHIES

Alexander Georgiev

Alexander Georgiev is a senior and alumnus of Florida Atlantic University High School who works as an undergraduate researcher at the Business and Economics Polling Initiative (BEPI) under the mentorship of Dr. Monica Escaleras and Mr. Eric Levy. Alexander has been interested in Business and Finance for several years and is currently pursuing a Bachelor's in Finance at FAU. He presented related research, titled "Intelligence on Investing" at the 2024 Florida Undergraduate Research Conference and 13th annual FAU Undergraduate Research Symposium.

Annalisa Tran

Annalisa Tran has a Bachelor of Arts in English and aspires to attend medical school and pursue neonatology or pediatrics. She presented her research on the relationship between self-reported maternal anxiety ratings and observer-rated maternal anxiety scores during mother-infant interactions at FAU's Undergraduate Research Symposium in April 2024. She has worked in the Infant Cognition Lab for a year and a half as a behavioral coder and eye-tracking technician and intends to continue her work in the lab until she is accepted into medical school.

Benjamin Crawford

Ben Crawford is a Bachelor of Arts in Anthropology graduate of the Wilkes Honors College with an interest in archaeology, material culture, and the peoples of the Andes. In 2022, he received the opportunity to excavate a late Manteno structure in Ecuador for an archaeological field school. He is currently working around the Southeastern United States as an archaeologist with the cultural resource management firm SEARCH Inc.

Camila Rimoldi Ibañez

Camila Rimoldi Ibañez is a graduate of the FAU Wilkes Honors College with a major in Marine Biology and minor in Environmental Studies. Being interested in the marine world, Camila gets involved in everything she can to help protect the oceans. She has conducted her own bachelor's thesis focusing on corals, serves on Earth Echo's Youth Leadership Council, is a marine science educator at Loggerhead Marinelife Center, has led several community projects within her campus (such as candy wrapper recycling, mangrove replanting, and beach cleanups), and has attended several conferences promoting the 30x30 United Nations goals for the ocean. She was awarded a Summer Undergraduate Research Fellowship in 2023 and Undergraduate Researcher of the Year in Spring 2024.

Emma Bermudez & Jacqueline Hammack

Emma Bermudez is an alumna of Florida Atlantic University with her Bachelor of Science in Biological Sciences. She was part of the Infant Cognition Lab as an undergraduate research assistant starting in Spring 2023. Her research in the lab explores infant-toy interactions in naturalistic free play settings. She plans to pursue a career in research and medicine.

Jacqueline Hammack, M.A., is a graduate student in the Infant Cognition Lab at Florida Atlantic University. Her research interests include infants' perception and comprehension of mechanical and social entities, the identification of cortical networks implicit in social processing, as well as infant-mother gesture use and movement coordination during free play.

Benjamin Cohen

Benjamin Cohen is a recent graduate of Florida Atlantic University. As an undergraduate, he participated in the Leon Charney Diplomacy Program, served as the Chief Justice of Florida Atlantic University Student Government, and was inducted into the Phi Alpha Theta and Pi Sigma Alpha honor societies. He has received academic awards from the Departments of History and Political Science and the Dr. Eric H. Shaw Undergraduate Research Symposium Excellence Award. Benjamin completed his research on the 1858 Government of India Act as an undergraduate in the Department of History's 4+1 B.A./M.A. program. He is currently pursuing his master's degree in History at Florida Atlantic University. His graduate thesis explores Andrew Johnson's 1868 impeachment trial. In addition to his academic work, he is a graduate teaching assistant and the Student Government Chief Financial Officer. After completing his graduate degree, Benjamin plans on attending law school. The work published in this manuscript was collected during his time as an undergraduate at Florida Atlantic University.

Claire Sanford

Claire Sanford is a Florida Atlantic University alumna who graduated in 2024 with her bachelor's in Behavioral Neuroscience. During college, Claire worked as an undergraduate research assistant in the WAVES Emotion Lab under the direct supervision of Samantha Gott and Dr. Nancy Aaron Jones. Claire has been interested in developmental psychology for several years and has been researching this topic as part of the WAVES Emotion Lab. She presented related research, titled "Emotional Development in Infants 6 Through 10 Months Old", as a poster presentation at the 22nd annual Symposium for Scholarly and Creative Research at the Wilkes Honors College in April 2024.

Ty Craaybeek

Ty Craaybeek grew up in Fort Pierce and is now an undergraduate student at FAU. He is currently studying for a Bachelor of Arts in Linguistics. Ty plans to continue studying at FAU for his masters, and he wants to work for a Ph.D. in Linguistics with a research focus in Austronesian languages. He wants to continue working in research and education with his degree.

Mia Michnik

Mia Michnik graduated from FAU in 2024 with a Bachelor of Science in Biology and is currently pursuing a career in medical research. As she prepares to attend medical school in the upcoming year, Mia currently volunteers as a tutor at teen centers and actively contributes as a medical assistant. Mia is passionate about scientific discovery and has enjoyed mentoring students while leading experiments, sharing her knowledge to help inspire and guide other students. She was a 2023 Summer Undergraduate Research Fellow and has also presented findings at the 14th annual Undergraduate Research Symposium. The work published in this manuscript was collected during her time as an undergraduate at Florida Atlantic University.

August Stone, Madison Rieth & William Burnett

August Stone is a recent graduate of Florida Atlantic University with a Bachelor of Arts in Anthropology. August began her research under Dr. Meredith Ellis in 2022 with a focus in bioarchaeology. After participating in an excavation in Sicily, she found a passion for the bioarchaeology of social age in subadults. After presenting her research at the annual Undergraduate Research Symposium, Life Sciences of South Florida's Undergraduate Research Symposium, and Florida Undergraduate Research Association's Posters at the Capitol event, August was awarded both Scholar of the Year and Undergraduate Researcher of the Year for the College of Arts and Letters. She is continuing her education in the Master of Arts program at FAU from Fall 2024 onwards, studying under Dr. Ellis in social bioarchaeology.

Madison Rieth is a December 2023 graduate from Florida Atlantic University with a Bachelor of Arts in Anthropology. She started the Anthropology Master of Arts program at FAU in Fall 2024 and is passionate about pursuing bioarcheology. She attended the Ecuador Archaeology Field School with Professor Valentina Martinez in 2022 and participated in skeletal analysis and archaeology with Dr. Brian McConnell and Dr. Meredith Ellis in Palike di Mineo, Sicily in 2023. Madison has co-authored a poster presentation and presented it at the FAU Undergraduate Research Symposium and the Life Sciences of South Florida Undergraduate Research Symposium, placing 3rd at the latter with her teammate. She has worked in cultural resources management.

William Burnett was a senior at Florida Atlantic University at the time of writing, pursuing a B.A. in Anthropology. He was accepted into the M.A. in Anthropology program for Fall of 2024, and has interests in biological and medical anthropology. He has authored/co-authored two undergraduate research posters, both of which were presented at the 14th annual FAU Undergraduate Research Symposium. He wishes to continue his M.A. research into biological anthropology such as skeletal analysis and archaeology or medical anthropology and the effects COVID-19 has had on antimicrobial resistance globally. Outside of school, William enjoys being outdoors and spending time with friends or his cat.

Ava Vignola

Ava Vignola is a Florida Atlantic University High School student. She is also a member of the Business and Economics Polling Initiative at Florida Atlantic University and has presented her work at the Florida Undergraduate Research Conference and the FAU Undergraduate Research Symposium. Her project was awarded a FAU Office of Undergraduate Research and Inquiry grant. Ava is currently studying business and plans on continuing her research throughout graduate school.

Stefan Tsapenko

Stefan Tsapenko is a third-year undergraduate student, pursuing bachelor's degrees in History and Political Science, and a pre-law concentration at the Dorothy F. Schmidt College of Arts and Letters. Stefan's academic and research interests are influenced by his background as a Ukrainian-American. His research includes a focus on Ukrainian history and legal studies. Stefan has presented his research on 17th century Indian history at the 13th annual FAU Undergraduate Research Symposium and has presented his research on 20th century Ukrainian history at the 14th annual FAU Undergraduate Research Symposium. Stefan has received the Traci Jill Edelman Award in European History for his paper on Ukrainian history, and the Harold L. Glasser Memorial Award in World War II history for his paper on WWII Ukrainian history. Stefan is a proud member of the Phi Alpha Theta history honors society.

Abhishikta Srigiriraju

Abhishikta Srigiriraju is a Medical Biology major who entered Florida Atlantic University in 2022 and plans to pursue a career in medicine. In addition, Abhishikta conducts research at the Max Planck Florida Institute for Neuroscience as part of the Stern Lab, where they study feeding behavior. Due to its potential clinical applications, Abhishikta developed an interest in genetic engineering prior to university and was inspired to investigate further after taking a course in genetics under the instruction of Dr. David Binninger.

Notes

FAURJ

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Published each Spring, the *Florida Atlantic Undergraduate Research Journal* (FAURJ) was originally established through the Council for Scholarship and Inquiry, a registered organization of FAU's Office of Undergraduate Research and Inquiry, and has continued under the direction of OURI.

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