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Quantum Entanglement
“Spooky action at a distance” – Albert Einstein
Bill Gibbons
(Featured on the Cover)

ABSTRACT

“Quantum Entanglement” is a 2D graphic produced using PhotoFiltre 7[®], a French software program similar to Photoshop[®], and GIMP (GNU Image Manipulation Program), an open source, 2D graphics software program. Basic forms, shading, and colors were created using PhotoFiltre 7[®], and GIMP was used to add more subtle and smoother distortions to imply depth and motion. Selected layers were then merged to render the final image.

The composition represents the artist’s visual interpretation of *quantum entanglement*, a fascinating phenomenon associated with quantum mechanics whereby two submicroscopic particles, for example, electrons or photons, are entangled across the space-time continuum. The implications of entanglement are mysterious, paradoxical, and counterintuitive to the classical mechanics of Newton and even to Einstein’s theory of special relativity. Characteristics of each particle (e.g., direction of spin, location, or momentum) are opposite each other. This can be imagined as two billiard balls that collide on a pool table and separate, each with spin opposite the other. In the case of entanglement, the relationship remains, whether the particles are in the same room, or one remains on Earth and the other travels millions of light-years away to the Andromeda Galaxy. However, the characteristics of each cannot be known until a measurement or observation of one particle is made, at which point the same characteristic of the other particle, even light-years away, can be inferred.

Although quantum mechanics is used today in cellular communications, LED displays, medical diagnostics, and other applications, such phenomena are not completely understood. Albert Einstein is quoted as dismissing entanglement as “spooky action at a distance” because it implied the transmission of information faster than the speed of light. Existing applications notwithstanding, future applications might include hyper-secure encryption technologies, teleportation (of, at least, data), and remote exploration of other celestial bodies.

In the image, the seemingly chaotic, unorganized forms inside the transparent spheres represent complex mathematical probabilities, termed wavefunctions, of the various characteristics of each particle. These wavefunctions collapse, or “gel,” only upon observation. The colorful swirls represent the uncertainty of interaction and unpredictability of observation while flashes of light represent the volatility of interaction and its effects. On a human scale, this representation is analogous to interactions between two students on campus or between a student and professor who meet and affect each other in some way. Like wavefunctions, there will be unknown probabilities of intellectual, emotional, or physical effects, regardless of the time lapse or the distance between the two people. Like quantum entanglement, the immediate effects cannot be determined until the event occurs and the results observed, even much later and thousands of miles away, when we can possibly infer the effect of the interaction on the other person.

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Letter from the Editor-in-Chief

As editor-in-chief and a founding member, I watched *Laridae* grow from a mere concept, into reality and was able to make it stronger in just four years on the Salisbury campus. I am extremely grateful for the experience. However, none of this would have been possible without Dr. Rhyannon Bemis, the director of the Office of Undergraduate Research and Creative Activity, this year's executive members Mackenzie Nickle and Madison MacDougall, our brilliant editors, our faculty reviewers and mentors, all of the previous members of *Laridae*, the Publications Office, the Office of Graduate Studies and Research, and of course our undergraduate authors. If it were not for the combined effort from the SU community, we would not be a journal today.

With that in mind, *Laridae* is pleased to present the third edition featuring 14 articles and six creative works in disciplines ranging from physics as an art form to the struggles in the medical field due to COVID. Out of 30 original submissions, 20 were selected and edited over the summer months to appear in the journal in your hands today.

Laridae will continue to represent our undergraduate students and strive to encourage explorations in future research and creativity in the hands of our future editor-in-chief, Mackenzie Nickle.

I once again challenge you, as the reader, to learn something new, think outside of the box, inspire, explore, and contribute to *Laridae's* legacy of celebrating and distributing knowledge.

Margaret Giggey
Student Editor-in-Chief



Letter from the Future Editor-in-Chief

As part of our mission statement, "*Laridae* seeks to facilitate and further augment SU's academic environment by offering a high-quality forum for the pursuit of knowledge."

Laridae helps provide students with an opportunity not easily accessible to them during undergraduate education: publishing their academic work. As an undergraduate researcher myself, my goal when I joined with *Laridae* in 2020 was to help advance undergraduate research and encourage creativity at Salisbury University. Throughout my time with *Laridae*, we have had over 35 authors and artists publish work in our second and third editions.

I hope to continue *Laridae's* mission of providing students with a means to share their research and contribute to the SU experience as the next editor-in-chief. But for now, please enjoy our third edition and learn from our wonderful scholars.

Mackenzie R. Nickle
Co Editor-in-Chief



HOW *SCHITT'S CREEK* AFFIRMS SEXUAL ORIENTATION AND GENDER IDENTITY

Chloe Birch

ABSTRACT

Schitt's Creek ushers in a refreshing portrayal of LGBTQ+ characters and relationships. This pattern starts early in the series and continues to the finale, culminating in a marriage between two men. Throughout the series, the characters explore their own sexual orientations, engage in conversation about sexual orientation and gender identity, and live in a healthy, supportive environment. These scenes position the audience to receive LGBTQ+ identities and relationships as normative and healthy. Instead of the ever-present tokenism of LGBTQ+ characters to be “diverse,” this show makes an effort to give the characters agency and individuality. Through David and Patrick’s romantic relationship, Patrick’s coming out story, and the various experiences about living as a queer person, the viewers are challenged to confront stigma, acknowledge the breakdown of stereotypes, and ultimately establish themselves as allies of the LGBTQ+ community. This paper will seek to critically examine how *Schitt's Creek* conveys the idea that queer* orientations and gender identities are valid, normal, and healthy through close readings of the episodes and detailed textual analyses.

* The author’s use of the term “queer” is intended to be inclusive of all identities within the LGBTQ+ communities and to orient the reader to the field of queer studies.

DESCRIPTION OF THE PROGRAM

Schitt's Creek, produced in Canada and the United States, portrays a wealthy family who lose all of their money and assets after their business manager embezzled from the family company, Rose Video. They relocate from their grand manor to a run-down town that the father, John, once purchased as a joke for his son, David. Living in a shoddy motel, they learn how to adjust to life without money and how to live with each other.

Eugene and Dan Levy, a father and son duo who play Johnny Rose and David Rose respectively, created the show after wondering how wealthy people would react to losing their money (Adalian, 2020). After selling to the Canadian Broadcasting Corporation (CBC), Not A Real Company, and Pop TV, the show received considerable positive feedback in Canada, while American audiences did not seem to be hooked as quickly. However, after Netflix picked the show up, views and rates skyrocketed (Jung, 2019). *Schitt's Creek* has been nominated and won various awards, including the Primetime Emmy Award, the Critics’ Choice Television Award, and the GLAAD Media Award (Bilefsky, 2020; Gardner, 2019).

Johnny Rose is the father and business guru of the family. His wife, Moira, was an actress and is fondly remembered by fans of the program for her extravagant outfits. Their daughter, Alexis, is the somewhat bratty and entitled daughter who learns to be selfless. Completing the family is their son, David, who learns the value of hard work and opens a business with his partner, Patrick Brewer, who eventually becomes his romantic partner throughout the series. Stevie Budd, the manager

of the motel, is David’s best friend in the unfamiliar town and is significantly involved with David’s life endeavors.

JUSTIFICATION OF THE STUDY

It is rare for TV programs to portray healthy LGBTQ+ characters and relationships. While they can be found in many series, those relationships and portrayals of them are at best tokenism, and at worst detrimental (Pandey, 2020). Writing a gay character for the sake of having a gay character just to claim representation and diversity is a far too common phenomenon in modern television. The characters have no individuality or arc. They are devalued to feminine stereotypes and only exist to be “the gay best friend” trope, such as Kurt Hummel’s character in *Glee* who was diminished to an effeminate gossip (Linnell, n.d.). They give fashion advice and the occasional word of counsel. These examples are evidence of the tokenism usually found in shows with LGBTQ+ characters and it is very disheartening to those in the LGBTQ+ community to see themselves reflected as surface-deep characters valued only for their contribution to “diversity.”

Contrary to popular TV shows that rely on stereotypes, *Schitt's Creek* was designed to be a program that affirmed and included the LGBTQ+ community. The creators wrote characters who were intended to be queer; they did not announce post-production that characters were gay without any evidence in the show itself or refer to a scene that could distantly be construed as queer. Instead, they had creative and informative demonstrations of life lived as a queer

person and more importantly, they showed these experiences in a positive and healthy light so often missing (Smith, 2020). The characters were met with full acceptance without a hint of questioning.

This portrayal is significant because members of the LGBTQ+ community live life in constant trepidation. As being queer is still viewed as abnormal by the majority, queer people are often “othered” and ostracized for their identities and orientations. The unquestioning acceptance from the small rural town, David and Patrick’s parents, and their friends is refreshing and is a message to the viewers that queer relationships are legitimate and worthy of embracing. The audience is challenged to confront stigma, acknowledge the breakdown of stereotypes, and ultimately establish themselves as allies of the LGBTQ+ community. This paper will critically examine how *Schitt's Creek* conveys the idea that queer orientations and gender identities are valid, normal, and healthy.

METHODOLOGY

After watching the entirety of *Schitt's Creek* on Netflix, three episodes were deliberately selected to examine the normalization and acceptance of queer characters and relationships. Every episode is from a different season to understand how the show consistently depicts queer characters and relationships as natural and healthy. The first episode that will be analyzed is from the first season and it serves to appreciate how the show introduces LGBTQ+ characters. The second episode is from the fourth season and it recognizes how important the acceptance of the LGBTQ+ community

is. Lastly, the third episode is from the fifth season and it considers the audience's witness of the very personal experience of coming out and how that should only be done on one's own terms. This is an incredibly positive and vital message to the audience – cisgender, heterosexual people, allies, and queer people alike. Close readings of the episode were conducted to examine the normalization and acceptance of queer characters and relationships. The section below contains a detailed textual analysis with support from Mittell (2010) to reveal how the creators of the show conveyed these messages.

ANALYSIS AND DISCUSSION

The first episode to be analyzed is titled "Honeymoon" from season 1, episode 10 (Levy & Ciccoritti, 2015). Following a one-night stand between David and Stevie, they go to the grocery store to browse the wines (09:20). Stevie covertly queries David's sexuality by explaining she only drinks red wine and that until their hookup, she thought David too only drank red wine. Stevie's tone and facial expressions suggest innuendo and invite David and the audience to read further into what she is saying. Understanding, David replies that he drinks red wine too, but he also drinks white wine, rosé, and once tried a merlot that used to be a chardonnay. Stevie concludes that David is open to all wines, to which David replies, "I like the wine, and not the label."

Stevie uses red wine as an analogy for men. By asking if David only drinks red wine, she is questioning if he has a singular interest in sleeping with men or if he is open to all genders. David's reply that he drinks red, white, and rosé wine indicates that he has sex with men, women, and nonbinary people, respectively. The merlot that used to be a chardonnay is a metaphor for a female-to-male transgender individual. When David says he likes the wine and not the label, he is expressing his pansexuality, which Johnny confirms later in the episode.

There are several significant events in this scene. First, Stevie respects David's personal life by not openly asking him about his sexuality in a public place. She has the wherewithal to make an analogy and have the discussion that way. Second, David's coming out is not riddled with humor at his expense. *Schitt's Creek* intentionally writes the scene to convey David's sexuality as a normal part of life, rather than a subject for ridicule. Neither Stevie nor anyone else ever criticizes David's pansexuality or laugh at him. They simply accept it with an "Okay, cool" and move on. Third, so much representation is packed into a few lines and includes everyone in the LGBTQ+ community.

It is rare to see a bisexual, pansexual, or transgender character depicted in a TV show who exists as a character with depth and emotion. This scene naturalizes the existence of queer folks and encourages the audience to not only accept them, but to ally with them as well. When Stevie moves on from the conversation without relentlessly interrogating David for his sexuality, she is portraying her allyship with the queer community. It is one thing to acknowledge the existence of something and another thing entirely to be at peace with its existence.

The second episode to be considered is titled "The Barbecue" from season 4, episode 7 (Read & Gunnarsson, 2018). The Rose family is hosting a barbecue and invites Patrick, David's partner, to come (08:11). While they are eating, Alexis introduces Rachel, a girl she met outside the motel who came to Schitt's Creek to win back her fiancé. Little does Alexis know, Rachel's fiancé is none other than Patrick. Rachel questions what Patrick is doing and that she has been texting him for two days without reply and David excuses himself upon learning that Patrick had a fiancé. Patrick then follows David to clarify that they are no longer engaged and that he had called the engagement off before he moved to Schitt's Creek.

He explains that their relationship had been on and off for a while and Rachel had been asking to get back together for the past several months, which he did not tell David. David is understandably upset by this and questions why Patrick would tell David to trust people – to trust him – when Patrick deliberately kept him in the dark about this woman and told him he had nothing to worry about. Patrick then proceeds to emphatically tell David that he truly has nothing to worry about:

'Cause no matter how hard I tried with her, it just never felt right. And up until recently, I didn't understand why. David, I've spent most of my life not knowing what right was supposed to feel like, and then I met you. And everything changed. You make me feel right, David. (19:08)

David is soothed, but still feels that he needs some time. Patrick offers to bring David a plate of food as the discussion started before they had time to eat. The episode ends with the couple suspended in this limbo, unsure of the direction their relationship will go. They do, however, get back together in a later episode and stay together for the rest of the series.

This episode was chosen to highlight the importance of legitimizing queer relationships. From what Patrick says, it is evident he has lived trying to be heterosexual, forcing himself to be straight with a woman he does not love. And he does not know why. It seems that his lack

of understanding stems from the culture of secrecy surrounding LGBTQ+ people. The topic tends to be taboo and kept that way in order to privilege the dominant heterosexual group. By ensuring that people view queer people and queer relationships as wrong or abnormal, the dominant group can continue to enforce their rules upon others and marginalize those who are not like them. Patrick could not even begin to contemplate his sexuality being different from the dominant view until he met David because he was not given the opportunity to witness healthy LGBTQ+ relationships and the endorsement of them. Finally, he feels right with David because he is allowed to do this. David shows him a fuller experience of queerness and helps Patrick know he is loved and accepted that way. The culture in Schitt's Creek itself also does this – never in the series do any of the townsfolk say anything derogatory or even peripherally negative about the LGBTQ+ community.

This scene conveys to the audience that trying to hide queerness is hurtful. It is impossible not to feel sorry for Patrick as he tells David that he never felt right until he could be in a queer relationship and identify himself as a gay man. It impresses the sense that people need to move toward a more accepting culture like that of Schitt's Creek and actively validate queer people and queer relationships so they do feel right. People deserve to feel normal and hearing Patrick's story inspires the audience to make it so.

The final episode chosen is titled "Meet the Parents" from season 5, episode 11 (Levy & Canning, 2019). David invites Patrick's parents to Schitt's Creek to throw a surprise party for Patrick's birthday. When they arrive at the motel, Johnny accidentally lets it slip that Patrick and David are in a romantic relationship. Up until this point, unknown to David or his family, the Brewers were operating under the understanding that Patrick and David were simply business partners. David assumes that the Brewers know the two men are romantically involved, but upon hearing about their confusion at Johnny's comment, he questions Patrick. Patrick reveals they do not know because he wanted to tell them in person and has not had the chance to do so. He then expresses his fear that his parents knowing he is gay will change everything, even though he knows they are good people.

David soothingly tells Patrick, "What you're dealing with is very personal. And it's something you should only do on your terms." He is then forced to tell Patrick about the surprise party and how he invited the Brewers to town, offering to be just Patrick's business partner at the party so that Patrick does not feel any pressure to

come out. Patrick cannot ask that of David because he wants his parents to know and says he owes it to their relationship to tell them. He plans to do so at the party, but is unaware that they already know about his sexual orientation.

At the party, Patrick is apprehensive about telling his parents and asks David what he will do if they do not react in the way he wants and thinks they will. David tells him that he is right there through it all and they will figure it out together. Emboldened, Patrick sits down with his parents, who almost reveal that they know. Patrick's mannerisms show his nervousness, but he is able to tell them about his relationship with David. His parents affirm their love for him and tell him that his happiness is all they care about. Patrick's relief is visceral and emotional as he expresses his love for his parents. The episode ends with David and Patrick slow dancing as they talk about the party and how the discussion with the Brewers went. Patrick has figured out that his parents already knew before he told them. David explains that he wanted to "make everything okay" and Patrick tells him that he did. They kiss as they continue to dance and a message giving resources for those struggling to come out closes the episode.

This episode deals with something very personal – coming out. Every queer person faces the prospect of this moment. If and when they come out, it is not a one time thing. They continue coming out to people for the rest of their lives because queer people are still seen as "other."

Patrick's fear, even though he knows his parents are good people, is a fear most queer people have felt, regardless of their situation. Fear of being disowned, ostracized, or cast out permeates their being and can be detrimental to their mental health. The fact that the creators of *Schitt's Creek* included this fear validates it and tells queer people that it is permissible to be afraid.

David's reaction to Patrick's fear depicts understanding and acknowledgement of it. He does not tell Patrick platitudes like "everything will be okay," or that his parents will accept him no matter what, or that there is no reason to be afraid. Instead, David reassures Patrick that he is there and they will deal with it. Furthermore, David does not get upset at Patrick for not telling his parents. He reassures Patrick that the decision to come out is one's own and only one's own, to do on their own terms. Many queer people are pushed to come out by their partners or friends, which even though well-intentioned, can be harmful. David's offer to be just Patrick's business partner is respectable and shows his genuine support for Patrick to come out to his parents when and how he wants to. The audience, upon seeing this interaction, is encouraged to ensure they do not push LGBTQ+ folks to do something they are not ready to do yet. They are positioned to learn to be supportive and present, as David is.

The fact that Patrick's parents fully accept him being gay is representative of the program's intention for *Schitt's Creek* to be homophobia-free. Often,

homophobia is used as a barrier or obstacle for the character to overcome and grow from. Though this can be the case in reality, using something as hurtful and dangerous as homophobia to foster character development is itself hurtful and dangerous. One could argue it also is lazy writing. *Schitt's Creek* is one of the only programs that queer people can watch without apprehension of a homophobic scene or discouraging event related to sexual orientation or gender identity. It is important that this show exists because entertainment should be for everyone and not at the cost of anyone.

CONCLUSION

These three episodes convey the way *Schitt's Creek* legitimizes queer people and relationships and additionally persuades the audience to view them as such. Further, the audience is influenced to be allies of the LGBTQ+ community. As the audience watches David and Patrick's relationship, they are invited to confront their own stigma and acknowledge stereotypes they may perpetuate. By doing this, they have the opportunity to actively change their mindset and become defenders of the LGBTQ+ community. With the world still being discouraging at best and dangerous at worst to queer folks, this program allows queer relationships to be acknowledged and validated as normal and healthy. As a popular TV show, it is hopeful that many people will be touched by it and come to a position of support and advocacy.

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CHINA, SOFT POWER, AND INFLUENCE OVER ASIA

Brigid O'Connor

ABSTRACT

China's soft power influence has become more dominant in recent decades and has increased China's influence on the international stage – especially with respect to surrounding nations. The purpose of this study is to analyze why and how China has used soft power to influence its relationship with other Asian countries – and the United States – in recent decades. First, to understand China's soft power influence, the definition and origins of soft power through the Beijing Consensus are examined. Numerous strategies are identified as being used either singly or in combination, including assimilation of Chinese culture and values into other Asian countries, influencing education in other countries and encouraging bidirectional study-abroad programs, business and trade, and providing significant economic aid and development assistance to developing nations. This paper contributes to a greater understanding of Chinese soft power on its neighbors and a wider international context, which is an area that has not been extensively explored by scholars.

INTRODUCTION

As the world's second largest economy, China remains a dominant force globally in both political and economic spheres. In recent decades, China has recognized the soft power influence of the United States in Asia, and – in response – has steadily been trying to increase its own soft influence over the region, particularly in East, South, and Southeast Asia. Several methods that are used by China to increase its soft power and influence have been identified and explained in this article's literature. These include the assimilation of Chinese culture and ideals in other Asian societies, the influence on domestic policies and economies in other nations, and attempts to overtake and diminish the United States' influence in the region. Therefore, it is key to understand the influence and use of Chinese soft power on other countries and economies to give further insight as to the dominance of China in a global context. This article aims to explore and describe why and how China has used soft power to influence its relationships and dominance over other Asian countries – and the United States – in recent decades.

LITERATURE REVIEW

The use of power generally in society can be both good or bad, depending on who uses it and to what end. According to scholar Joseph S. Nye Jr., power is generally defined as a relationship between two parties where influence is involved, with one party having control over the other (Nye 1990, 154). In a political context, power usually involves a myriad of factors, such as a country's population, economy, military forces, territories, and politics (Nye 1990, 154). Whether a nation in this context has any of these factors in their favor over another nation, it can be

assumed that they will have a significant amount of influence and power over them.

Political scholars have further delineated two specific types of power that have coexisted for centuries: soft power and hard power. Hard power – according to scholars Young Nam Cho and Jong Ho Jeong – can be defined as power in the form of political, economic, and militaristic force (Cho and Jeong 2008, 455). Soft power, on the other hand, emphasizes power through coercion and attraction rather than more forceful means (Cho and Jeong 2008, 455). Similarly, scholars Neil Renwick and Qing Cao define soft power in China as being able to attain one's desires not through the use of force, but by the power of persuasion (Renwick and Cao 2008, 69-70). The rise of Chinese soft power comes from three main areas, according to Cho and Jeong: the establishment of the Beijing Consensus (which is a variation of the United States' "Washington Consensus"), Chinese culture and cultural development, and China's foreign policy endeavors (Cho and Jeong 2008, 455). These main factors have made China successful in its soft power pursuits rather than its use of hard power, which other global powers must respect (Renwick and Cao 2008, 70).

The definition of the Beijing Consensus is imperative in understanding how soft power and Chinese influence are impacted by the Beijing Consensus. The Beijing Consensus is a derivative of the Washington Consensus, the latter of which argued for a more stable economy and financial reforms in order to promote the liberalization and economic growth of the market (Huang 2011, 3). The Beijing Consensus, in turn, was an alternative approach to the American doctrine which would create a more liberalized and free market economy, while maintaining

China's authoritarian rule (Huang 2011, 3). The doctrine consists of five main pillars: gradual implementation of reform plans, economic growth through exports to other regions, experimentation of policies, a capitalist economy, and maintaining authoritarian rule (Williamson 2012, 435-436). More specific interpretations of the Beijing Consensus would only state the importance of the latter two pillars, yet all five aspects of the doctrine are important for China's rise in power. Particularly, China's success in maintaining a free market economy alongside the long-lasting authoritarian rule of the Chinese Communist Party (CCP) allows China and the CCP to be influential in other areas outside of China and establish its dominance through soft power.

China is a dominant force in a global context, in part, because of its economic prowess, demographics, huge mainland mass, territorial claims – such as Hong Kong, Singapore, and Taiwan – and significant influence over other generations through provision of education, study abroad for Chinese citizens, and reciprocal opportunities for foreign exchange students. China maintains its sheer, continued dominance and control over the world's supply chains, manufacturing sectors of many goods and technologies, and markets; and has abundant cheap labor. China's influence also disproportionately extends to its nearest neighbors. Due to its population of over 1.4 billion people and huge land mass, China naturally has a significant amount of influence over other Asian countries across South, South-East, and East Asia (Lee 2008, 128). This is through implementing Chinese education programs in these regions through CCP-approved curriculums and Confucius education – providing economic aid

to further develop these countries and encouraging Chinese students to study abroad and international students to come to China.

Although China and Korea have had a rocky relationship politically for decades, China is beginning to use its influence to rectify relations and encourage Koreans to have a more favorable view of China (Lee 2008, 128). Offering Korea aid by way of educational programs, economic development, and investments, in hopes of furthering its soft power, have all led to an increase in the number of Koreans who view China positively (Lee 2008, 128). According to scholar Jung-Nam Lee, anti-American sentiment in Korea is also growing, whereas Koreans are beginning to view China in a better light than decades before (Lee 2008, 128). Furthermore, in a 2004 poll, 63% of Koreans believed China to be Korea's most impactful and influential partner, both commercially and diplomatically (Lee 2008, 128). This is a result of foreign investment and economic development of the region as well as the promotion of educational programs (Lee 2008, 128). Other ways in which China has increased its favorability in Korea have been through joining regional and international partnerships along with vowing to settle territorial disputes with its neighbors without the use of hard power or force (Lee 2008, 128).

Another example of Chinese influence on Korea is a cultural shift in the way ethnicity is interpreted by the Korean minority in China. Ethnic Korean minorities in China regard China as their fatherland, rather than seeing North or South Korea as their country of origin (Woo-Gil 2001, 130). This is due to the historical immigration between China and Korea, which gave Korea a large Chinese influence through language and culture, with some Koreans in China seeing themselves as having a dual identity (Woo-Gil 2001, 130). According to a study conducted in 1999, the Korean minorities in China consider themselves more different from ethnic Koreans living on the peninsula in terms of language and cultural backgrounds, as a result of being educated in Chinese classrooms to regard China as their ancestral land (Woo-Gil 2001, 130). China wields this influence to establish its presence over Korea, and with Korean minorities in China, through coercion of Chinese ideals into the psyche of these populations with the use of propaganda and appealing to emotional ties and nationalism (Woo-Gil 2001, 130).

As the scholarship suggests, Korean minorities in China had begun to show animosity toward South Korea due to the adoption of Chinese ideologies and political influences stemming from the

Cold War (Woo-Gil 2001, 132). Korean Chinese who visit South Korea have been discriminated against and an inability to adapt to South Korean culture, which furthers the animosity toward the country (Woo-Gil 2001, 133). Because of China and North Korea's close alliances during the Cold War, Chinese education and propaganda would be skewed toward anti-American sentiment. Seeing as South Korea was a close ally of the United States, the anti-American sentiment among the Chinese and ethnic minorities in China would extend into anti-South Korean sentiment as well, through the aforementioned use of propaganda and educational institutions. China historically was also able to increase its soft power influence over South Korea and Korean-Chinese minorities through the People's Republic of China's communist influence on North Korea (Woo-Gil 2001, 124). During the Korean War, ethnic Korean minorities fought with the Chinese People's Army, which gave the Korean minorities in China a sense of kinship and belonging with the Chinese and North Koreans fighting against the South Koreans and the United States (Woo-Gil 2001, 124). Furthermore, since China and North Korea were closely allied against the United States and their ally South Korea during the Korean War, ethnic Korean minorities in China were coerced and influenced to believe that their ties were closer to North Korea than South Korea (Woo-Gil 2001, 130-131). The use of education and propaganda rather than brute force are key reasons for this, which extends China's soft power into Korea by promoting Chinese culture to its minorities and coercing their ethnic minorities to accept China as their true fatherland, rather than South Korea.

The Korean wave – known as “Hallyu” to the Chinese – that made way for the spread of contemporary Korean culture began in the late 1990s in Asia and exploded globally in the 2010s (Jeong 2012, 88). As Jong-Ho Jeong points out, the products that Korea brought to China became a massive success in the country, with K-dramas and K-pop being the most notable imports (Jeong 2012, 88). Chinese television stations were quick to embrace South Korean television, and the relatively inexpensive cost to import these phenomena further boosted the wave's popularity in Chinese society (Jeong 2012, 88). However, after it was revealed that the Korean imports were causing Chinese production companies to lose work and employment, an anti-Korean wave sentiment in China began to grow (Jeong 2012, 88). Due to this, China was able to establish propaganda against the Korean wave and against Korea as a whole. This could also partially stem from

the aforementioned propaganda associated with the ethnic Korean-Chinese against South Korea.

Since China has had a global stronghold politically for decades, it naturally has impacted the daily lives of many nations around it. Through Chinese policies in Hong Kong, China has been able to significantly influence the city and subtly attempt to censor any exposure of negative policies (Sciutto 1996, 133). Furthermore, the Belt and Road Initiative (BRI) is a massive influence both culturally and economically, which harkens back to the Silk Road. Through these nations in Asia and the wider trade network, China is able to expand its culture and ideologies in order to maintain political and economic influence across the East Asian region.

China's influence over Hong Kong is widely known in the modern day. After being handed back from Britain following the expiration of its 99-year lease, China has kept a watchful eye over the region and has continued to establish Hong Kong as part of the mainland. This is in addition to the continued assimilation of Chinese culture into Hong Kong's society. Due to the strict policies of the CCP, Hong Kong would be subject to much censorship and overwatch from the Chinese government. For example, journalism in Hong Kong is heavily favored toward the Chinese, and reporters will frequently be paid off to keep quiet or to bend to Chinese pressure due to low pay (Sciutto 1996, 133). This interference and coercive, under-the-table tactics of censoring the Hong Kong media further demonstrate the dominance of Chinese soft power in areas outside the mainland.

China's influence and attempts to overtake U.S. soft power has culminated into what is known as the “Belt and Road Initiative” – also known as the BRI. The BRI, in essence, aims to increase global correspondence when it comes to Asian economies, trade, and cultural influences (Salamatin 2020, 1434). Scholar Mikkaela Salamatin has examined the impacts of the BRI in terms of Chinese influence, which describes how China has taken both direct and indirect actions to ensure the BRI's success and China's continuous influence over East Asia (Salamatin 2020, 1432). She explains that it was no single action that led to China's soft power rise, but rather a culmination of actions that would cause the effects of China's influence on the BRI to be bothersome, likening the situation to a bucket where what is inside only becomes a threat after it is almost completely filled (Salamatin 2020, 1432). Although there are negatives to the Belt and Road Initiative, it remains a key part in promoting Chinese soft power in East Asia and diminishing U.S. soft power through the goals of the initiative.

China's take on the BRI would be to bring back the spirit of the Silk Road that lasted from the 2nd century BCE until the 18th century (Salamatin 2020, 1434). This outcome would be through a dedication to peace and mutual connections within East Asia (Salamatin 2020, 1434). China uses the BRI to create a meaningful and positive connection to other East Asian nations and uses the connections to influence them through trade and cultural connections. China can spread its ideologies and products through the BRI just as it did during the Silk Road era, which, in turn, spreads China's cultural influence and allows China to establish a stronger presence in East Asia.

The Belt and Road Initiative was further emphasized by China's soft power endeavors in Cambodia. The spread of Chinese culture and the language were a product of the BRI, which influenced Cambodia by assimilating Chinese culture into their citizens' everyday lives (Kobayashi 2020, 17). For example, China sends teachers to Cambodia to teach students about the Chinese language, and friendly competitions are offered – sponsored by the PRC – for non-Chinese students to become more familiar with Chinese culture and the language (Kobayashi 2020, 19). Similarly, the Cambodian government only allows textbooks that are approved or supported by the PRC (Kobayashi 2020, 19). Steps like this will create more of a blend of Chinese culture into Cambodian society, which is beginning through the education system.

With this example of Cambodian education in mind, it becomes clear that education is a large reason for China's growing influence in Asia and on a larger international scale. China has been routinely sending many students abroad, with international students coming to study in China as well (Pak 2007, 59). Confucian ideology has significantly impacted the political legitimacy of China's rule, which not only ensures domestic stability through religion and governmental policies (Pak 2007, 59), but further extends into foreign education endeavors. Confucianism is the idea of ruling by virtue and education. To that end, China has developed a partnership with other nations in order to promote Chinese education. For example, "Confucius Institutes" are organizations and partnerships set up between Chinese and foreign universities with three main goals: to teach Chinese, have cultural exchanges between the two countries, and promote business within the region (Paradise 2009, 651). With the exchange of students and teaching of Confucian ideals, China has been able to increase its international influence, spread its culture

and teachings to other countries as well as promote inclusivity.

ANALYSIS OF CONFLICT

China influenced the wider population of several East Asian societies through soft power and assimilation of Chinese culture into the region through education and religion. Furthermore, it has had significant influence on other nations' economies and domestic politics. The large-scale soft power of the United States had convinced China to attempt to overtake American influence and establish China as the dominant soft power impact in Asia. To that end, China has used its wealth to implement strategies such as economic aid to its neighbors and demilitarization to increase positive relations with other Asian countries.

China has been using soft power more recently to ensure it remains a dominant force in East Asia and around the globe, but not necessarily as a threat. A primary goal of China's competition with the United States is to acquire and develop foreign technology in order to increase its foreign and regional influence (Lewis 2020, 1); although some nations have accused China of intellectual property theft. China also is attempting to diminish its dependence on foreign technology and is enacting its "Five-Year Plan" to reach new goals for technological advancement and economic development (Lewis 2020, 2). Although this point is not specifically referring to East Asia itself, China's economic and technological influence go far beyond China itself. The United States is currently the most dominant power in terms of influence on East Asia, and one area where China is trying to further strengthen itself is in economic and technological development. For China to have more influence on East Asia and diminish American influence in the region, it must first have significant influence on its own borders. Growing domestic influence will naturally reverberate to other nations within its region and extend globally.

DIAGNOSIS OF FOREIGN POLICIES

With economic development comes significant monetary costs, and in its soft power pursuits, China has begun investing heavily into the wider Asian region and its partnerships with other nations. For example, China has the goal of investing \$1.5 trillion in worldwide economic ventures by 2025, such as investing a combined \$65 billion for Silk Road ventures, or \$91 billion in various regional and international banks (Shambaugh 2015, 100). China has been collaborating with other countries to propose both regional and international institutions such as the New Development Bank,

Free Trade Area of the Asia-Pacific, or the Asian Infrastructure Investment Bank (Shambaugh 2015, 100). With its practices in spending money on infrastructure and economic collaboration with other countries, China will influence the areas which are impacted by the partnerships and organizations with the soft power tool of its sheer wealth.

In recent years, China has been developing a "grand strategy" in order to assume more political influence in East Asian countries, attempting to cement its place as having a stronger foothold in the region than the United States. According to scholar Jin H. Pak, China's grand strategy for a stronger and more influential mix of hard and soft power can be summarized in four major goals (Pak 2007, 56). The first goal is to develop a stronger military – an area in which the United States has dominated China for decades, although China in recent years has attempted to shift focus toward economic development and de-emphasize the hard power of the military (Pak 2007, 56). The second is to increase China's political influence in a global context (Pak 2007, 56). The third is to establish territorial integrity, and ensure that China's borders are secure from use of force from other nations (Pak 2007, 56). Despite the protection from invasions and maintaining its territorial integrity, it is still shifting toward a soft power emphasis because of the economic implications with its neighbors through economic partnerships such as the Association of Southeast Asian Nations (ASEAN) and the East Asian Community (EAC). Lastly, the fourth point is to maintain the domestic stability of China (Pak 2007, 56). These factors will ensure China remains competitive with the United States for positive influence in East Asia, although whether China will ultimately succeed still requires research and time to watch their strategies unfold. Furthermore, there are two distinct outcomes for China's grand strategy being put in place, one of which would be beneficial to China's pursuit of soft power by reducing the United States' influence in the region (Pak 2007, 68). Meanwhile, the second outcome could potentially see China return to its hard power habits during the era following World War 2, which would complicate relations in the greater Asian region.

The first of China's grand strategy plans is arguably the least influenced by soft power, as an increased military presence is more hard power in nature. However, there are still ways in which China uses its soft power to increase its military prowess. China's army – the People's Liberation Army (PLA) – would focus its efforts on attempting to coerce Taiwan into independence agreements or

settlements that would be more in Beijing's favor (Pak 2007, 62). Furthermore, China would also develop nuclear weapons and strengthen its air force to compete with U.S. technological advancements (Pak 2007, 62). Doing this will ensure China's influence over East Asian nations through both soft and hard power. For example, China used coercion to try and settle Taiwan into remaining under China's rule, thereby creating a more legitimate soft power strategy for Chinese influence in the region (Pak 2007, 62).

Influence on the territories of East Asian nations is also evidence of China's impact and soft power in the region. Historically, China was seen as a hegemon in the region, in part due to its tributary system that dominated East Asia during the Ming and Qing dynasties of Chinese rule (Pak 2007, 62). East Asian nations were required to pay tributes to China – the more dominant power in the region and had to regularly send representatives of East Asian nations to China to pay said tributes (Pak 2007, 62). This would include Korea, Thailand, as well as parts of Vietnam and the Philippines, for instance (Pak 2007, 62). Should envoys refuse to pay tribute to China, their home countries would be subject to political and economic sanctions (Pak 2007, 63). These policies ensure China's dominance over East Asian nations by requiring nations in the region to legitimize China's rule, as tributes typically show a sign of dependence of one nation on another. Confucian ideals were also paramount in establishing China's soft power influence, as Confucianism would teach to rule by virtue. Now, with China's pursuit of soft power, it has called back to its original Confucian ideals of ruling by moral excellence, rather than hard force.

Territorial integrity and a desire for security of China's borders has caused technological marvels and attempts to secure China's borders from foreign invasions, seeing as it is difficult to completely defend a country so massive from invasions. As Pak points out, the Great Wall of China is a culmination of this desire for border security (Pak 2007, 60). Due to constant invasions during China's early years from the hostility of the Turks, Manchus, and the Mongols – as well as internal kingdoms and tribes fighting with each other (Pak 2007, 60), China has become increasingly protective over its territories. For example, when Taiwan campaigned for independence after World War II, the response from China was less than enthusiastic due to its desire to maintain control and influence over its territories (Pak 2007, 60-61). Although China had used hard power in the past to keep its competitive edge in East Asian affairs, in recent decades, it

has been resorting to soft power to ensure territorial integrity – as can be seen with more coercive tactics for keeping Taiwan and the overall grand strategy being put in place to increase China's soft power presence.

In an attempt for China to shift focus from authoritarianism and forceful strategies into becoming a partner to East Asian regions and aid with economic challenges. China has been increasing its trade with East Asian nations and with the United States in order to give a more positive outlook for those regions about China and Chinese policies (Holyk 2011, 225). For example, China has entered trade deals and partnerships such as the East Asian Community (EAC) and the Association of Southeast Asian Nations (ASEAN). Both partnerships between East Asian nations were created in an attempt to diminish or cease the occurrences of financial crises in the region, and particularly to prevent a similar crisis to the 1997 financial devastation to the region (Pak 2007, 56). In modern day, these partnerships exist mostly to develop security in the region and address issues related to security (Pak 2007, 56). As mentioned by Pak, China's grand strategy may have two different outcomes when it comes to its success or failure (Pak 2007, 68). First, the success of China's soft power pursuits in East Asia would undermine American influence in the region, causing U.S. soft power in East Asia to decrease (Pak 2007, 68). Alternatively, China could be unsuccessful in its pursuit of soft power in the region, causing it to return to its hard power roots and become more forceful, particularly when it comes to military endeavors (Pak 2007, 68). It remains to be seen exactly which route will be taken by China, and if the continued use of its soft power will be as effective, while simultaneously dealing with challenges to maintain it. However, it becomes clear that this will increase the nation's soft power influence on the region due to a sense of partnership, rather than forceful agreements that seek to mostly – if not only – benefit China.

CONCLUSION

China's influence in East Asia and on a wider global scale have more so been established through hard power since the CCP's rise. However, due to challenges faced by the CCP in relation to the rise of soft power influence by the United States, China is now attempting to maintain a stronghold of the East Asian region and establish itself as the foremost political, economic and cultural influence. Currently the United States is seen as the most impactful, with China as the second. China in recent years has been establishing more soft power influence

through several avenues. First, there is the cultural assimilation of Chinese ideals and culture into other East Asian countries. Another major way is the rush to diminish the soft power influence of the United States in the region. Lastly, China has begun to establish an influence on the daily lives of other East Asian societies and their economies through economic aid and the spread of ideology. It remains to be seen whether China will be completely successful in its grand strategy of maintaining soft power, but whether China succeeds or not – and whether the United States remains the forefront of soft power influence – will have a significant impact on East Asia for decades to come.

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EVERYTHING WITHERS IN THE ARMS OF FALL

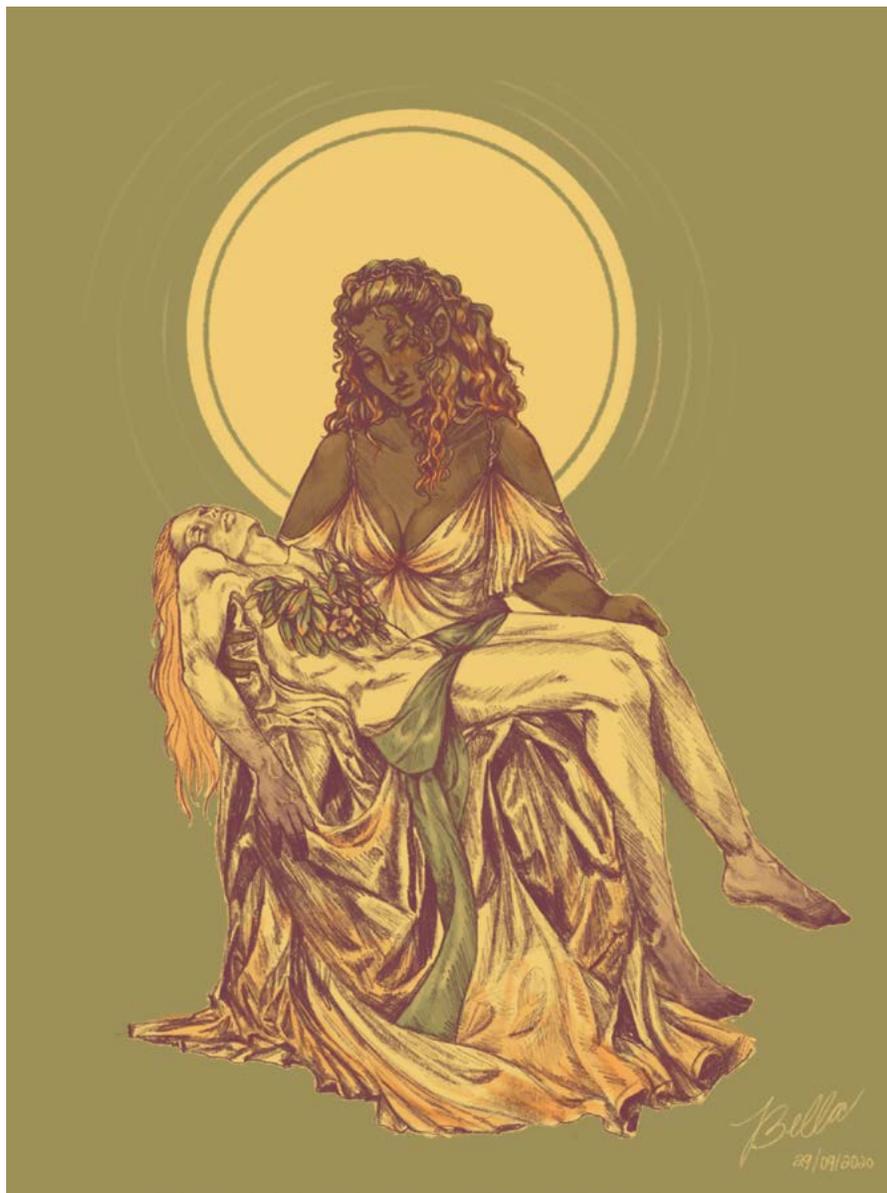
Isabella Rose Garrison

ABSTRACT

“Everything Withers in the Arms of Fall” Procreate on iPad Pro 2400 x 3300px. This digital piece was inspired by the Oscar Wilde quote: “All at once summer collapsed into fall.”

The color palette was chosen to reflect the colors during the transition between summer and fall. ‘Summer’ is depicted as the androgynous draped figure with necrotic appendages and diseased leaves strewn across their chest, while ‘Fall’ stands above them looking healthy, as if they are glowing with life. Each year, summer fades away into the grip of fall with vibrant green leaves withering into shades of orange. This transition is represented by ‘Summer’s’ gaunt figure becoming slowly enveloped in the folds of ‘Fall’s’ dress. The viewer’s eyes are immediately drawn to the halo around ‘Fall’s’ head that overshadows Summer. This is the moment where Summer ceases to be and Fall reigns.

The drawing itself is a take on the Pieta statue carved by Michelangelo in 1498. The statue was used as reference for the piece since Christ, who lays dying in the Virgin Mary’s lap, is a symbol of resurrection. Just as Christ was said to have resurrected in the Bible, ‘Summer’ may be fading for now, but next year they will return.



THE NATIVE VOICE IN THE NATIONAL NARRATIVE: NATIVE PRESENCE ON THE EASTERN SHORE OF MARYLAND

Anna M. Long

ABSTRACT

U.S. history shows bias towards the colonial narrative as it portrays the white settler as the protagonist in the country's history. As a result of this skewed focus, the Native American storyline has become overlooked by the public. Colonial-age misconceptions regarding the fate and presence of the American Indian survive today and have contributed to the misrepresentation of Native Americans in history. Furthermore, scholars agree that this distortion of history has affected the perception and existence of Native voices in communities around the U.S. today. The public's unfamiliarity with indigenous history on the Eastern Shore of Maryland reflects the nationwide struggle to reassert Native relevance in American history. This paper analyzes the state of U.S. historical presentation today, more specifically through public art, to determine both the causes of and solutions for Native neglect in history and communities. Sources confirm that efforts are being made at multiple levels to address Native American misrepresentation and underrepresentation as well as to reestablish indigenous narratives in history. Providing local, interactive, and creative educational opportunities that encourage willing acceptance of alternative perspectives on the Eastern Shore and across the country could help successfully reintroduce indigenous voices in public history as the Native and settler narratives are intertwined to give the public a more complete understanding of the country's past.

INTRODUCTION

An imbalance of power exists behind the voices that recount U.S. history. The history of this country is widely presented and interpreted from a colonial-centric perspective that encourages people to identify with the white settler throughout history. One voice that has been silenced through the glorification of the colonist's story is that of the Native Americans. The indigenous people of this nation have been underrepresented and misrepresented in public history to conveniently fit into the white colonist's narrative (Daehnke, 2012; D. G. Lewis, 2014; McCue-Enser, 2020; Miles, 1992; Silliman, 2005). Scholars have traced this disregard for the American Indian story to colonial-age misconceptions that persist today, portraying Native Americans as an isolated, relic people who carry no current relevance (D. G. Lewis, 2014; McCue-Enser, 2020; Miles, 1992; Rowse, 2014). Public historical parks and art reinforce this separation of narratives as they present the colonist's story as central to American history while indigenous history remains in the periphery (Daehnke, 2012; McCue-Enser, 2020; Miles, 1992). However, scholars have recognized a growing resistance to the country's colonial-centric presentation of history. Efforts have been and are being made at multiple levels to reestablish Native voices in communities in a way that encourages acceptance of additional historical perspectives. The Eastern Shore of Maryland, with its rich Native

American history that is widely unknown by communities today, reflects the national problem of colonial bias and Native underrepresentation. Reintroducing indigenous voices on the Eastern Shore via creative, interactive education that intertwines the narratives of the Native American and the white settler allows people to embrace additional perspectives, contributing to a more complete and meaningful understanding of U.S. history.

A REVIEW OF HISTORICAL PRESENTATION IN THE U.S. TODAY

Before exploring the scope of Native presence on the Eastern Shore of Maryland, it is necessary to understand the state of U.S. historical presentation today. Jürgen Osterhammel, author of *Colonialism: A Theoretical Overview*, describes colonialism as "a relationship of domination between an indigenous ... majority and minority foreign invaders" (as cited in Miner, 2015, p. 221). U.S. history, however, offered from a colonial-centric perspective, conveys colonialism in a more favorable light. As a result, colonialism is then portrayed as "cultural contact," a form of interaction between indigenous and settler peoples that emphasizes exchange and positive communication (Silliman, 2005, p. 58). Public knowledge of the country's past tends to follow this storyline of the colonist. Incorporating additional points of view into the U.S. narrative threatens the idealistic portrayal of the white settler that has been branded

into public thinking today. In contrast, Native history reveals centuries of violent interaction as well as Native displacement, genocide, and coerced assimilation that more accurately follow the definition of *colonialism* (used in this essay according to the definition provided above). As a result of this skewed focus on the white colonist, the historical narrative of the Native Americans is pushed to the background, receiving little acknowledgment in U.S. history.

To simplify history and to mitigate the impact of Native American voices on the protagonist characterization of the colonist, history manipulates representation in two ways to ensure colonial dominance. The first is to control the exposure of the Native narrative in history. U.S. history tends to present only aspects of the Native American story that serve and support the white settler's history (McCue-Enser, 2020, p. 182; Miner, 2015, p. 219). Furthermore, history separates the Native and European settler's stories, posing indigenous society and life as the "precursor to white settlement" (McCue-Enser, 2020, p. 182). Miles (1992) explained this separation well, stating that "Indian history remains an interesting sideshow worthy of a preliminary chapter ... after which the main act of white history takes the stage" (p. 54). Presenting Native American history solely as a narrative that predated colonialism and that benefitted the settler narrative reinforces colonial dominance in history and further hinders people's ability to

recognize that a more complex Native history exists.

This ahistorical portrayal of Native Americans in history today dates back to the white settlers' interpretations of their indigenous neighbors in the colonial era. In fact, basic historical terminology reflects the degree to which colonialism has affected historical representation. Miner (2015) and Rowse (2014) called out the European colonial origin of the term "Indian" (p. 219; p. 17); the title "Native American" shares a similar background. However, "[b]efore [white settlement they] were Shoshone or Mohawk or Crow" (Miner, 2015, p. 219). This preference U.S. history has for colonial vocabulary reveals a reliance on and bias towards an avenue of public understanding that is based upon the colonist's perspective. Moreover, such identification overgeneralizes Native identity and makes it easier to misrepresent and underrepresent Native Americans in history. Nevertheless, for the sake of consistency with existing literature and comprehension for the colonial-wired mind, "Indian," "American Indian," "Native American," "indigenous American," and terms of a similar nature will be used in this essay.

Along with clustering many indigenous tribes under a single, umbrella term, the settlers also tended to emphasize what distinguished them from the Native Americans. Many of these distinctions continue to keep both narratives separate today. Colonists were accustomed to a definite and defined social structure that stressed the importance of settler expansion and development. Conversely, Native Americans lived in more egalitarian societies and maintained conscientious relationships with the environment (Rountree & Davidson, 1997). Their more environmentally conscious culture was quite foreign to the settlers who, consequently, perceived this Native way of life as "inarticulate" (Miles, 1992, p. 53), primitive, and in need of colonial reform. To many colonists, American Indians were considered "savage and uncivilized" and "not fully human" (D.G. Lewis, 2014, pp. 420, 422). Author D.G. Lewis (2014) stated that Native Americans were even "equated with wolves" by some of their white counterparts (p. 420).

As a result of these biased interpretations, many settlers assumed a sense of superiority that they felt entitled them to invade and take over Native land. Claiming the role of the "heroic pioneer," colonists worked to civilize and "save" indigenous peoples through Christianity, literacy, and assimilation into colonial life (D.G. Lewis, 2014, p. 416; Miner, 2015, p. 222). Any indigenous resistance was met with violence and

coercion. Declaring that Native peoples needed "settler governments" (Miner, 2015, p. 222) has served as justification for colonists' harsh actions and fueled incorrect beliefs about American Indians all the way into current times. Conveying Native American dependency on colonial governments in history today reiterates the ideal, selfless persona of the white settler and misrepresents Native populations, further contributing to the imbalance in historical presentation and the separation of narratives.

This disconnect between the Native American and colonial stories runs deeper as the Native narrative is restricted to the past. History has remained colonial-centric by upholding the centuries-old idea of the "last Indian" (D.G. Lewis, 2014, pp. 414-416) and the "dying Indian" (Rowse, 2014, pp. 15-16). Daehnke (2012) noted that European settlers arrived at a time when many indigenous populations were in recovery from outbreaks of disease. Moreover, settlers witnessed a decrease in Native American populations that were later attributed to colonial coastal interaction. The colonists, who felt animosity towards Native Americans, whom they viewed as "stumbling blocks in the development of the West" (D. R. Lewis, 1996, p. 213), came to interpret the demise of American Indians as inevitable. This misconception led colonists to sensationalize the unlucky fate of their Native neighbors to better justify colonial efforts to remove indigenous populations from the land. A "sad but true" attitude towards this aspect of U.S. history is expressed today as "the genocide and removal of Native Americans are seen as an unfortunate though nonetheless inevitable aspect of statebuilding" (McCue-Enser, 2020, p. 180). This public acceptance of Native death has ensured that people view indigenous Americans solely as a relic people of a separate past who possess no current relevance.

THE IMPACTS OF HISTORY ON PUBLIC ART

Public art and historical parks today are not immune to colonial bias. They have actually been known to reinforce these ahistorical beliefs, deepening the separation between the Native and colonial narratives. The Vancouver Land Bridge in Washington state is just one example of public art that shows how much control the colonist's point of view possesses over historical representation in the U.S. This land bridge, which overlooks the Columbia River, was one project of seven under the *Confluence Project*, an artistic endeavor aimed to increase public awareness of Pacific Northwest tribes and Native interaction with Lewis and

Clark (Daehnke, 2012). However, the art on the land bridge presented Native Americans in a manner that played into selective representation of indigenous history as it served the narrative of the white settler (Daehnke, 2012); the panels on the bridge depicted American Indians on barren, undeveloped land, supporting the misconception that Native Americans needed white settlement to advance as a society and that they were part of the American story primarily *before* colonial development. Both Native stereotypes and exclusion can also be seen in Maynard Dixon's 1912 painting titled *What an Indian Thinks* as the American Indian is shown in a natural, secluded, and seemingly uninhabited setting (Miles, 1992). Depicting Native Americans as intangible and isolated makes relating and connecting to their stories more difficult for the public. This inability to find commonalities prevents people from discovering the relevance of Native Americans in history and the world today and distances them from a more complete account of history.

NATIVE PRESENCE ON THE EASTERN SHORE OF MARYLAND TODAY

The Eastern Shore of Maryland is a prime example of a region whose modern-day indigenous presence reflects Native American underrepresentation in history. The region has a rich Native past; however, it receives very little public recognition (Rountree & Davidson, 1997). Rather, historical commemoration is catered towards the white colonist's narrative. For example, Pemberton Hall Historical Park, a colonial plantation in Salisbury, MD, receives visitors daily and is well-known by members of the community; conversely, there is limited acknowledgement of both the Rockawakin and Tundotank American Indian reservations which were also in the area (Rountree & Davidson, 1997). Kyle (1991) further articulated this issue, stating that the names of ESMD rivers and the Chesapeake Bay are some of the only indicators of the area's indigenous roots. What local efforts do remain to keep Native memory alive are quite contained, not garnering wide public attention or attendance. Such efforts include the annual Nanticoke Indian Powwow in Millsboro, DE (<http://www.nanticokeindians.org/>) and Chicone Village Day, which is located on an old Indian reservation in Vienna, MD.

Moreover, the Indigenous World's Tribal Directory, which helps locate active Native tribes in North America today, recognizes the Nanticoke tribe of the Eastern Shore but does not

provide any data about other tribes in the region such as the Pocomoke, Wicomico, and Assateague Indians, indicating their underrepresentation (<https://indigenousworld.org/indigenous-directory/>). Yet, according to Rountree and Davidson (1997), “descendants are [here], all over the Eastern Shore” (p. 215). The lack of active Native American voices on the Eastern Shore of Maryland is representative of the national problem of historical underrepresentation. The Native past of the Eastern Shore is currently advertised as a separate history whose public relevance is measured by the scope of its presence. Therefore, the Eastern Shore must take measures to teach the general public about its more complete history in a way that encourages acceptance and the mingling of narratives.

DEFINING RESISTANCE

“The endurance of exclusive narratives of white people and places... depends on the maintenance of silences” (McCue-Enser, 2020, p. 193). This quote from McCue-Enser’s (2020) analysis of colonial dominance in public art reveals both the need for and the method of reinstating Native voices in the country’s history and communities today. Decolonization, “the process of dismantling settler colonialism’s social, political, intellectual, and spiritual hegemony,” (Miner, 2015, p. 225) has been undertaken in the country today at multiple levels with a unified goal of restoring Native control over their own narrative in history (McCue-Enser, 2020). The Idle No More movement, which allies Indigenous and non-Indigenous people together to fight for Native rights and sovereignty, is a prime example of activism that has spawned from the suppression of indigenous voices at an international level (<https://idlenomore.ca/about-the-movement/>; Miner, 2015). The movement holds peaceful protests, marches, ceremonies, and more to bring awareness to Native history as well as environmental issues (<https://idlenomore.ca/about-the-movement/>). However, while larger-scale activism such as this is certainly impactful, many scholars have also indicated that providing localized educational opportunities that showcase Native American history in a creative and interactive way can be just as successful in reaching the people. The following methods could be adopted by the Eastern Shore of Maryland in their process of reframing local history.

INTERACTIVE, EDUCATIONAL SOLUTIONS FOR SUCCESSFUL REINTRODUCTION OF THE NATIVE NARRATIVE

One of the most powerful acts of “talking back to settler colonialism” (McCue-Enser, 2020, p. 180) is simply the acknowledgement of Native resilience through assimilation and their ability to adapt to the changing, developing world while still maintaining their indigenous identity (Rowse, 2014). Silliman (2005) observed this balance of “change and continuity” as being vital to Native survival and a more peaceful coexistence (p. 66). Miner (2015) agreed that both past and contemporary realities must be considered for American Indian voices to endure through a history that tends to favor the white, European settler (p. 226). Moreover, Smith (1999) noted that celebrating the survival of Native American culture through the centuries rather than solely recognizing their suffering aids in reintroducing the Native narrative in a positive, hopeful manner rather than in a penitent way (p. 77). For example, tribes all over the U.S. today choose to commemorate their heritage and strength through festivals, cultural awareness days, and historical demonstrations. When communities decide to embrace Native life rather than lament past suffering, others are able to see and understand the current relevance and prevalence of indigenous histories.

As mentioned earlier, a few small events are hosted annually on the Eastern Shore to keep the Native American presence alive and celebrate its resilience. However, they, like many other Native events around the country, are not well attended by members of the general public. Therefore, to gain more attention and support from the broader community, these interactive Native historical experiences must be better advertised and made more accessible to the public. For example, reaching communities through the public school system via school field trips to the Chicone village in Vienna, MD and the Nanticoke Indian Museum and annual powwow would help introduce this additional point of view to children at a younger age which would, according to Corntassel (2012), assist in broadening historical perspectives for future generations (p. 93). Providing people with opportunities such as these to interact with history’s many viewpoints helps them establish stronger connections with their homes and their place in history.

Another innovative technique that has integrated Native history into communities today is the establishment of what Miner (2015) called “reversed English-language text” signs (p. 232), a method

also supported by Corntassel (2012) and Smith (1999). Native American artist, Edgar Heap of Birds designed road signs that featured the names of “Native hosts” in a geographic area. Terms and names of places recognizable to the general public appear backwards on the signs to remind people that their perspective is not always a given (Smarthistory, 2020). Heap of Birds’ work encourages people to take a step back from their colonial-based perspective and look at the world from an outside point of view, allowing them room to realize the multitude of narratives land and history possess. Similar methods could be implemented on the Eastern Shore. In fact, efforts are already being made to acknowledge the region’s Native presence through signage. For example, providing educational plaques inside the dorms at Salisbury University that explain the indigenous relevance of the building names is one step towards increasing public awareness of the area’s Native past.

When one reconsiders alternative historical perspectives, one may also discover commonalities between narratives and beliefs. Calling upon community support over shared concerns can also encourage greater acceptance of the Native American narrative in an engaging, interactive way. Coincidentally, both Corntassel (2012) and Daehnke (2012) observed local projects that aimed to establish bonds between community members and Native ancestors of the land by acknowledging mutual beliefs regarding the environment. For example, one of the major goals of the *Confluence Project* (discussed earlier) was to increase awareness of the Native presence in the Columbia River region during the time of the Lewis and Clark expedition through acknowledging shared environmental worries (Daehnke, 2012). Though the project ended up reinforcing the “white savior” persona of the settler, its original intention was based on establishing common ground between two separated narratives. A more successful effort, the “Water Walkers” movement in Ontario, Canada, exemplifies successful unity and community engagement with Native history through united environmental concerns. The Water Walkers hold spiritual walks around Lake Superior to bring awareness to the pollution of bodies of water in the region (Corntassel, 2012). Native and non-Native people alike attend and participate in this event, acting in solidarity to overcome the separation of narratives and time.

Communities on the Eastern Shore of Maryland could host similar events that unite people over collective appreciation for the environment. The Eastern Shore is defined by its many waterways and forests,

many of which are named after Native tribes. Therefore, encouraging community participation in events that bridge the gap between indigenous and settler histories through current, shared environmental beliefs is quite possible on the Eastern Shore. In short, showing how people can interact with and relate to their homelands and other peoples in history encourages greater acknowledgement and acceptance of a more complete historical narrative.

Providing creative and interactive experiences is paramount to the successful reestablishment of Native voices in communities, as such involvement gives people the opportunity to voluntarily consider other voices in history. Miner (2015) noted an “intangibility of art” that grants it the power to more objectively and effectively introduce alternative perspectives to the public (p. 227). Art garners an innate respect from people by providing another lens for interpreting life without creating pressure. It encourages people to consider other viewpoints on their own account, resulting in more willing acceptance of additional narratives into history. Moreover, *sharing* history is necessary for people to understand the importance of Native history and presence today (Smith, 1999). And so, whether prompting a passerby to take a second glance at a road sign to interpret its meaning or providing younger generations with an Eastern Shore Native American cultural experience at a Native-curated historical park, powwow, or museum, educational opportunities that teach history in a creative, interactive, and innovative way encourage greater acceptance of Native perspectives. Further, they reveal the relevance of these narratives as they help establish a deeper connection between communities and the history of the land.

A WILLINGNESS AND WISH TO CONSIDER ALTERNATIVE HISTORICAL PERSPECTIVES

It is inevitable that such efforts to reframe American Indian voices in history will be met with some skepticism regarding their effectiveness. The odds of undoing the country's dominant colonial narrative to make room for the Native point of view, a storyline which contradicts the idea of the “heroic pioneer,” (D.G. Lewis, 2014, p. 416) are low. The reality is that history cannot be wholly undone; decades of history lessons and both explicit and implicit submission to colonial-centrism would have to be nullified. So, rather than change history, these suggestions are meant to provide a more complete version of history to the people. In fact, scholars have recognized a willingness in people to get history right, even if it risks muddying the pristine narrative of the white settler.

This desire can be witnessed in the revision of public art that once misrepresented Native Americans. For example, McCue-Enser (2020) observed a historical park in Minnesota called Murphy's Landing that had neglected its Native history. However, the park eventually changed its name from Murphy's Landing to simply The Landing to acknowledge the other people who had inhabited the area prior to and during colonial settlement (p. 188-189). McCue-Enser (2020) also reviewed *Scaffold*, a gallows structure that was rebuilt in 2017 to acknowledge the thirty-eight Dakota Indian lives lost in a mass execution in 1862 in Mankoto, Minnesota (p. 179). While the piece was meant to increase awareness of the area's Native history, many descendants of the Dakota tribe felt greatly pained having such a dark part of their history insensitively displayed without their input (McCue-Enser, 2020, p. 189). Native protesters immediately fought for the dismantlement of the structure out of respect for the Native Americans in the community. Eventually, the art center where the structure was located tore down the piece and began to host panels with American Indian artists to ensure adequate and accurate indigenous representation in future projects (McCue-Enser, 2020). Inclusion of both Native and non-Native voices in discussions of historical presentation ensures more accurate representation in U.S. history and provides the public with a fuller historical perspective.

People around the country are increasingly realizing the importance of acknowledging marginalized voices in history and are willing to work for a more collective historical narrative. Scholars have noted this elevated interest in the Native American story in academia. For example, D.G. Lewis (2014) recalled that “[o]ver the past forty years, more has been written about the tribes of Oregon than in the previous 130 years, with many authors utilizing Native perspectives and oral histories in their narratives” (p. 417). D.G. Lewis (2014) also predicted greater incorporation of this writing in public school systems (p. 417). The passion and concern for this effort is mounting across the country. In recent years, the fight to rename Columbus Day as Indigenous Peoples' Day has grown significantly. Eight states currently officially observe Indigenous Peoples' Day. The first state to adopt this change was South Dakota in 1990, and Virginia most recently recognized the new title in October 2020; eight more states have proclaimed their acknowledgement of the new name (Andrew et al., 2020). Likewise, individual communities around the country are embracing the switch in perspectives by

changing the name of the holiday. In fact, Salisbury, MD became one such town in October 2020 (Cechini, 2020). In an age where human rights and equality are prominent topics in the news and in the world, people are becoming more sensitive to the perspectives of others that have been overlooked and underrepresented for so long. So, in the words of D.G. Lewis (2014), “change is possible” (p. 419).

CONCLUSION

A historical account that focuses solely on the story of the white settler is unfinished. Separating the narratives of the American Indian and the colonist has only resulted in an ahistorical retelling of the past and has prolonged the battle for Native Americans to regain control of their own story. Miles (1992) observed that despite mounting evidence regarding the importance of the Native voice in U.S. history, many colonial-era historians failed to include these findings in their studies because presenting the indigenous and settler perspectives separately was almost impossible to convey to the public (p. 54-55). Therefore, it is paramount that the Native and colonial narratives be intertwined in historical presentation to successfully bring indigenous voices to the public consciousness and help people recognize Native importance in history. As Zimmerman (2010) aptly argued, “No group has a monopoly on truth itself” (p. 34). Scholars agree there is a need to compromise on a collective account of history to move forward peacefully, knowing a more complete and accurate history (Miner, 2015, 232; Silliman, 2005). McCue-Enser (2020) eloquently summarized the value in weaving narratives, calling attention to “[the] deeper understanding of place [that] emerges when focus is placed not on a singular narrative of place but rather on a collection or cacophony of voices” (p. 194). This can be achieved through creative, local acts of resurgence that *encourage* rather than *coerce* people to consider additional points of view in history by overlapping the narratives. By acknowledging multiple historical perspectives, people from the Eastern Shore of Maryland and across the country are given the opportunity to develop a deeper connection with the history of their homes and all of its voices.

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NAVIGATING MATERNITY CARE: AN ANALYSIS OF DOULAS' EXPERIENCES DURING COVID-19

N'Tumahawa Kamara

ABSTRACT

A doula is a trained childbirth assistant who provides emotional, physical, and informational support to a pregnant woman before, during, and shortly after childbirth. This professional has been around for centuries, initially assuming the role of the laboring woman's lay companion during her home birth. In recent years, doulas have broadened their scope of practice outside of the home and into hospitals and birth centers, with an added commitment to advocacy. Before COVID-19, doulas were making strides toward improving maternal and infant health outcomes, such as lowering cesarean section rates and low birth weight (LBW) births and increasing the woman's satisfaction with her birth experience. However, there is insufficient data on the impact of COVID-19 on doulas' work within the birth setting. This qualitative study examines the context in which doulas provide maternity services and the professional barriers they faced during the COVID-19 pandemic. Snowball sampling was used to recruit prenatal/birth and postpartum doulas who were over 18 years old and practicing in Maryland. Participants took a 14-item survey on their professional doula history and discussed 17 open-ended questions on Zoom about their experiences during COVID-19. This study provides insight into the state of maternity care in Maryland and the challenges doulas faced in supporting pregnant and laboring women during COVID-19.

BACKGROUND

While we are familiar with the impact of COVID-19 on the larger population, the direct and indirect effects on pregnant women remain largely unknown. Findings reveal pregnant women are at increased risk of severe illness from COVID-19, especially women of advanced maternal age, those who are overweight/obese, or have pre-existing comorbidities (Ellington et al., 2020). At the height of COVID-19, infection control policies and procedures were instituted in maternity care to limit exposure between patients, visitors, and staff (Arora et al., 2020). While such policies have been effective mitigation strategies for public health, the consequences to pregnancy and birth outcomes are undetermined.

Reported changes to maternity care included reduced frequency of prenatal visits from approximately every four weeks to every 8-10 weeks (for a normal low-risk pregnancy), with interim telehealth check-ins (McSpedon, 2020). Many labor and delivery units also instituted a modified "no-visitor" policy, restricting each patient to having one adult visitor (Arora et al., 2020; Weiner et al., 2020). While some locations allowed trained labor support professionals such as doulas in addition to a support person (Weiner et al., 2020), others banned visitors entirely (Arora et al., 2020; Weiner et al., 2020). Thus, many women were forced to choose between having familial support and professional labor support or none (Pfeiffer, 2020). Such restrictions may have significant implications for low-income women and

women of color who are at increased risk of adverse birth experiences and outcomes (Thomas et al., 2017).

The literature details the effects of receiving professional support during labor on maternal well-being and birth outcomes. This evidence shows that labor support, exclusively provided by doulas, reduces operative vaginal delivery and cesarean sections (Muza, 2017), reduces pain and need for analgesics (Fortier, 2015; McGrath, 2008), and increases satisfaction with the birth experience (Wen et al., 2010). Doula care also lowers the incidence of low-birth-weight babies and increases breastfeeding initiation (Gruber et al., 2013). As clinical and ethical guidelines evolve during COVID-19, pregnant women are in significant need of support while navigating motherhood (Davis-Floyd et al., 2020). Doulas are ideally suited for this task as they provide continuous, individualized care to birthing persons and their partners to meet their informational, physical, and emotional support needs (Dekker, 2013). Nevertheless, there is limited data on the capacity of doulas to practice during COVID-19.

LITERATURE REVIEW

HISTORICAL PERSPECTIVE OF CHILDBIRTH: FROM THE HOME TO HOSPITALS

Until the 20th century, births took place mainly in the home and were attended by female relatives and friends

and a midwife with no formal training (Dye, 1980). Historically, midwives were a diverse group of local women who were usually married with children and acquired their skills through informal apprenticeships with more established midwives (Dye, 1980; Kaplan, 2012). During labor, midwives employed pharmacological comfort measures to alleviate pain and speed labor (Dye, 1980), assisting the delivery in its natural course. With this minimal approach to birth, morbidity and mortality rates were high, and labor was a dreadful ordeal for the woman (Dye, 1980; Kaplan, 2012).

American women first invited male physicians into birthing rooms in the 1760s (Kaplan, 2012). Previously, male physicians were rarely present at births except in life-threatening emergencies (Kaplan, 2012). The development of obstetrical science transformed childbirth into a standardized process, which was subsequently transferred to hospitals (Kaplan, 2012). This move effectively barred out traditional midwives, although they still managed a large percentage of births well into the 1900s (Kaplan, 2012). From the turn of the century, women increasingly opted to deliver with physicians over midwives, with in-hospital physician-assisted births becoming the dominant source of maternity care (Dye, 1980).

TRADITIONAL MATERNITY CARE VS. DOULA MODEL OF CARE

Skilled care at childbirth is a vital aspect of maternity care occurring at

the culmination of pregnancy. This care comprises integrated medical efforts intended for the fetus' safe delivery and the mother's return to a non-pregnant state. Childbirth is managed primarily in hospitals by physicians and Certified Nurse-Midwives (CNMs) or Certified Midwives (CMs) (MacDorman & Declercq, 2019). Fewer childbirths occur in out-of-hospital locations, but this number is increasing as more women seek to control their birth experience (MacDorman & Declercq, 2019). The shift from hospital-centered births coincides with the professionalization of alternative models of maternity care that emphasize low-intervention approaches and informed decision-making for the birthing person and family.

The doula model of care fosters a patient-centric approach to maternity care in which doulas provide continuous, non-clinical support to the woman so that she achieves the childbirth she desires (DONA International, 2016). At the core of this model is a commitment to advocacy and empowering women to control their birth. Doula support is associated with improved maternal health outcomes and lower rates of medical intervention during childbirth (Essex & Pickett, 2008). Despite the benefits, doula-assisted births are relatively few in the U.S. In a survey of 2,400 American mothers, 6 percent of women reported having received doula support between 2011 and 2012 (Declercq et al., 2013). Of those who did not have a doula but were aware of what they were, 27% would have liked to have a doula (Declercq et al., 2013).

Women of color are significantly less likely to utilize doula support services, although they may benefit meaningfully from receiving such care (March of Dimes, 2019). According to Kozhimannil et al. (2013), limited health insurance coverage is a likely barrier to doula care. While Medicaid funding is a potential means for making doula care accessible to pregnant people, there are overriding concerns over a loss of autonomy and exclusion of socially marginalized birth workers if the government or health institutions become involved (American Progress, 2020). Additionally, there is no federal regulation of the doula profession, and certification is not required to become a doula (Lantz et al., 2005). The lack of required certification allows more women to become doulas without financial hardship but may make it challenging to integrate into official birth settings and become accessible to more patients. Regardless of the reason, this study found that doulas are experiencing even more resistance to their inclusion within birth spaces.

MATERNAL HEALTH IN MARYLAND BEFORE COVID-19

In Maryland, the maternal mortality rate was 31.3 deaths per 100,000 live births in 2017 (Hogan et al., 2020). This rate was above the Healthy People 2020 Objective MICH-5 target of 11.4 maternal deaths for every 100,000 live births. A considerable racial disparity exists in maternal death rates, with Black women four times more likely to die from a pregnancy-related complication than White women (Hogan et al., 2020). During 2013-2017, the maternal mortality average for Black women was 44.7 compared to 11.3 for White women (Hogan et al., 2020). The rate of Black maternal death was also higher than that of all racial groups in Maryland (24.8 per 100,000 live births). Black women's high risk of mortality is indicative of a general lack of health equity spanning income and education levels (Wint et al., 2019). The ongoing legacy of racial injustice, bias, and healthcare disparities also persists in shaping Black women's reproductive life course and healthcare experiences.

Since COVID-19, some racial and ethnic groups have experienced higher infection, hospitalization, and death rates (Rubin-Miller et al., 2020). The national infection rate for Hispanic patients was over three times higher than the rate in White patients and over two times as high for Black patients (Rubin-Miller et al., 2020). Lopez et al. (2020) attributed these disparities to the socioeconomic conditions of ethnic minorities, which is usually typified by challenges including inadequate healthcare coverage, greater comorbidities, and financial insecurity. COVID-19 magnifies and combines ongoing maternal health issues with the vulnerabilities of certain racial and ethnic groups to public health emergencies. There is a potentially transformative role of doula support when considering the current state of maternal health in Maryland and the current and future impacts of COVID-19.

RESEARCH PURPOSE

This qualitative study examines the context in which doulas offer maternity services and the professional barriers they encounter during COVID-19. The effects of COVID-19 on pregnant women are evident and require the integrated efforts of birth professionals to address. Also, as COVID-19 persists in shaping the care process, doulas are increasingly essential to women's navigation of maternity care. By understanding the challenges within the doula profession and the barriers to rendering services, recommendations can be made to support doulas during COVID-19 and future pandemics. Findings might also aid in further integrating doulas into the birth setting for

improved birth outcomes.

The following research questions guided this study:

RQ1: How has COVID-19 impacted the doula's provision of maternity services?

RQ2: Has COVID-19 affected the integration of doulas into the birth setting?

METHODOLOGY

TARGET POPULATION

This study was a survey of prenatal/birth and postpartum doulas in Maryland. The study population consisted of doulas who were over 18 years old and practiced in Maryland during the COVID-19 pandemic. Fluency in the English language was an additional qualifier for participation.

RECRUITMENT STRATEGY

Participants were recruited between February and March 2021 through snowball sampling. Snowball sampling is a nonprobability sampling technique in which primary research participants refer future participants to be included in the study (Tenzek, 2017). While snowball sampling cannot be generalized to a population, it can be used for conducting qualitative research with a specific or relatively sparse population that is hard to locate (Kircherr & Charles, 2018).

In late January 2021, the principal investigator sent an email with a recruitment flyer to potential study participants in her network. The email solicited doulas to contact the student researcher if they were interested in the study and share the attachment with acquaintances who met the study criteria. In addition, the student researcher reached out to doula organizations in Maryland via their Facebook pages and asked them to post the recruitment flyer on their page or forward it to doulas within their organization. The recruitment flyer and a short message were posted on the Facebook group page, Doulas Serving All Clients in MD, DC, and Virginia, a group with which the student researcher is affiliated. The PI also made an announcement during a meeting with a Prince George's County breastfeeding coalition, garnering additional participants.

SAMPLE SIZE AND DATA SATURATION

Data saturation is a methodological principle for arriving at a sample size in qualitative studies (Creswell, 2009). Guest et al. (2006) defines data saturation as the point in data collection and analysis when the ability to obtain additional new information is unlikely. The authors also

affirm that saturation occurs within the first twelve interviews, with new themes emerging less frequently thereafter (Guest et al., 2006). Thus, a sample size of 12 participants was determined to be adequate for this study.

Uniformity was maintained in this study using semi structured interviews comprising 17 opened-ended questions specific to four key areas: changes in practice, COVID-19 policies, interactions with maternity care providers, and access to professional development resources. Participant homogeneity was also attained through the standard criterion that participants were doulas who practiced in Maryland during COVID-19 (Guest et al., 2006). These similarities exhausted new apparent themes within less than twelve interviews.

DATA COLLECTION INSTRUMENT/SOURCES OF DATA

Data was collected through a 14-item online survey and semi-structured interviews conducted via Zoom. The student researcher developed the survey after reviewing the literature for the meaning of a doula and their role in maternity care and childbirth. The survey was administered in Qualtrics, an online survey software. Survey questions were close-ended and open-ended and inquired about the doula's sociodemographic characteristics, practice setting, and clients' demographic characteristics. Similarly, the literature was consulted in the development of interview questions, which explored the impact of COVID-19 on doulas providing maternity services in Maryland.

At the start of the study, participants were emailed a link to a survey and a unique five-digit identifier used in survey Item 2. Upon completing the first phase, participants were emailed to schedule a 45-to-60-minute interview to discuss 17 open-ended questions about their experiences during COVID-19. All interviews were recorded for transcription and data analysis.

A consent form was presented both visually and verbally with details to maintain confidentiality to the highest level. Participants were informed of potential risks. They were also provided with the choice to opt out of the survey and interview.

DATA ANALYSIS

Survey data were analyzed using basic descriptive statistics to summarize the characteristics of the doulas who participated in the study. Coding and thematic analysis were used to extract and report themes from interview transcripts following the six phases stipulated by Braun & Clark (2008), which are (1)

TABLE 1
Socioeconomic Data of Participants

Participant	Race	Ethnicity	Gender	Level of Education
D1	Black/African American	Non-Hispanic	Female	Master's
D2	Black/African American	Non-Hispanic	Female	Bachelor's
D3	White	Non-Hispanic	Female	Master's
D4	Black/African American	Non-Hispanic	Female	Trade School
D5	Bi-racial/Multiracial	Hispanic	Female	Bachelor's
D6	White	Non-Hispanic	Female	Bachelor's
D7	Black/African American	Non-Hispanic	Female	Master's
D8	Bi-racial/Multiracial	Non-Hispanic	Female	Other
D9	Black/African American	Non-Hispanic	Female	Master's
D10	Black/African American	Non-Hispanic	Female	Other

TABLE 2
Participant Practice Characteristics

Participant	Type of Doula	Years as a Doula	Birth Setting of Work	Certification Status	Geographic Area Served
D1	Both	4.5	All	Yes	Urban
D2	Prenatal/birth	3	Hospital	No	Urban
D3	Both	4	All	Yes and no	Urban
D4	Both	5	Hospital	No	Urban
D5	Prenatal/birth	2	Hospital	Waiting for certification	Urban
D6	Both	5	Hospital	No	Urban
D7	Prenatal/birth	2	Hospital	Yes	Urban
D8	Both	34	Hospital	Yes	Urban
D9	Both	3	Hospital	Yes	Suburban
D10	Prenatal/birth	4	All	Yes	Suburban

Familiarization with the data, (2) Coding, (3) Generating themes, (4) Reviewing themes, (5) Defining and naming themes, and (6) Producing a report. Interviews were transcribed through intelligent verbatim transcription, which involved the removal of filler words, repetition, and false starts from the transcript. This level of transcription was utilized to improve the readability of participants' responses. Since thematic analysis is employed in identifying common ideas from data, it was determined that a verbatim transcript

was not needed (Halcomb & Davidson, 2006). For interviews where the PI was the lead interviewer, the student researcher took notes and compared those notes to the recording to look for dependability. Descriptive analysis was conducted using SPSS Version 26.

RESULTS

SURVEY DATA

Sociodemographic data were

collected on participants' biological sex, race/ethnicity, and highest level of education completed. Information was also collected on practice characteristics, including the type of doula services provided, the birth setting worked in, health professionals primarily worked with, certification status, county/ies of practice, race/ethnicity of clients, and geographical area served. Table 1 displays the sociodemographic data of the study participants. Table 2 displays participant practice characteristics.

INTERVIEW DATA

Six emergent themes were found after the analysis of transcripts from doula interviews. The results are presented in alignment with each research question.

RQ 1: How has COVID-19 Impacted the Doula's Provision of Maternity Services?

COVID-19 impacted the provision of services by all doulas. Most reported a change in the type of support they provide, noting an effect on their interactions with clients. Doulas also reported having a greater dependence on information resources provided by national organizations for navigating the COVID-19 environment. The following themes emerged.

Changed Interactions with Client

Some doulas reported providing increased guidance and reassurance to clients during COVID-19. D2 reported that there was *"a change in support in terms of families needing more [support] because they don't have their own support circle to pull from as easily as we have before the pandemic."* She also noted that many clients *"need more hand holding than before"* because of the unprecedented nature of COVID-19 and the uncertainties accompanying the changes within maternity care. Likewise, D1 reported providing a lot of COVID-19 related reassurance regarding unknowns about transmission while birthing in hospitals and the protocols clients were expected to comply with inside the labor and delivery room. D3 noted that change in support was dependent on her clients, asserting *"Some clients have had more anxiety and stress around the pandemic than others, and so, just like normal doula care, people require different amounts of support."*

Similarly, D4 and D6 reported providing virtual support in the labor and delivery for clients birthing in hospitals restricting access to doulas. D8 also offered partial virtual packages to clients who wanted in-home care before or after they labored in the hospital. D9 formed a partnership with a maternal psychotherapist. D9 explained:

I have a partnership now with a maternal psychotherapist just to discuss how the pandemic has been like if my clients are interested in discussing how the pandemic may have had an effect on their pregnancy, how it had an effect on their relationship, relationship with their partner, relationship with their other children, how they feel about being pregnant during a pandemic, just so they can hash out any traumas or things that they need to work through.

Wider Access to Information

All participants reported obtaining information from several national and international organizations. The most named were Evidence-Based Birth, the American College of Obstetricians and Gynecologists, National Black Doula Association, World Health Organization, and DONA International. Most doulas used a combination of these organizations to obtain COVID-19 related information and evidence-based information for supporting their clients. Doulas also reported using community knowledge, such as social media and other maternity care professionals, to inform their practice. D9 explained, *"I utilize a lot of different well known social medical profiles so maybe midwives, they may be other doulas, it may be lactation consultants, just depending on what they are, but social media helps a lot..."* D7 reported partaking in educational seminars after noting a rise in stillbirths at the beginning of COVID-19. D10 also mentioned turning to her doula family network for guidance, stating, *"I am blessed to have so many doula family members that I can reach out to and look up to and they have all been mentors to me..."*

Initial Difficulty Obtaining PPE

Except for D2, most participants reported unavailability of personal protective equipment, such as masks, gloves, and sanitary products. D3 also reported being discouraged with obtaining N95 masks because of recommendations that they should be kept for providers who had to be around people with COVID-19. Some doulas were able to supplement their doula PPE with PPE from their other line of work. For instance, D6 stated, *"I also at the time worked part time in a clinic, and so I was able to get surgical masks for myself."* D6, D1, D7, and D8 reported receiving donated masks or creating their own to offset the scarcity. D7 stated, *"When cloth masks started being more consistently used a lot of the team started having people sew them. So, people sewed us masks and sent the doulas masks."* As the pandemic progressed, most doulas reported having greater access to PPE as online stores began to restock. However, a few doulas noted an uptick in the prices. D7 shared her experience buying gloves

once they became available, stating that one pack of gloves cost 40 dollars to purchase.

RQ 2: Has COVID-19 Affected the Integration of Doulas into the Birth Setting?

All 10 participants reported being restricted in the birth setting, especially in hospitals. Limiting policies prevented some doulas from providing in-person support at hospitals. Also, doulas reported that a strained relationship with some maternity care providers in the hospital setting further marginalized doulas, specifically through hospital-adopted policies. The emerging themes are highlighted below.

Restrictive Hospital Policies

Most doulas reported being restricted in their access to hospitals at some point during COVID-19. Respondents were initially banned from the labor and delivery room under a no-visitor policy and were later inhibited by the institution of the one support person policy by some hospitals.

D2 stated:

In the beginning, it was pretty much a full shut down where it was like the only people present or can be present is the person's partner, and it had to be one partner. And it's even worse if that person was positive for COVID because they didn't allow their partner to be present either.

D9 also explained:

Some hospitals are going back and forth between allowing doulas and not allowing doulas. Some hospitals have strictly been no doulas from the beginning, and some are like slowly opening back up to allowing doulas to come in just based on the [rise and fall of] cases.

When they could be physically present in labor and delivery, some doulas faced additional limitations. D3 and D7 reported not being able to leave the labor and delivery room once inside.

D3 stated:

A few [hospitals] had policies where we couldn't change so if a doula came—if I came to a birth and then I was at the birth for 30 hours, I couldn't have another doula come, which is typical practice in doula care.

D7 reported an internal policy change by her group practice in response to the ban on backup doulas. Her group practice changed their policy to providing 18 hours of in-person support and switching to virtual support with a backup doula if the doula on duty can no longer function.

A few doulas reported policies that targeted certain doulas from being admitted by some hospitals. D3 stated, *"Quite a few [hospitals] have required certification*

and not only just certification, but certification from a group they know, and think is legitimate, which is obviously really problematic in terms of gatekeeping for the profession.” Although D10 was not directly affected by the certification requirement, she knew family members without certification who were hindered by the new policy. D1 and D4 also mentioned the establishment of in-hospital doula programs in selected hospitals, both noting that the development was a conflict of interest. D1 asserted, “the hospital doula program is contradictory because as doulas we work for the birthing person, we don’t work for the hospitals.”

Varying Acceptance by Maternity Care Providers

Some doulas reported a lack of acceptance in general from maternity care providers. D8 reported, “I’m [going to] say from my experience, about 75% in the medical field still are not accepting of doulas.” D8 goes on to explain, “the role of the doula has been seen as adversarial because we are giving people back the right to control their own body and I think nurses, midwives, and doctors, they go about things very methodically.” Respondents also indicated acceptance by maternity care providers depended on the provider and birth setting. D6 explained, “I think midwives are a lot more open to doulas and generally have a more positive outlook on doulas than obstetricians do. Nurses are like hit or miss; I don’t know.” A few doulas speculated on the reason for this reaction from some care providers, with D7 stating, “I know some OBs have had doulas who are very opinionated and pushy and so they have a bad taste [of doulas].”

Others expressed that COVID-19 may have been used as an excuse to keep doulas out of hospitals. D6 noted, “With COVID it’s created this opportunity for hospitals to create biased policies that are anti-doula and I think some hospitals have been wanting to do this for years, and finally the opportunity has presented itself.” D4 also stated, “I feel like COVID may have been used I guess kind of as an excuse to keep doulas out of the birth space because of whatever preconceived notions or whatever some providers have about us.”

Altered Communication with Maternity Care Providers

Respondents mentioned changes in their communication with maternity care providers during COVID-19. A few reported contacting hospitals to inquire about policies. D7 stated, “At the beginning we did a lot of checking in with nurse administrators to try to figure out what the policy was that day, because there was like an entire month where policy changed every day.” D8 also explained, “We would call doctor’s offices that we had clients coming up for and say, ‘hey, so we have so and so due in 3 weeks, and we want to make sure we’re still welcomed or what the PPE policy is.” D2 noted a change in the tone

TABLE 3
Summary of Main Findings

Changed interactions with clients	Wider access to information	Initial difficulty obtaining PPE	Restrictive hospital policies	Varying acceptance by maternity care providers	Altered communication with maternity care providers
D4 A lot of the support has moved to virtual platform	D1 Look to bigger organizations	D3 Discouraged trying to find N95 masks	D5 Initially restricted from being in person	D2 Challenges between doulas and medical staff in general	D1 A lot more communication during COVID
D5 Prenatal appointments had to be almost completely through Zoom	D2 Shared information gathered from different settings	D10 Challenging to get a lot of the equipment	D7 All hospitals in Maryland are single person	D1 Home birth setting was a more inviting environment during COVID	D7 Communicating more with other parts of the care team
D2 Clients need more hand holding	D8 About any set of information online	D8 Short for a little bit	D6 One visitor only policy	D6 Don’t think OBs love doulas	D2 More contact than before in terms of needing to know updates
D4 Prepare them to not have me present	D7 Community knowledge	D9 At first it was really hard to find masks	D2 Specific doulas with specific certifications	D7 Administrators don’t really think about us	D6 Communicating with staff through clients
D9 Physical support has changed the most	D10 Access to the world wide web	D6 Received cloth masks from donations	D6 No clear timeline for allowing doulas back	D9 Some are really doula friendly	D8 Midwives and OBs never seem to know hospital policy
	D9 Have to comb through to find general baseline information	D7 Obtaining them was expensive and difficult	D5 Initially restricted from being in person	D10 Some providers are extremely welcoming and thankful	D2 Making people aware of bias, rudeness, abuse

of her conversations with maternity care providers. She stated, “We are not in a great position where people are being cared for anymore, because we’re limited, and so we really have to have those very direct and open conversations about the implications of the care that we’re giving.”

A few doulas also reported being unable to build a relationship with staff during COVID-19. D1 stated, “Before I would be able to you know do some research on the provider, talk to them a little bit more, develop a relationship, but with COVID-19 a lot of things are restricted, and people are really pressed for time.” In contrast, D7 experienced an increased communication with other parts of the care team. She stated, “So, lactation consultants, [I] talk to postpartum doulas a lot more often than I did pre-pandemic and more of that is just trying to make sure people have a support network around them when they’re ready to go home.”

Table 3 outlines a summary of the main findings.

LIMITATION OF STUDY

This study has several potential limitations. First, participants were recruited from a convenience sample of doulas practicing mainly in urban areas in Maryland. Because the geographic scope of participants was limited, the findings largely exclude doulas working in suburban and rural areas, who may have had different experiences during COVID-19. Findings are therefore not generalizable to all doulas in the State of Maryland. There is also a potential that respondents answered interview questions based on what they felt the interviewer wanted to hear instead of what occurred. This is known as social desirability and is considered a threat to external validity. Lastly, interview transcripts were coded by the student researcher alone. The final themes were subjected to the purview of

the student researcher and the limits of the coding methods employed. Other pressing themes may have been less apparent in the reading of transcripts. Consequently, themes may be interpreted differently by another person reviewing the transcripts. Despite these limitations, this study can be used as a steppingstone for a more extensive survey of the doula population in Maryland to provide an in-depth view of maternity care during COVID-19.

CONCLUSION

This study sought to answer two research questions 1) How has COVID-19 impacted the doula’s provision of maternity services, and 2) Has COVID-19 affected the integration of doulas into the birth setting. Doulas confronted professional barriers within the birth setting even before COVID-19. Ballen & Fulcher (2006) observed an adversarial relationship between doulas and maternity care providers stemming from a misunderstanding of doulas’ role. Kozhimannil et al. (2013) also noted a lack of third-party reimbursement for doula care, despite the documented benefits of continuous labor support. Also, as doulas are an unregulated entity, they are susceptible to policies at all levels of the birth setting. The certification requirement implemented by some hospitals in Maryland is a notable example. Several study participants asserted that this emerging criterion was ostensibly discriminatory and a representative attempt to gatekeep the profession.

Barriers to doula care have significant implications for both pregnant women and doulas. For pregnant women, the inability to have a doula present with them at birth is an additional way in which control over their birth is diminished. Having a doula in the labor and delivery room allows

the birthing woman to raise important questions and adequately meet her birth plan. It also brings informed consent and the rights of birthing people into the equation so that the doula can facilitate a positive birth outcome and satisfying birth experience. The restrictions of doulas in labor and delivery may also affect doulas' professional autonomy and satisfaction as they tackle barriers to supporting their

clients during birth. The return of control to the pregnant woman is fundamental to the key values held by doulas. During COVID-19, many doulas were unable to accompany their client in the hospital, which essentially stripped away the doula's advocacy role.

The value of doulas to maternal health is even more apparent as families deal with the aftereffects of COVID-19

and the changing birth space. While not the only answer to improving maternity care, doulas are crucially important to birth outcomes and experiences. The findings of this study demonstrate the marginalization of doulas, specifically in hospitals. To combat this, policymakers across all birth settings must recognize the importance of doulas and include them in future policy decisions.

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URBAN CULTURE

Eric Johnson Jr.

ABSTRACT

The two photos collectively titled “Urban Culture” were shot with a Nikon D3400 camera and edited using Adobe Photoshop. Urban Culture is the heart that makes fashion so unique, and its urban style tends to be a mixture of casual and hip. It is about sub-culture rather than major trends and has roots in the sports and music movements. In other words, it is not always about where you purchase your clothes, but rather how you put things together to best fit you.

I consider the urban style to be a reflection of your lifestyle, attitude, and individuality. In life, these are the main characteristics that people tend to search for in an individual. The graffiti wall was the perfect background for this photo shoot, because all the different colors, shapes, and art compliment the unique styles and accessory combinations found within urban culture. Jeans, jewelry, sneakers, t-shirts, and so much more are used. Unlike mainstream fashion, anything comes into play in the urban fashion world.

Fashion photography allows me to showcase feelings, integrity, and dedication in my work and everything else that I produce. I never wanted to be part of any table, so I decided to build my own. This is just the beginning. Please enjoy my take on urban culture.



ZACK SNYDER'S JUSTICE LEAGUE: A PARATEXTUAL HERO'S JOURNEY

Jacob Beaver

Excerpt from Honors Thesis:

“Zack Snyder’s Justice League: A Case Study of the Auteur in Contemporary Cinema”

ABSTRACT

How does an auteur director establish relevance through a marketable strategy in today’s entertainment environment, and how is Zack Snyder’s *Justice League* an example of a modern auteur platform? Research to evaluate this phenomenon and situate director Zack Snyder within this environment included exploring film history to establish the past dynamic between auteurs and studios, investigating the events surrounding 2017’s *Justice League*, defining Zack Snyder as an auteur, and evaluating modern cinema. The research methodology includes a review of scholarly sources regarding the studio system, auteur theory, and the evolving entertainment landscape. I drew upon news sites to construct a general timeline of events regarding *Justice League*. Screen captures of films, graphs containing quantitative data, diagrams constructed to represent key concepts, and social media posts are included for visual reference. Results of research included the identification of Snyder’s auteur signature and a paratext use by the director to popularize the Snyder Cut of *Justice League*. The current entertainment era is defined within this thesis as being separated from past film history thanks to new mediums that have expanded their cultural relevance. Findings have led to a clear methodology that may be used by auteurs to remain relevant when the idea of a solo artist is challenged by the success of an executive creative process. Furthermore, my findings have spurred a paradigm in which auteurs and popular films can be examined and placed within the new status quo set by franchises fueled by a new studio system.

Entertainment has reached new heights of technological advancements and narrative scope in the 21st century during a golden age of superhero films. The genre has evolved from standalone entries into culturally celebrated cinematic events that have taken various characters from comic books and brought them to general audiences. *Justice League* (2017) is a film that spawned from this new era, adapting the DC Comics superhero team of the same name in live action for the first time. The movie’s director was Zack Snyder, who helmed previous films in an interconnected cinematic universe leading up to *Justice League*. However, the filmmaker left production when family tragedy struck. The creative team ordered by Warner Bros. Pictures, which included *The Avengers* (2012) director Joss Whedon, made significant changes to the film before it was delivered to theaters (“Zack Snyder Steps Down”). Audiences loyal to Snyder responded to what they perceived as alterations by forming an online movement. Campaigns on various platforms called for a version that aligned with what they believed the filmmaker intended to deliver.

In an era where cinema has a heightened cultural presence thanks to an increased focus on a robust and executive-led studio system, what place or platforms exist for the lone artist? The theory of the auteur is a concept involving artists who incorporate personal signatures in their films, resembling the individual

creativity of writing. The theory arose in the cultural aftermath of World War II after longstanding cultural institutions and myths in various artforms, including cinema, were rejected (Corrigan, *Film and Literature* 25). According to critic Andrew Sarris, an auteur’s personal expression could be crafted through two schools of thought (Sarris). The first was thematic motifs, which involved narrative themes repeatedly appearing in a director’s filmography. For example, their films may often focus on the theme of death. The second was style and mise-en-scène (scenery and surroundings). For example, a director’s films may often take place in an urban setting (Wollen 590).

During the early days of cinema, an auteur implemented their personal signature within films in resistance towards the studio system. This was a leadership concept that arose in the 1920s alongside scientific management, which introduced executive hierarchies seen in modern film studios. During this time, directors were not the head of production, but rather part of a “coordinated and well-regulated apparatus” (Regev 597). However, the auteur concept dramatically shifted in the blockbuster era of the 1970s. Film scholar Timothy Corrigan argued that in such a commercial age, the theory of the auteur became less focused on the romantic ideal of the individual artist and rather a concept used in the promotion of films. Instead of being an authored personality staged against the confines

of a studio system, auteurs in this new context were now stars themselves within the Hollywood industry (Corrigan, “The Commerce of Auteurism” 48- 49).

Zack Snyder is a modern auteur with a signature that uses visual techniques and trademarks to alter the audience’s experience with his films. For example, his 2006 film *300* was shot at no slower than fifty frames per second, allowing for slow motion and speed-ramping to be inserted into any part of the film during the editing process. Rather than using simple slow motion to extend the temporal experience of shots, speed ramping alters the framing of action sequences. This replicates the panel-to-panel reading process within graphic novels where readers are meant to connect the actions that happen in each still image per panel to develop the overall story. Snyder, who oversaw the entire editing process and closely collaborated with the visual effects team for the movie, selected single shots that were slowed down before then being sped up again and followed by another slow-motion shot. The way audiences experience these slowed-down shots compared to the sped-up ones is meant to imitate the way a comic book artist would select single images in panels to be presented to readers (see fig. 1) (Jeffries, 274-276, 280).

There is a journey associated with Snyder’s personal interpretation of heroism that can be traced back to the works of mythologist Joseph Campbell. The hero’s journey is a concept



Figure 1: A constructed diagram showing how Snyder's use of slow motion and speed ramping mimics the panel-to-panel structure of comic books.

developed by the scholar that focuses on an archetypal narrative endured by fictional characters throughout ancient and modern stories. The journey can be simplified down to four stages. The Natural Environment or State of Being is a character's center and original purpose or existence. The Call to Adventure introduces a problem or opportunity which presents a quest that will carry the hero away from the Natural Environment. The Threshold of Adventure introduces a mentor for the hero and a series of challenging lessons and tests. The Resurrection allows the hero to return to his or her Natural Environment with newfound lessons learned from the mentor and challenges (Lee 155). This concept is one Snyder is known to admire and apply to his films, especially ones with heroes from comic book literature (see fig. 2).

The hero's journey as a storytelling template is not revolutionary. It has impacted popular franchises such as Harry Potter, The Lord of the Rings,

and interaction, separate his use of it from other directors.

Justice League, Zack Snyder, and the culture surrounding the Snyder Cut forms one cohesive narrative constructed by various elements encouraged by Snyder himself and his fans. There are heroes, villains, and a driving plot. It is a story that allowed Snyder to separate himself from the 2017 theatrical cut. Furthermore, this narrative allowed for the subsequent marketing of his version of the film, and the movement around it, into a product produced by Warner Bros. Pictures and HBO Max. While the commercial value of the auteur director was introduced as a concept by film scholar Timothy Corrigan, there is a paratext that transformed *Zack Snyder's Justice League* (2021) into a product beyond a single film. Paratext in its most literal form is defined as any item attached to a novel that is not the textual body of literature itself. Simple examples include the author's name or publication date (Hobbs 21). These details can be closely

of the actual watching experience for audiences (22). For example, a movie such as *Raiders of the Lost Ark* (1980) could have a special edition Blu-ray released years after its theatrical run with a completely different design from original promotional material. Paratext has helped redefine what cinema as a medium and product contains beyond a film itself, as well as how audiences interact with motion pictures.

Zack Snyder and *Justice League* evolved the cinematic paratext concept. Merchandise is an element that can exist alongside film and allows the audience to form a relationship with it by interacting with a physical representation of on-screen characters, props, or locations (Hobbs 22). Following the release of the theatrical cut of *Justice League*, Snyder designed t-shirts sold on the Ink to the People website that occasionally included cryptic messages (see fig. 3). While cryptic elements are essential to the paratexts of *Zack Snyder's Justice League*, the context here is that merchandise was being produced for the version of a film never released. All monetary proceeds from sales went towards either the apparel manufacturer Ink to the People or the American Foundation for Suicide Prevention, but Snyder's intentions were clear. The Snyder Cut went from the faint wish of a small fandom to a well-branded movement cheered on by the director himself. In

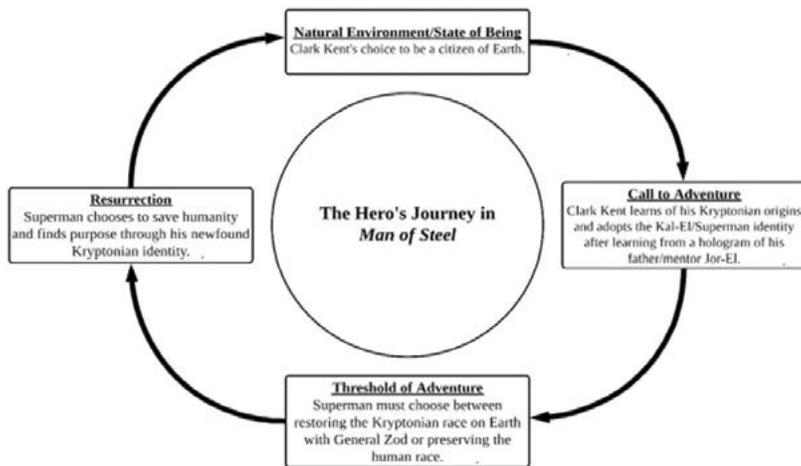


Figure 2: A constructed diagram of the hero's journey as seen in *Man of Steel* (2013).

and Star Wars. Joseph Campbell himself acknowledged that "George Lucas [used] standard mythological figures" when crafting his action-packed genre movies. According to the scholar, Luke Skywalker was not simply a lightsaber-wielding hero, but a character who fights against an impersonal system that the villain Darth Vader embodies ("Ep. 1: Joseph Campbell"). Snyder's visual and narrative representations of the journey, which require heightened audience attention

related to the book but are alongside (para-) the text. Applied to film, a paratext could include a theatrical release date or poster. However, as entertainment evolved through the late 1990s and into the early 2000s, paratext in cinema expanded. The introduction of DVDs and Blu-rays allowed for aesthetic packaging of films, which could influence the perception

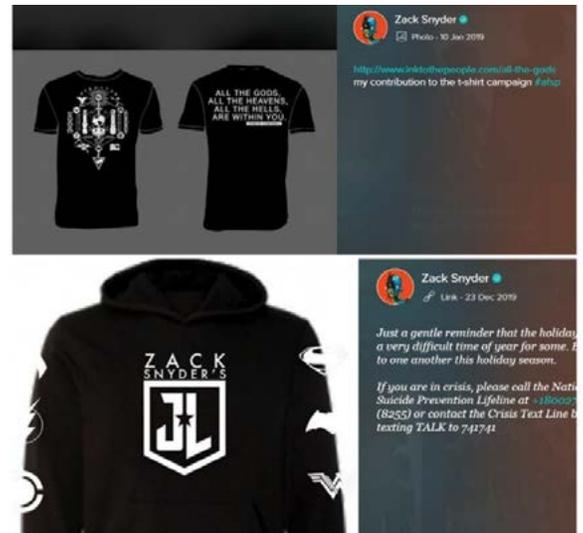


Figure 3: Shown on top is a t-shirt depicting a cryptic design that maps out Zack Snyder's 5-part arc for his DC films (from @zack Snyder "my contribution to the t-shirt campaign ..."). The bottom is a jacket that served as the first merchandise from Snyder with the name "Zack Snyder's Justice League", the title that would eventually officially label Snyder's cut of *Justice League* (from @zacksnyder "Just a gentle reminder that the holidays ...").

fact, the charitable aspect of the merch is its own text. By contributing to a nonprofit dedicated to suicide prevention, the purchasing of merchandise and

engagement in the movement became elevated to a higher purpose. A Snyder Cut shirt represented the filmmaker's work and honored the memory of his daughter who had passed away due to suicide. The relationship between Snyder and his audience shifted thanks to the involvement of the text of merchandise. A t-shirt, hat, or wrist band became a signifier which harbored a variety of meanings and memories related to Snyder (Hobbs 28).

Zack Snyder used a singular tool to help gather his various other texts surrounding *Justice League* onto one accessible platform. Before leaving the project, Snyder used the lesser-known social media application Vero to unveil marketing material for 2017's *Justice League* after the director's collaborating photographer Clay Enos introduced him to the app. Updates for the film included everything from behind-the-scenes pictures to the first official trailer, with the latter being later released online elsewhere but premiering on Vero (Alexander). The shift to the app appeared to be out of the director's admiration for its lack of ads and privacy claims (Enos). Snyder's personal choice and influential marketing points towards the habits of an auteur because there is still an element of authorship in his methodology. The aesthetics, design, and release cycle of this film's promotional material are texts controlled by Snyder.

After *Justice League* released following Warner Bros.' intervention, the filmmaker began using Vero to post images of what appeared to be scenes from his DC team-up film that did not appear in the theatrical version (see fig. 4). This allowed for a set of expectations and constructs to be developed regarding the Snyder Cut. The images led to fans comparing scenes shared by Zack to what had been released in theaters, and discovering elements not seen in the official version altogether. He interacted with fans by answering questions about the unseen film on a regular basis in the comments section of posts. This created a reciprocal flow of dialogue around the Snyder Cut. The filmmaker could clarify, refute, validate, or confirm any theories, misconceptions, or opinions a fan had regarding *Justice League* (see fig. 5). Throughout every campaign, including the two-year anniversary event where Snyder enthusiasts collectively organized to get the hashtag #ReleaseTheSnyderCut trending worldwide (Bennett), the filmmaker vocally encouraged the



Figure 4: Screenshot from Zack Snyder's Vero account showing an unseen scene from *Justice League* featuring Jason Momoa as Aquaman (from @zacksnyder "This guy").

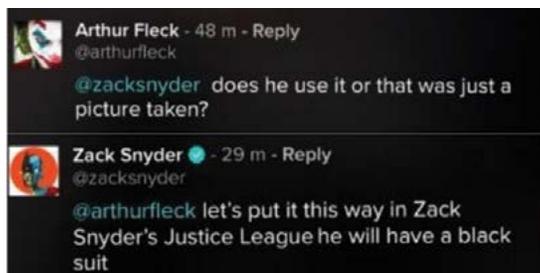


Figure 5: Screenshot of Zack Snyder answering a question from a fan about Superman's black suit, not featured in *Justice League* (from @zacksnyder "let's put it this way in Zack Snyder's Justice League ...")

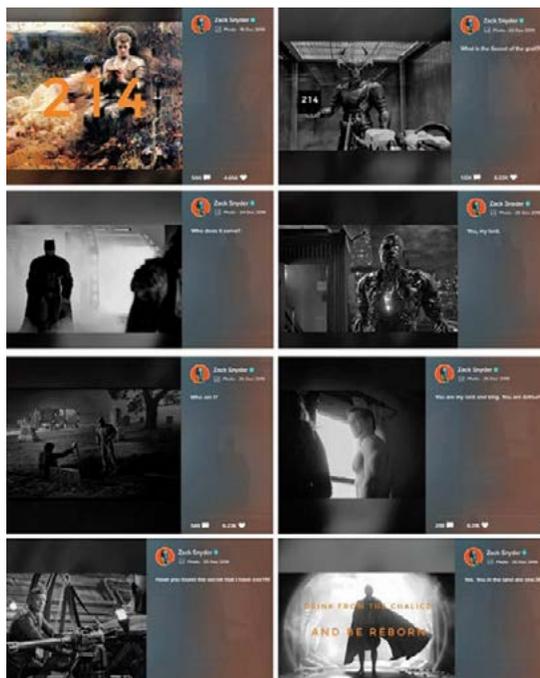


Figure 6: At the top left is a post from Zack Snyder's Vero account showing the cryptic number 214, referring to the Snyder Cut's alleged runtime of 214 minutes (from @zacksnyder 214 image). All remaining images are posts from Zack Snyder's Vero account showing a cryptic campaign for the release of "Zack Snyder's Justice League," featuring unseen images from the film and a quote from *Excalibur* (from @zacksnyder "What is the Secret of the grail?", @zacksnyder "Who does it serve?", and @zacksnyder "You, my lord.", @zacksnyder "Who am I?", @zacksnyder "You are my lord and king. You are Arthur", @zacksnyder "Have you found the secret that I have lost?", and @zacksnyder "Yes. You in the land are one.").

movement's efforts via social media. This served as fuel for the movement. On its own, fans asking for a film is not unusual. However, a director using a less-known social media platform to encourage such campaigning allowed his interaction to serve as another text he influenced.

Vero sponsored an in-person event for screenings of Snyder's director's cuts that was also captured via live stream. This underscores the idea of a tangible experience in correlation with Snyder's films. Unofficial holidays and days of remembrance were created to honor the release of the 2017 version on November 17 and celebrate the supposed two hundred fourteen-minute length of the Snyder Cut on February 14. Even traditional holidays such as Christmas featured a flurry of Vero activity from Snyder developed specifically for his audience. While the official word that the movement had accomplished its goal would not come for another six months, Snyder intrigued his audience with a sense of victory as 2019 ended. From December 18 to Christmas morning, Snyder posted a series of unseen images from *Justice League* with captions that featured dialogue from a scene in *Excalibur* (1981) involving the heroic acquisition of the Holy Grail, with the appearance of the cryptic number two hundred fourteen (see fig. 6). During this campaign, the director also posted a link showing a new merchandise design for apparel that included the phrase 'Zack Snyder's Justice League.' It is known that by the time these posts were published in December, negotiations had begun regarding the official release of the film with the same title.

In early 2020, a poster contest was held where the director himself served as a judge to determine which submission he saw as the best representation of his artistic vision for *Justice League* (see fig.7). This interaction with thousands of submissions, each with a fan's individual stylistic interpretation of the Snyder Cut, allowed for an open dialogue regarding the visual appearance, symbology, and aesthetics of the unseen film.

These interactions were a move by the director to brand his version of the film ahead of release. To those unfamiliar with Snyder's social media activity, these posts and other social media festivities were likely meaningless. However, to devoted fans of the director's filmography, these posts strung together, along with the new merchandise, meant one thing: the Snyder Cut was real and on its way. Half a year later, Snyder's Holy Grail



Figure 7: Screenshot from Zack Snyder's Vero account showing the winning design from a poster contest for his version of Justice League. Design by Victor Ku aka Mighty Pegasus Art (from @zacksnyder "Thank you all again ...")

was revealed. To the rest of the world, it was a surprise. To fans following the director's clues, hints, and purchasing his customized merchandise, it was simply the inevitable coming to pass. Before the official announcement, Zack Snyder's Justice League already had branded merchandise, a host of still images, and official posters mimicking the black and white aesthetic in the still images posted by the director. The film had even generated more tweets than any other Warner Bros. Pictures film at the time, yet it was never released or even confirmed to exist. A speculative movie became a franchise with a fandom that supported a selection of films that presented comic book characters in cinematically subversive ways.

Following the announcement, the director utilized another text in the form of press exposure through popular YouTube channels associated with the movement. In addition to attending interviews and Q&As on traditional platforms, Snyder specifically chose to interact with ones that supported his filmmaking. This drastically alters the discourse and atmosphere involving Snyder's exposure to the public. It also refines the scope and characterization of the director's selective audience. Like his primary use of the smaller social media application Vero, the filmmaker's interaction with unverified YouTube channels exhibits a closer and active relationship with his audience. Film professionals who have worked alongside the director also interacted with this audience via interviews and live streamed events on YouTube. This includes cinematographers Larry Fong and Fabian Wagner, stunt performer Richard Cetrone, assistant director Damon Caro, photographer Clay Enos, stunt choreographer Matthew Rugetti, and more. This allowed Snyder's audience to be exposed to and engaged in an exploration of techniques, practices, and other aspects and roles involved in the

creation of a film beyond directing. This does not contradict the idea of a cinematic signature being born from a lone artist. Instead, it expands who and what is involved in the perception of the signature. To Snyder's audience, these professionals are not parts of a studio machine. They

director's chair, but he was still personally attached to the project. Second, content was only released on Vero for a time, a platform that does not boast nearly the same number of users as Instagram or Twitter ("Ayman Hariri's Vero Social App"). The motivations behind this choice

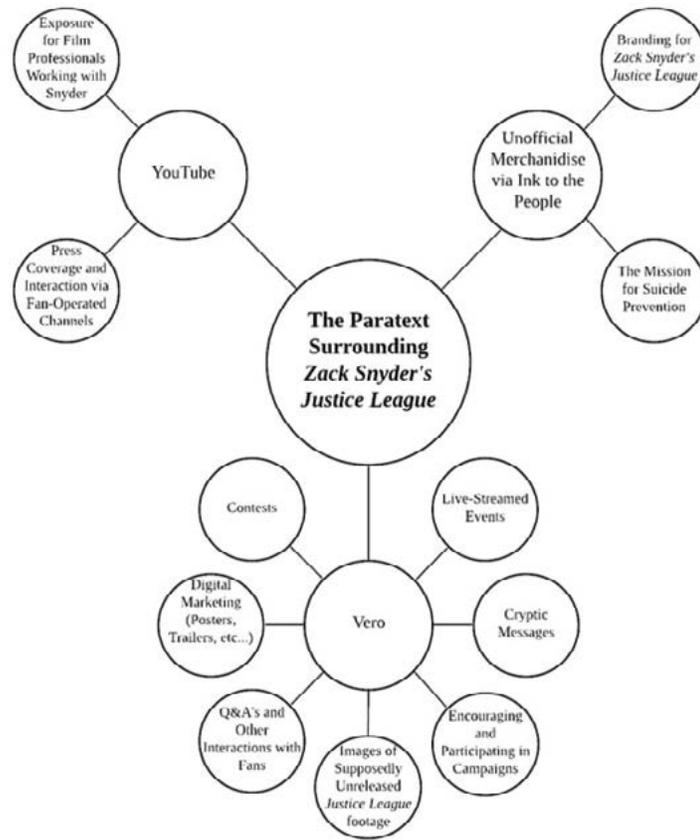


Figure 8: A constructed diagram showing the paratext around Zack Snyder's Justice League.

are viewed as artistic collaborators bringing about Snyder's personal auteur expressions. Furthermore, they are another piece of the paratext surrounding Zack Snyder's Justice League (see fig. 8).

Snyder's activity and interaction with fans on Vero and YouTube revealed a variety of purposes for the cryptic messages, unofficial merchandise, and other texts that branded his version of Justice League. First, they encouraged discourse around an unreleased product. Fans were already delivered the on-screen debut of the DC team, regardless of perceived quality, via the 2017 film. However, Snyder gave little attention to the released version that lists his directing credits. Instead, he expressed multiple times that he never saw the theatrical cut ("Zack Snyder Steps Down"). With posts showing behind-the-scenes pictures and never-before-seen screen captures, it is almost as if the 2017 version was never released, and promotion is simply continuing as intended from the beginning. Snyder may have stepped away from the

likely involve the criticism of his films from general audiences and publications, and the creation of an exclusive atmosphere for his audience.

Zack Snyder's Justice League was announced on Vero with the filmmaker's name front and center. There is significance in the choice of the Snyder Cut's official title and the initial promotional art. The auteur has already been classified as a commercial product. One interpretation of Zack Snyder's Justice League could be that the new title is an attempt to clearly differentiate his version from one he did not have total creative control over. The inclusion of a director's name in the film's branding allows for an artistic presence in its aesthetic. The name Zack Snyder comes with recognizable signifiers such as thematic and marketable traces, as well as cultural presence. Therefore, the large size of the filmmaker's name in promotional material graphics is intent on promoting Snyder's distinct controlling influence on his films, as well as the mentioned marketable traces

(Hobbs 28). During the online Justice Con event held by fans of the director in July 2020, Snyder claimed the title was chosen because "it's really a pure sort of vision unencumbered by anything other than what we think is awesome" (Justice Con). Such verbiage clarified that the Snyder Cut was not intended by its creator to simply be a director's cut with a few extra scenes added onto the 2017 film. It was to be Snyder's personal envisioning of DC Comics' prize characters. Elements including color correction, visual effects, characters, character designs, alternate versions of scenes, subplots, extended scenes, and new sequences would be included under the direction of Snyder and his collaborative team.

While Warner Bros. had intended to deliver a film that celebrated the history of these heroes, Snyder's loyal followers were more interested in screenshots, storyboards, and answers to questions about a film Zack Snyder intended to offer, rather than what filmmakers and artists had produced for DC decades before him. Even if the movement's efforts had not been enough for the cut to be released, it is likely that campaigns would have continued with no end in sight if Snyder continued to wield his paratext. However, the opposite occurred. Not only did the Snyder Cut's official announcement accomplish re-releasing a film already shown in theaters only years prior, but tens of millions of dollars were to be invested to transform a rough director's cut into a fully finished product.

While HBO Max was chosen to house Zack Snyder's *Justice League*, it was years of unofficial merchandise, social media campaigns, Q&As, charitable donations, and online contests that formed the idea of a Snyder Cut. All these texts were ones that Snyder had a hand in regulating. The fans, or readers of the texts, should not be underestimated. However, it is worth noting the meticulously involved role Snyder held in the film's unofficial marketing. Each of these texts on their own holds value in expanding upon the relationship a director and their fans can have with one another. However, it is a narrative formed from the Snyder paratext that holds the most potential for a director's promotional tool, creative defense, and a marketable product for studios.

The hero's journey is a concept Snyder has heavily integrated in his DC films, so it was expected that *Justice League* would include those elements. However, since the film with Snyder's auteur signature had not yet surfaced, the narrative journey of heroes uniting to fight a common enemy was one that would be manifested in real life before

being cinematically realized. Snyder's trials in his own journey as a creator began following poor audience and critical reception towards *Batman v Superman: Dawn of Justice* (2016). The film was the second entry in DC's response to the Marvel Cinematic Universe, known as the DC Extended Universe (DCEU). Snyder had been handpicked in 2011 by director Christopher Nolan to jumpstart the DCEU because of his unique visual storytelling (Bernardin). This is an example of Warner Bros. Pictures allowing a director to have a larger role in major decisions that would affect the studio's financial future. While executives gave the approval for Nolan's choice, the filmmaker's role in selecting Snyder is significant because it shows a status quo that somewhat contradicted the established studio system. While his first film *Man of Steel* (2013) saw lukewarm reception, *Batman v Superman* was rejected by critical publications and general audiences ("*Batman v Superman* Draws Mixed Reviews").

When the 2016 film featuring Batman and Superman did not live up to Warner Bros.' expectations, DC Films underwent a series of changes that altered the course of

Snyder's plans for the DCEU. First, rather than existing as a brand under Warner Bros. executive leadership, DC Films would purposefully mimic the executive and operating structure of Marvel Studios under the Walt Disney Company, which aligned with the traditional studio system. DC Films would now be overseen by its own leadership, starting with executives Jon Berg and Geoff Johns ("*Batman v Superman* Fallout"). Second, DC movies going forward were to be lighter in tone, specifically away from the darker themes explored in Snyder's previous films (Fritz). For an auteur such as Snyder, these changes would mean that elements of his filmmaking signatures could be managed or removed altogether from his films by executive leadership. The status quo that existed when Snyder first began the DCEU had reverted towards the studio system's perception of the director as a piece in the production machine rather than an artist.

Despite the studio shakeup, production on Snyder's next film *Justice League* began as scheduled in 2016. Executives Berg and Johns were on set frequently, and critics who previously expressed distaste towards Snyder's films

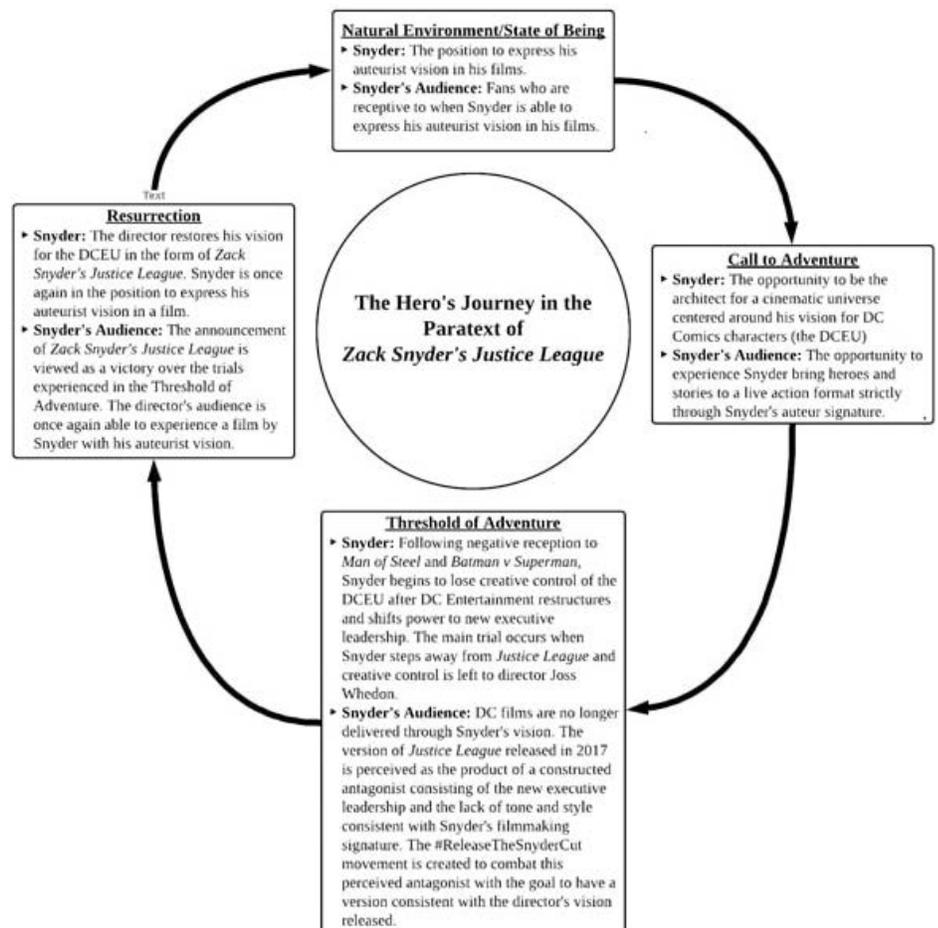


Figure 9: A constructed diagram showing the hero's journey applied to the narrative of Zack Snyder and his audience surrounding Zack Snyder's Justice League.

were invited to see the changes being made to appeal general audiences who were turned off by *Batman v Superman* (Buchanan). While Zack's diminishing authorship as an auteur continued, 2017 brought more personal challenges for the director. In March, Snyder's daughter Autumn passed away due to suicide. Although he finished filming in late 2016, Snyder would step down from directing duties for *Justice League* during the post-production process. *The Avengers* director Joss Whedon would oversee a series of reshoots and finish assembling the film in his absence. While it was initially reported that Whedon's directing would adhere to Snyder's style, the auteur's audience quickly recognized the absence of his signature (and the inclusion of Whedon's) upon the premiere of *Justice League* in 2017.

This hero's journey with *Justice League* is one that Snyder himself and his loyal audience embarked on for over

three years. Steps taken towards Snyder's creative absence on the 2017 version of *Justice League* were the trials endured in the Threshold of Adventure. Each campaign, new merchandise release, and unseen image of the film created the perception of a narrative arc leading towards the Resurrection stage of this journey with the Snyder Cut's release. When applied to the hero's journey model, *Zack Snyder's Justice League* becomes more than just a movie. It is the culmination of Snyder and his fans fighting a battle, enduring trials, and overcoming obstacles through acts of charity, branding, campaigning, and discourse surrounding the unseen cut. Like King Arthur and the Holy Grail, the Snyder Cut could not simply be released, it had to be earned and obtained. Like quests of ancient mythology, this journey involved the search of an object, a transformation, and established a communal mind and collective authorship

(Auerbach et al. ix). In keeping with the Grail's symbolism of the fulfillment of physical and spiritual needs (Kenney 47), the Snyder Cut symbolized Snyder's pure artistic vision, the restoration of an auteur's creative control, the voice of a movement, and heroic charitable actions. It resembled the journeys in Snyder's films where the hero must face trials and transformation before obtaining his or her reward. This journey becomes clearer when events surrounding the Snyder Cut are filtered through it (see fig. 9). However, the most relevant discourse is not what happened, but rather how Snyder and his fans authored a story with these events to serve their interests. What is most significant is that a filmmaker's auteur signature has reached beyond the theatrical medium while retaining a tangible audience.

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STUDENTS WITH HIGH FUNCTIONING AUTISM SPECTRUM DISORDER: THE IMPACT OF INDIVIDUALIZED EDUCATION PROGRAMS AND GENERAL EDUCATION INTEGRATION

Elliana Larsen

ABSTRACT

The world may be in upheaval, but students with autism still deserve the right to a balanced education where their voices can be heard. While much research has been done on educational barriers for students with autism, more research is needed to examine challenges faced by students with High Functioning Autism Spectrum Disorder (HFASD). One practice often used as part of a student's Individualized Education Program (IEP) is general education integration, which places those with HFASD into general education classrooms. Although this practice is common, the question of where to correctly place students with HFASD is nearly impossible to answer, as they are on the precipice of being labeled "normal" while also struggling with the ability to effectively communicate with their peers, paraprofessionals, one-on-one aides, and instructors. To solve this dilemma, more information is needed surrounding the reevaluation of IEPs to include best practices for the integration of students with HFASD. This paper will discuss solutions that center around additional parental involvement, aide training, the inclusion of conversational and social goals in IEPs, and increased peer interaction. The proper balance of support planning by IEP teams, along with a dual focus on social and academic goals in the IEPs will positively impact students with HFASD. This shift in practice is essential for IEP teams to understand the diversity of HFASD and allows students to have necessary continuity both in school and at home, especially during a global pandemic when both of these places have become one and the same.

When one is mid-conversation, the facial expressions and tonal cues of the speaker are commonly studied by the listener to understand the meaning behind what is being said. However, individuals with High Functioning Autism Spectrum Disorder (HFASD) struggle with these nonverbal aspects and often have to be taught conversational norms. This form of interpersonal communication education is an important factor in the success of students with HFASD, making it a primary facet of many Individualized Education Programs (IEPs). IEPs allow students with HFASD to flourish in an academic environment through guided planning and additional support throughout the school day. Students with HFASD require an IEP to plan which goals their educators and parents want them to achieve during the school year, but even with these plans, individuals with HFASD often remain underserved in the public-school system.

Throughout the education sector, students with HFASD are often deemed "less disabled" than other individuals with disabilities. Instead of being placed in special education classrooms with their peers, these students are commonly

integrated into general education classrooms (Sansosti & Sansosti, 2012, p. 917). In recent years, there has been a call to eliminate the term HFASD from use, as "policymakers have used the term to decide which individuals should receive services or funding rather than carefully evaluating individual needs" (Singh Chawla, 2019). Due to numerous challenges faced by individuals with HFASD, the proper balance of support planning by IEP teams, along with a dual focus on social and academic goals in the IEPs themselves is necessary to positively impact students with HFASD. This shift in practice is essential for IEP teams to understand the diversity of HFASD and allows students to have continuity both in school and at home.

UNDERSTANDING AUTISM

Autism Spectrum Disorder (ASD) cases are on the rise in the United States, making discussions about this disability more important than ever in today's society. In the United States, "one in 54 8-year-old children have been identified with autism" as demonstrated by a recently released 2016 study from

the Centers for Disease Control and Prevention (2020). One reason for this upswing in autism cases is due to the fact that many parents are having their children tested for autism earlier than in previous years (Centers for Disease Control and Prevention, 2020). Because of the increase in diagnoses, there has been a simultaneous increase in news coverage about ASD.

Individuals with ASD strive to be successful in their daily lives but can encounter roadblocks when they are treated unfairly. Although their symptoms and behaviors vary drastically, students with ASD are often treated similarly, which can be misleading due to the diversity of this disorder (Wilczynski et al., 2007, p. 653). According to the American Psychiatric Association (2013), a child is deemed to be on the autism spectrum if they struggle with communication in a range of scenarios and repeatedly perform specific, atypical, and often unproductive actions. These actions may include agitated hand fluttering, intense focus on body positioning, repetitive swaying whilst sitting or standing, fixation on a restricted number of specific topics or interests, and

a strict adherence to routine (Wilczynski et al., 2007, p. 657). Once an individual diagnosed with ASD enters the education system, decisions have to be made in relation to how much assistance each student needs in a classroom setting.

HFASD is one type of autism, but students are often placed in classrooms based on their “high functioning” label instead of the “autism spectrum disorder” portion of their diagnoses. HFASD diagnoses differ greatly between each individual, but most have the ability to “speak in full sentences” and communicate with those around them while performing “inflexible behavioral responses” (Cravalho et al., 2020, p. 31). HFASD can also be differentiated from other forms of autism. Compared to individuals with Low Functioning Autism Spectrum Disorder (LFASD), those with HFASD have higher levels of communication, as well as greater intellectual and academic capabilities. This is why students with HFASD are commonly placed in general education classrooms with students who do not have disabilities, while students with LFASD remain in special education classrooms. However, instead of being discounted, students should be accommodated individually to meet specific IEP goals, regardless of their diagnoses.

In addition, HFASD is routinely grouped with Asperger’s syndrome, but Sharma et al. (2012), as cited in de Giambattista et al. (2019), states that the distinct differences between these two disorders are important enough that they should be referenced when creating communicative growth plans (such as IEPs) for these individuals (p. 139). Although those with Asperger’s syndrome now receive autism diagnoses, HFASD stands on its own as a distinct disability and should be treated as such. Those with former Asperger’s syndrome diagnoses and those with HFASD both have average or above average intellectual abilities, but those with HFASD have more severe challenges in the social domain, displaying additional restrictive and repetitive behaviors and interests.

GENERAL EDUCATION INTEGRATION PREPARATION

As previously discussed, integrating students with HFASD into general education classrooms is a common practice, but this is not always the right decision. One important aspect in determining IEP goals is whether or not general education integration is the best fit for the student (Slade, 2018, pp. 244-245). Factors to consider in making this decision include the “student’s age, academic strengths/weaknesses, communication skills, behavioral support needs, personal preferences, and degree of independent

functioning” (Sansosti & Sansosti, 2012, p. 923). In order for students with HFASD to be successful in a general education classroom, all involved parties need to be aware of the incoming student and prepared to make changes, thus reducing external stimuli that could contribute to challenges they may face throughout the school day (Boutot, 2007, p. 158).

Sansosti & Sansosti (2012) explain that general education integration fosters a positive environment where students can create relationships with their teacher and peers (p. 924). This form of social skill building is essential because many students with HFASD struggle with making friends. By allowing students with HFASD to become members of general education classrooms, socializing becomes easier during group peer-bonding activities. Special education classrooms commonly have smaller class sizes, and between paraprofessionals, one-on-one aides, and teachers, individual attention is an easy thing to come by. While this may be helpful in some circumstances, students with HFASD can benefit from real-world examples of independent learning.

Many students with HFASD strive for social independence, as their daily lives are typically overseen very closely by parents, paraprofessionals, one-on-one aides, and educators. General education integration, in a sharp contrast to a routine special education classroom, can allow individuality to develop. Lloyd (2019) explains that general education integration contributes to student growth because it allows them to flourish in a more interactive environment that is less monitored than a typical special education classroom (p. 21). In fact, studies have shown that “children with autism in inclusive settings are able to meet their IEP goals at faster rates compared to children with autism in non-inclusive settings” (Kurth & Mastergeorge, 2010, as cited in Koegel et al., 2019, p. 2434). However, preparation is the key to success, and educators need to strive to engage in strategies that will make integration more successful for students with HFASD.

For successful integration to occur, the relationship between student and teacher needs to be fostered to establish boundaries and allow opportunities for educational experiences with an adult who is explicitly aware of the student’s goals. General education teachers should be encouraged to work with the student with HFASD before class begins, either in the summer or during the year before planned integration (Lloyd, 2019, p. 20). If this is not possible, the teacher should, at minimum, meet with the student at the start of the school year to get to know them and establish a trusting relationship. Without directly spending time with the

student in a one-on-one manner, general education teachers may struggle with understanding the behaviors of that individual. Since the teacher knows the details of the student with HFASD’s IEP, and is involved in IEP meetings, it is important for the student to spend time with an educator who understands all aspects of their situation.

If there is a lack of teacher understanding about how to work with the student with HFASD, a sense of isolation can occur, separating the individual from the rest of the classroom. In fact, “research suggests that the failure of children with HF-ASDs to engage in successful conversations with peers may contribute to their higher-than-normal rates of social isolation and depression” (Bauminger & Kasari, 2000; Bauminger et al., 2003; Stewart et al., 2006, as cited in Müller et al., 2016, p. 192). This lack of socialization can set some students with HFASD further back in their progress toward reaching IEP goals and can also be detrimental to their health and well-being.

Acceptance of the student with HFASD is another essential aspect of individuals reaching their goals and effectively moving forward toward social and educational benchmarks. One way to prepare general education students for the arrival of an individual with HFASD is by “emphasizing how much the students are alike versus how the students are different” (Boutot, 2007, p. 159). In doing so, the teacher becomes a positive role model for general education students by demonstrating inclusion and understanding instead of just leaving the individual with HFASD to fend for themselves upon arrival. Typically, however, instead of spending time with their teachers or peers, students with HFASD interact extensively with their one-on-one aide or paraprofessional.

PARAPROFESSIONALS AND ONE-ON-ONE AIDES

Another important facet of the school days of students with HFASD is their paraprofessionals or one-on-one aides. Many students with HFASD have a paraprofessional they are paired with when they integrate into a general education classroom, and these individuals assist students with things they may struggle with throughout the school day, such as reading, writing, social skills, or even something as simple as staying focused and on task (Sutton et al., 2018, as cited in Koegel et al., 2019, p. 2427). Paraprofessionals typically have a fair amount of training in working with students with disabilities, as they have to earn an Associate Degree, at minimum, or pass an examination before working with students. One-on-one aides tend to vary more greatly in their

training, depending on the school district or classroom they are working in.

Although these positions sound similar, they can be differentiated from a legality standpoint: “There is no federal legal definition for an ‘aide’... (but) when you use the term ‘paraprofessional’ in the IEP, you refer to a federal legal definition and a quality standard” (Whitney, n.d., as cited in Center for Parent Information & Resources, 2014). This is important for IEP creators to note, as one-on-one aides may not have as much experience as paraprofessionals, possibly lacking the skills necessary to assist students with HFASD in reaching their IEP goals. Additionally, when paraprofessionals are going to be out of the classroom for extended periods of time, or they move to a new position, long-term substitutes are commonly brought in to assist students with special needs. Since the timetable for the gap between paraprofessional to paraprofessional is unknown, substitutes may work with individuals with HFASD for months at a time with little to no specific training on their student’s disability.

A lack of training in any setting can lead to a range of issues, but when combined with a student with HFASD who experiences challenges understanding social cues, the potential misunderstandings can be extremely problematic. Brown and Stanton-Chapman (2017), as cited in Koegel et al. (2019), explain that without training, many one-on-one aides fall into the habit of becoming overly helpful in students’ activities and conversations, which can be detrimental to IEP goal completion (p. 2427). Constant contact with an untrained aide can force students to form a dependence, limiting the freedom a student with HFASD needs to grow and improve (Lloyd, 2019, p. 18). Although these aides typically only have the best in mind for the student they are working with, they need to be trained to ensure the correct balance of assistance and skill-building is occurring for the student with HFASD. Explicitly trained one-on-one aides, however, can be beneficial for students with HFASD and have been shown to be especially helpful during the summer months when many students are enrolled in programs to help maintain their IEP goals throughout the year (Koegel et al., 2019, p. 2434). Paraprofessionals and one-on-one aides are just one important piece of the puzzle in a student with HFASD’s IEP team, and the collaboration of all adults involved in the individual’s life is necessary for them to succeed.

IEP TEAMS AND PARENTAL INVOLVEMENT

The IEP team for a student with

HFASD involves all of the adults in the individual’s daily routine, including teachers, paraprofessionals, one-on-one aides, and especially the student’s parents. The effectiveness of the IEP team as a whole is necessary for a balanced education, and teamwork is essential to ensure the best possible outcome for a student with HFASD. According to a recent study, “60% (of parents) felt their children’s IEP teams were mostly effective or effective” (Slade, 2018, p. 248). This effectiveness comes from an understanding of the perspective of the student, which is needed before they can be given improvement goals, as these goals may vary depending on the severity of the disorder (Wilczynski et al., 2007, p. 653). Parents are best equipped to understand this perspective, as they spend the most time with their child, and their input is needed to create balanced and reasonable IEPs. Because of this important factor, another aspect of IEP success is increased parent interaction (Boutot, 2007, p. 160).

Without parental involvement, educators are left to make decisions by themselves about the next steps for students with HFASD, which can lead to serious parent complaints. IEP meetings involving parents are essential for members of the IEP team to discuss progress and next steps for the student with HFASD. However, parents often have issues with the various scheduling hurdles that arise in the planning of their IEP meetings (Yell et al., 2003; Zirkel & Hetrick, 2017, as cited in Slade et al., 2018, p. 243). Without scheduled IEP meetings that involve everyone, students with HFASD can become confused and frustrated at the lack of continuity in their daily routine. Educators must consider parental opinions when creating IEPs, as parents are the primary monitors of their child’s progress while they are at home.

Parent opinions of student IEPs also have a large impact on their success. Research has shown that parent belief in their child’s IEP functionality was not based around their student’s abilities, but instead on school involvement, teacher education, and funding (Slade et al., 2018, p. 254). A high level of school involvement in the student’s educational assistance is vital for parent satisfaction, as schools can vary greatly in how much support is given to each individual. Teacher education has also been shown to be a factor in parent satisfaction, as educators with more advanced degrees may have additional strategies for how to work with students with HFASD. Funding is yet another large predictor of how successful each individual will be in school. If there are enough funds to support the student with a paraprofessional or technological learning supports, for example, the student’s

experience may improve and parental views on IEP success may undergo positive change.

Balance is an important term to keep in mind when structuring the IEP of a student with HFASD. In finding a proper balance between the roles of parents and educators in creating a student’s IEP, connections between school and home can be increased, allowing students to feel supported in all aspects of their lives (Lloyd, 2019, p. 20). With the proper support in place, IEP teams can then begin to work toward goals to enhance individual abilities. In order for IEPs to improve students’ lives, instruction needs to focus on academic and social goals simultaneously to foster growth (Wilczynski et al., 2007, p. 655). These goals can be taught using peer modeling strategies, allowing students with HFASD to feel more comfortable in social settings they may be faced with throughout the school day.

SOCIAL AND CONVERSATIONAL GOALS

Social and conversational goals need to be taught to students with HFASD so they can feel less of a disconnect when conversing with both peers and adults in the classroom. Scholars agree that students with HFASD struggle with social interaction and the differences between these students and their peers can be overwhelming (Koegel et al., 2019, p. 2426). This struggle stems from the fact that students with HFASD often have to learn conversational cues from scratch. The complex social construct of understanding discussion-based norms can be difficult to learn while also trying to undergo educational improvement. Because of this, a common strategy used by educators to foster social goals is facilitated peer interaction, which works to “increase participants’ spontaneous, meaningful, and naturalistic conversation” (Müller et al., 2016, p. 193). By interacting with those around them under the guidance of an instructor, students with HFASD can learn by example without the stress that can occur when interacting with peers on their own. Facilitated peer interaction also allows those with HFASD to learn skills necessary for maintaining conversations that will help them succeed in their daily discussions both inside and outside the classroom.

Increased peer interaction is an important goal for many students with HFASD, and peer modeling is one way to promote positive student behavior (Wolfberg, 1999, as cited in Boutot, 2007, p. 156). This type of education allows students with HFASD to see how they should be both acting and reacting in the classroom. Students with HFASD respond

well to examples of appropriate behavior in an educational setting and can use these examples to improve their responses in general education classrooms (Cravalho et al., 2020, p. 38). These appropriate socialization guides give students with HFASD the ability to model their behavior after successful students, furthering their abilities in the classroom.

Students with HFASD often flourish in a setting where they can improve their socialization, but specific communication education has also been shown to improve student behavior. Studies have shown that discussion-based education is necessary to ensure students with HFASD can have appropriate conversations with those around them (Müller et al., 2016, p. 194). Discussion-based education is not centered around social and conversational etiquette, but instead helps the individual understand necessary talking points needed to hold a successful conversation, allowing them to converse in a comfortable setting without fear of judgment. By focusing distinctly on specific communication skills, educators can “provide direct instruction in both the *how* and the *why* of conversation” (Müller et al., 2016, p. 193). In contrast to other forms of social education, which often give students conversational strategies without explaining the meaning behind them, discussion-based learning can help individuals with HFASD grow.

Students should be taught how to incorporate conversational strategies to help improve their memory and integrate skills, adding to the length and diversity of discussions they may have throughout the school day (Müller et al., 2016, p. 194). For example, students are instructed to “select topics of mutual interest... demonstrate active listening...and...repair simple conversational breakdowns,” just to name a few (Müller et al., 2016, pp. 193-194). Cravalho et al. (2020) states that individuals with HFASD drastically increase their social skills with this type of instruction (p. 32). Overall, integration typically has a positive impact on students with HFASD, but educators must carefully monitor their classrooms to ensure that negative emotional displays are not being replicated by the general education students in the classroom (Sansosti & Sansosti, 2012, p. 926). This unfortunate effect of general education integration may lead some educators to believe that students with HFASD may learn better in a special education classroom instead of being introduced to the general education realm.

INTEGRATION ISSUES

Although peer interaction typically helps students with HFASD learn necessary social skills to meet IEP goals and improve daily conversations with both

the peers and adults in their lives, “the increasing presence of special education students in general education classrooms has raised concerns...about its potentially adverse impact on the general education population (Nat’l Ctr. For Educ. Statistics, Digest of Educ. Statistics 2010, 2011, as cited in Keaney, 2012, p. 830). For example, too much focus may be placed on the integrated student with HFASD in the general education classroom, leading the general education students to feel left out, as less attention may be given to them throughout the school day. This potential downside to general education integration is important to discuss before the student with HFASD has been integrated into the classroom.

Educators have made it clear that the integration of students with HFASD “should not compromise the learning, safety, or overall well-being of GE (general education) peers” (Sansosti & Sansosti, 2012, p. 926). This is where a properly trained paraprofessional or one-on-one aide is needed to manage a student with HFASD, especially in situations where they may be having an emotional outburst due to increased stressors or distractions. In doing so, the paraprofessional or one-on-one aide ensures that the teacher can keep working with the general education students without being interrupted. If the student with HFASD becomes too disruptive, action must be taken to work with the individual outside of the general education classroom.

Another unfortunate issue that occurs in many classrooms as a result of general education integration is the bullying of students with HFASD. Because the student with HFASD’s behavior may differ, general education students may take advantage of opportunities to point out these differences. Holzbauer (2004) and Shaw et al. (2012), as cited in Hartley et al. (2014) explain that “unless the problem of bullying is addressed, the experience of victimization may lead to negative social roles with potential lifelong consequences, such as never attempting to seek gainful employment for fear of future harassment in the adult workforce” (p. 177). This possible bullying can be discouraged before the student with HFASD ever steps into the classroom. One way to do this is by reading the general education students a novel “depicting someone who learns, acts, or moves differently from others” (Boutot, 2007, p. 159). By assisting the general education students with understanding the behaviors of the student with HFASD, general education teachers can foster an inclusive environment, allowing students with HFASD to feel comfortable and supported throughout their school day.

CONCLUSION

While there is an extensive amount of research into the impacts of general education integration on students with Autism Spectrum Disorder (ASD), there needs to be an increased focus specifically on integrating students with High Functioning Autism Spectrum Disorder (HFASD) into general education classrooms. Many educators lack the proper training needed to understand students with HFASD, and instead may rely on outdated or incorrect information. This lack of understanding can contribute to Individualized Education Program (IEP) planning that does not cover the full range of the student with HFASD’s disability, leading to confusion and frustration for both the student and their parents. More emphasis is needed on the balance necessary for students with HFASD to work toward their IEP goals while simultaneously improving social interaction. In doing so, students with HFASD can be appreciated for their individuality and given the educational equality they deserve.

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CLOSING THE TREATMENT GAP IN BLACK MEN WITH MENTAL ILLNESS

Carly Nascimbeni

ABSTRACT

People with mental illness tend to face discrimination in multiple facets of their lives, contributing to internalized stigmatization. According to a 2019 study, Black people experience higher levels of discrimination than any other minority population group in the United States (Lee et al., 2019). In addition to being historically marginalized and discriminated against, Black men experience further self-stigma when having mental health challenges that may require treatment and diagnosis. Self-stigma reduces self-esteem, empowerment, and a sense of recovery, while also exacerbating psychiatric symptoms and reluctance to engage in treatment. The combination of these factors creates significant barriers when considering treatment options. The implications of leaving a mental illness untreated can be seen in ruined relationships, job loss, substance abuse, unnecessary medical procedures, psychiatric hospitalization, and suicide. Evaluating the disparities Black men with mental illness face through studies and statements suggests that the combination of socially and culturally relevant approaches, information, and resources will reduce self-stigma in Black men with mental illness and result in narrowing the treatment gap.

More than 50% of people will be diagnosed with a mental disorder in their lifetime (Center for Disease Control, 2019). Even so, mental illness is one of the most highly stigmatized diseases. People with mental illness are not only challenged by the symptoms and disabilities that result from their disease, but also from the stereotyping, prejudice and discrimination that manifests from misconceptions about mental illness. These experiences often create a social identity that leads to negative self-concept¹ or self-stigma.²

Black men with mental illness experience these factors in addition to being historically marginalized and discriminated against. Racism and discrimination alone often lead to poorer self-concept, internalized racism, and negative affective responses, as well as the abuse of illegal substances (Chae et al., 2016; Goodwill et al., 2019; Molina & James, 2016). The combination of mental illness stigma, discrimination, and racism is associated with reduced intentions of seeking treatment, less treatment engagement, and less compliance with treatment recommendations (Fox et al., 2018).³ Untangling the complex interactions between stigma, discrimination, and racism will result in the development of effective methods to ameliorate self-stigma in Black men with mental illness. Additionally, identifying the factors that contribute to stigma may be useful in the creation of necessary intervention methods, such as systemic therapy.⁴

STIGMA

The co-occurrence of labeling, stereotyping, separation, status loss, and discrimination is what comprises stigma (Ezell et al., 2018). Stigma is a multifaceted

conception and depends mostly on subjective perceptions. The perceptions are (i) the stigmatizing views a person has regarding others, (ii) the stigmatizing views a person has regarding themselves (self-stigma), (iii) perceived stigma beliefs regarding the views others hold, (iv) stigma that occurs through media, and (v) stigma that occurs through experiencing the discrimination firsthand (Wright & Mackinnon, 2011).

Stigma fuels social inequalities, reduces individuals' opportunities, and invites varieties of discrimination, all of which ultimately denies the individual or groups full social acceptance (Stangl et al., 2019). Stigma received from public sources affects the recipient's life in various situations, including housing, education, and employment (Ezell et al., 2018). When people begin to perceive devaluation, they tend to avoid situations where discrimination and stigmatization may occur (Carrara & Ventura, 2018). Avoidance of these situations can often result in blocked avenues of treatment and a decrease in financial stability and social support (Carrara & Ventura, 2018). For example, if someone with depression feels devalued by their peers when they are going through "low" periods, then they may avoid going to work and attending social functions, which results in the loss of income and the opportunity for socialization.

One of the ways in which people with mental illness receive stigma is through micro-aggressions. Micro-aggressions are overt and non-overt, recurring degrading and demoralizing indignities directed at historically marginalized populations (Ezell et al., 2018). Micro-aggressions are often subtle snubs or insults. Identifying micro-aggressions and how they are interpreted

by people with mental illness will assist in creating specific interventions aimed at reducing and confronting micro-aggressions.

Themes often described by people with mental illness experiencing micro-aggressions include stereotyping phrases or behaviors which allude that people with mental illness are dangerous, weak, incapable, or lack intelligence. Some people with mental illness have described experiences where they felt as though friends and family have doubted the severity or even existence of their mental illness. Quotes to support these themes include, "Well, he does have a mental illness... he tends to be violent" and "You'll be fine, just get over it!" (Gonzales et al., 2015; McCue, 2016).

Friends, family, and professionals are amongst the biggest perpetrators of micro-aggressions toward people with mental illness, rather than strangers or acquaintances. Mental illness is more easily concealed than physical disabilities, and thus it is usually revealed most to close friends and family (Barber et al., 2020). Research shows that daily indignities are significantly higher among people with mental illness who exhibit more than two psychotic episodes during a three-month period (Ezell et al., 2018). One may conclude that the individual's identification with mental illness is less concealable with an increase in symptoms. Furthermore, the research indicates that the more severe or persistent symptoms may be, the more likely one is to experience or perceive public stigma (Ezell et al., 2018).

A variety of factors contribute to the ways in which some people in the public develop their unfavorable views in relation to people with mental illness. One way in which the public view is misguided is

through negative portrayals of people with mental illness in popular media outlets (e.g., television, music, movies, and video games). The mischaracterizations and stereotypes that are bolstered through these sources portray people with mental illness to be violent, weak, and unsophisticated (Ezell et al., 2018).

Ironically, mental health providers commonly endorse negative stereotypes about people with mental illness, which include perceptions of danger, unpredictability, and blame (Corrigan & Niewegłowski, 2019). There is a fear amongst healthcare providers of the mentally ill that associating with people with mental illness will result in a loss of prestige and status (Kohrt et al., 2020). People with mental illness are often excluded from social gatherings and community groups, and healthcare providers fear receiving the same treatment. One study demonstrated that psychiatrists who perceived themselves as “socially closer” to people with mental illness were more likely to endorse the stigma (Corrigan & Niewegłowski, 2019).⁵

People with mental illness often internalize the public’s negative stereotypes, which results in low self-esteem and self-stigma. Self-stigma begins to occur when the person with mental illness becomes aware of the negative stereotypes others attribute to them, begins to agree with them, and even endorses the idea of stigmatization (Carrara & Ventura, 2018). Consequently, the person with mental illness begins to exhibit hopelessness and reduced levels of self-esteem and self-efficacy, which contribute to the unlikelihood of treatment seeking.

There is a direct relationship between high levels of self-stigma and low adherence to treatment, including both medicated and psychosocial (Carrara & Ventura, 2018). A possible reason for high levels of self-stigma and low levels of adherence to treatment may be that people with mental illnesses prefer to avoid treatment to hide their mental health concerns for fear of being stigmatized or discriminated against. Research also suggests that people with mental illness may have poor insight into the beneficial effects of treatment; it is possible that people struggling with mental illness may feel the burden of the stigma outweighs the benefits of receiving treatment (Carrara & Ventura, 2018).

DOUBLE BURDEN

Black men with mental illness face a situation referred to as a “double burden,” in that they have “a stigmatized mental illness condition” and are “a member of a historically marginalized or disadvantaged racial or ethnic group” (Ezell et al., 2018, p. 31). Specifically, the

burden should not be looked at as being a part of the marginalized population group or having a mental illness. The burden is the discrimination and stigmatization that is typically received as a result. Intersectional theorists argue that when individuals belong to a marginalized population group, they are more likely to be marginalized for other nonmainstream identities, such as having a mental illness (Lui, 2019).

Similar to the micro-aggressions that are received by people with mental illness, micro-aggressions toward Black men display themselves in the same subtle ways; however, there are two defining differences. First, experiences of racial micro-aggressions are “the daily common experiences of racial aggression that characterize aversive racism: commonplace verbal or behavioral indignities, whether intentional or unintentional, that communicate hostile, derogatory, or negative racial slights and insults” (Lui, 2019). Therefore, racial micro-aggressions are negative insinuations based on one’s outward appearance, differing from that of mental illness. Second, micro-aggressions toward people with mental illness are usually made by those closest to them, whereas micro-aggressions toward Black men can appear interpersonally and structurally. Microaggressions toward Black men most often happen at work, but also on the street, in stores, and in businesses (Hall & Fields, 2015).

Micro-aggressions toward Black men have the tendency to fall into the following categories: ascribing intelligence, viewing Black men as second-class citizens, pathologizing cultural values, making assumptions of criminality, believing that Black men are foreign or alien, claiming colorblindness, and denying racism (Sue, 2010). Sue (2010) states, “Microaggressions have the lifelong insidious effect of silencing, invalidating, and humiliating the identity and/or voices of those who are oppressed. Although their lethality is less obvious, they nevertheless grind down and wear out the victims (p. 66).” The experience of receiving micro-aggressions two-fold may exacerbate symptoms of mental illness.

Black men face disproportionately high rates of exposure to adverse social and economic environments (e.g., discrimination, unemployment, poverty, and violence) that generate and aggravate physical and psychological distress (Britt-Spells et al. 2018). The physical health effects of racism and discrimination include weight loss, insomnia, hypertension, gastrointestinal (GI) problems, tension, and neck and shoulder pain (Hall & Fields, 2015). The psychological effects of racism may result

in poorer self-concept, internalized racism, self-devaluation, suicidal ideation, poorer mental health outcomes, health damaging behaviors and negative affective responses (Chae et al., 2016; Goodwill et al., 2019). The combination of these factors may account for the steady rise in suicide rates reported amongst Black men over the past several decades (Britt-Spells et al., 2016).

TREATMENT GAP

The treatment gap for mental illness refers to the difference between those who need to seek care for mental illnesses and those who receive care (Jansen et al., 2015). In 2018, 50.1% of Black adults (ages 26-49) with serious mental illness did not receive treatment (CDC, 2019). Both systemic and psychological barriers contribute to the mental illness treatment gap in relation to Black men. For the purpose of this essay, there will be further elaboration on the psychological treatment barriers and how those interact with systemic barriers.

There are a number of psychological treatment barriers that are specific to Black men. In a study by Ward et al. (2013), it was reported that Black men are particularly concerned with stigma and self-stigma, which puts them at a higher risk for disengagement. The same study also revealed that Black men are apprehensive about seeking professional help for mental health issues. In addition to the stigmatizing beliefs about mental illness, the treatment gap may be due to a mistrust or lack of confidence in medical and mental health services (Maura & Mamani, 2017). Campbell and Long (2014) reported that Black patients’ specific reasoning for the lack of trust in medical and mental health services included a belief that White providers would not understand their experience, and a belief that they would be discriminated against because of their race. Moreover, this may explain why Black men have less improvement post hospitalization than Caucasians (Maura & Mamani, 2017).

Ward et al. (2013) discovered that Black men were not open to acknowledging psychological problems due to the fear of receiving a mental illness diagnosis, but were somewhat open to seeking mental health services. Smith and Applegate (2018) consider receiving a psychiatric label a possible barrier to treatment. Black men are already highly concerned with stigma and self-stigma in addition to reluctance to acknowledging psychological problems. Therefore, having a direct association with a psychiatric label would prove to be another challenge in seeking treatment.

It is important to further reiterate the social and cultural factors behind the treatment barriers faced by Black men.

These factors can be best understood through the following quotes from Black men discussing mental health with the Black Mental Health Alliance (Bryant et al., n.d.).

- “We are not supposed to seek help for our mental illness.”
- “There is still a lot of stereotyping of Black men in all of the medical professions.”
- “We have a problem asking for help from folks who we think are the reasons for our mental illness.”
- “No Black man in America is ever mentally healthy.”

The implications for leaving mental illness untreated are severe and wide-ranging; the effects include financial issues, public and individual health concerns, and social costs. Patients who are hospitalized for serious mental illness are much more likely to be readmitted within thirty days if they fail to receive follow-up treatment (Heslin & Weiss, 2015). The average hospital cost for a patient being readmitted for a mood disorder is \$7,100 (Heslin & Weiss, 2015). The National Alliance on Mental Health estimates untreated mental illness costs the nation as much as \$300 billion each year (Hunt et al., 2016). Additional implications for leaving mental illness untreated include ruined relationships, unnecessary medical procedures, job loss, substance abuse, and suicide (National Alliance on Mental Illness, 2018).

Currently, people with mental illnesses are more likely to experience police encounters than receive medical attention during a mental health crisis (National Alliance on Mental Illness, 2018). This is alarming, particularly for Black men, who already face a 33% chance of being incarcerated within their lifetime (Wang et al., 2014). Moreover, people who are incarcerated are considered to be a part of a “vulnerable population” group. Therefore, there are strict guidelines and regulations in order to permit them to participate in research. While the restrictions were placed after decades of unethical research in correctional facilities, they may also inhibit valuable insights into the relation between incarceration and mental illness. For example, if a researcher is following a community-based participant and they are incarcerated during the study, these laws prohibit the researcher from following up with them during incarceration and after their release (Wang et al., 2014). Black men are already disproportionately incarcerated and now performing research in order to examine its relation to mental illness is extremely difficult, if not impossible.

EFFECTIVE TREATMENT METHODS

Identifying the causes and developing treatment methods that address self-stigma in Black men with mental illness will result in narrowing the treatment gap. Black men with mental illness experience physical and mental symptoms from the “double burden” that they face. The components of the double burden break down into subgroups of micro-aggressions, discrimination, and stereotyping. Sociocultural beliefs also play a part in fostering or discouraging treatment engagement and adherence (Ward et al., 2013). Additionally, Black men with mental health concerns are at a higher risk of leaving mental illness untreated and for disengagement due to a mistrust and lack of confidence in services (Hankerson et al., 2015).

Numerous studies suggest training and awareness campaigns be administered in order to reduce perpetrations of micro-aggressions and their impact (Barber et al., 2020). Micro-aggressions should be thoroughly examined because research suggests that everyday discrimination appears to have a greater impact on mental health than lifetime discrimination (Britt-Spells, et al., 2018). “Lifetime discrimination” refers to events that impact one’s socioeconomic status over a wider time frame, whereas everyday discrimination reflects more recent events that are primarily concerned with attacks on one’s character. With that being said, intentionality is an area that should be further explored in the study of micro-aggressions. Training and awareness campaigns may only be effective if the micro-aggressions are unintentional. However, if one is intentionally a perpetrator of micro-aggressions toward Black men, perhaps further education directed at this target group would be helpful. Participants of a racial micro-aggression study often said White people denying racism were lying (Hall & Fields, 2015). Additional research should also focus on the intentionality of micro-aggressions toward people with mental illness.

Furthermore, family involvement is a rare occurrence in the treatment of mental health, but it is also crucial to the patient’s recovery. This is of particular importance amongst members of minority population groups, who are more likely to live with family members (Maura & Mamni, 2017). The exclusion of family members further discourages patient engagement and reduces adherence to treatment (Maura & Mamni, 2017). Fox et al. (2018) confirmed through a longitudinal data set that the more likely someone is to anticipate stigma from their friends and family, the

less likely they are to receive treatment for fear of appearing weak. Including family members in mental health treatment will aid in educating family members about the effects of stigma and lead to a reduction in self-stigma amongst Black men.

There are a considerable number of studies that examine the role social and cultural beliefs play in facilitating or impeding progress in the treatment of mental health. Incorporating social and cultural perspectives into mental health treatment has been deemed a moral and ethical responsibility of providers because of the overwhelming evidence of its impact on treatment seeking and adherence (Maura & Mamni, 2017). There are simple interventions providers can take in order to assist their patient in building trust and confidence. Examples include asking the patient what will help them feel respected, inquiring about their cultural history and identity, and involving community members or religious/spiritual support (Maura & Mamni, 2017). Studies have also shown that involving patients in the treatment decision making process by asking their opinion and preferences increases adherence to treatment (Maura & Mamni, 2017).

The lack of trust in providers may correlate to the sparse number of Black psychologists. As of 2015, only 4% of psychologists in the U.S. were Black (U.S. Census Bureau, 2015). Increasing the diversity of mental health care providers may aid in increasing the trust and confidence in health care services amongst Black men. Providers are still able to provide empathetic communication and collaboration with their patients by engaging in culturally sensitive practices and continuing education in this area.

Although receiving a mental illness diagnosis has been noted as a treatment barrier, research suggests that not only does it increase available treatment options, but ironically it works to combat stigma (Klik et al., 2019). Receiving a mental illness diagnosis allows patients to align themselves with groups, organizations, and communities that share the same diagnosis or concerns about stigma. Participating in groups leads to higher adherence to treatment by combatting and challenging ideas of stigma (Klik et al., 2019). Specifically, groups available for Black men with mental illness include Black Men Heal or Black Mental Health Alliance. Additionally, research has shown that identifying with a mental illness support group does not align with the patient’s preferences, the likelihood of adherence to treatment is still heightened as a result of the patient’s desire to disassociate with the group (Klik et al., 2019).

CONCLUSION

Unpacking all the layers that go into the mental health concerns Black men are facing can seem daunting. In order to decrease the consequences of leaving a mental illness untreated such as ruined relationships, job loss, substance abuse,

and suicide, Chin et al. (2012) suggests organizing the target areas by priority in order to decrease one disparity at a time. The target areas, such as provider mistrust or micro-aggressions, should be individualized for the patient since the specific causes of disparities will be unique

to their environment. Using a combination of socially and culturally relevant approaches, information, and resources geared specifically toward Black men and their unique challenges would help to close the treatment gap.

ENDNOTES

¹ Self-concept is the mental image one has of oneself (Merriam-Webster, n.d.).

² Self-stigmatization has been defined as the process by which a person with a mental health diagnosis becomes aware of public stigma, agrees with those stereotypes, and internalizes them by applying them to the self (Corrigan et al., 2010).

³ Treatment engagement refers to being committed to the therapeutic process and being an active participant in a collaborative relationship with a therapist to work to improve one's condition (Lizardi & Stanley, 2010).

⁴ Systemic therapy is a perspective that emphasizes treating the patterns that connect the problem behavior of one person with the behavior of other people (Loràs et al., 2017).

⁵ Socially closer refers to having a similar socioeconomic status.

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IN THEIR OWN WAY

Philippa Roberts

ABSTRACT

“In Their Own Way” is a collection of images shot using a Canon camera with a fast shutter speed and a wide aperture. Editing was performed using Photoshop and Lightroom, and sunlight was used as the backdrop to outline the natural beauty of women. In our society, women are expected to look, dress, and act in a particular way. These expectations are continuously seen on social media, in the entertainment industry and, unfortunately for some women, are even set by their families. With this topic in mind, I reflected on how I could use my position to help the young women of my generation. Through these images, I wanted to highlight that every woman is beautiful *in their own way*.

To emphasize this theme and to tie the images together, warm colors such as orange, red, green, and yellow were used in post-production editing. The yellow and orange tones were meant to signify the warmth of the glow that each model exudes from feeling comfortable in their own skin. The red undertones enhance this warmth by adding more energy and attraction for the audience. Lastly, the green tones (from their natural surroundings or on their clothing) work as a balance for the warmer colors while also emphasizing acceptance and growth from within. Additionally, posing the models in a central composition demands the attention of the viewers and enables them to observe the models in their surroundings, and experience each model’s individual aura.

Part of my job as a photographer is to empower the women I capture with my camera and not only show them, but also the world, how unique they are. With this uniqueness, they can stand tall. Everyone has their own trait that makes them stand out. It could be simply putting on an outfit that makes them feel like the best version of themselves – just like the young ladies in my images did. And so, with these images I wish to remind young ladies and women alike that they are unique *in their own way*, powerful *in their own way*, and beautiful *in their own way*.

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REPRESENTATIONS OF ARAB WOMEN IN THE MEDIA

Tharaphy Khin

ABSTRACT

The media can influence our perceptions and beliefs about various ethnic and racial groups. Consequently, misrepresentations in the media can exacerbate racial othering by propagating and perpetuating deleterious stereotypes. While the media itself does not cause inherent prejudicial ideas or effects, its vehicles can create disadvantages for vulnerable groups. This paper examines how common stereotypes of Arab women in the media can intensify racial othering and impede their fight for gender equality. The media's misrepresentations can flatten the complexity of Arab women's lives and produce the monolithic view of their existential landscape. Therefore, scrupulous analysis of media representations is necessary to diminish the problematic depictions of Arab women. Being mindful of the multiplicities in Arab women's stories reveals universal themes that can engender interconnectedness and eliminate the narrative that Arab women's struggles are mysterious and unique.

When I was a freshman chemistry major at a University in Burma, I failed one of my finals because I could not enter the exam room on the first day. The long commute on a crowded bus as a young woman is a stressful journey in Yangon. The ability to freely kick men in their groins when they "accidentally" press their bodies against me requires wearing jeans. However, I was not allowed in the exam room while wearing said jeans. The question occupying my mind while standing outside that exam room was, "How do I make the four-hour trip to change into my traditional clothing so that I can take an exam that ends in two hours?" The dress code is not explicitly stated in the university's official rules and regulations. So, I decided to be obnoxious to my heart's desire in public. Of course, that action landed me at the dean's office only to hear the words, "You look like a Western whore. You should have known better." Back then, I did not bother to correct the frequent misinterpretations that my defiance to injustice and oppression are signs of Westernization. Such imprudent assumptions might seem logical if one were to exclude the private dimensions of my life. Growing up, I never once heard "You cannot do it because you are a woman" uttered at home. My father was the one who told me stories about hidden influential female figures in Burmese history. Before I could read well in English, he would often translate the works of feminist writers to foment female empowerment. Therefore, even recurring unpleasant public encounters never led me to espouse reductive notions like 'all Burmese men are inherently sexist,' or 'all Burmese women are powerless victims of sexual oppression.' Upon reading Arab feminist literature, my own experience allowed me to comprehend why eliminating specific influences in

representing Arab women is detrimental to understanding the diversity and intersectionality of our lives. This paper presents that we should be considerate in our media representations of Arab women to avoid flattening the complexity of their lives. Media misrepresentations can reinforce stereotypes of Arab women as subservient vessels filled with victimhood, exacerbate racial othering of Arab women, and impede our common goal toward gender equality.

Accurate representations in the media are essential since most of us learn about people from other countries through our media consumption. For instance, media representations of Saudi women are readily accessible and sometimes the only medium available for some Americans to understand Saudi women (Mishra 2007, 260). In Smeeta Mishra's research article, the author made a salient point that, out of the 83 pieces from the American press, 53 of them focused on how oppressive the lives of Saudi women are in their country (267). Concentrating primarily on the oppressive dimension of Arab women's public life might mislead the audience into believing that one must liberate or rescue Arab women from their oppressed victimhood. Articles that emphasize how Arab women cannot drive or travel without a male relative's permission did not mention whether it is challenging or relatively effortless for Arab women to gain approvals from their male relatives for such activities. While the American press praised Saudi women's achievements in the public sphere, reports of their efforts in the private sphere were rarely mentioned in the media (268). Saudi citizens confront the same limitations as Americans in this sense. A total of 23 articles from various Arab news were chosen to analyze media representations of Western women. Arab news media

focused on probing the value of the freedoms that American women claim to possess (271). An Arab news article named "Funny Ideas About What Makes a Woman Happy" depicts the freedoms that American women cherish, such as having lunch with a male colleague or watching soap operas to demonstrate the superficial nature of American women's liberty (270). Several articles remarked that the American press mainly emphasized the restrictive lives of Arab women and also dictated how Saudi women should be conducting themselves more like western women; it highlighted the hegemony and intolerance of Arab religious faith and culture (270-271). Mishra noted that the United States and the Arab news media share the common trait of disciplining women's bodies in their dialogues (273). The media representations from the American press reflect the inclinations to transform the Saudi women's bodies to fit the Western narrative and values (273). In comparison, the Arab press media uses American women's representations to inspire resistance to Westernization and endorse cultural preservation (273).

Unequal access to media is problematic for Arab women in pursuing gender equality (Sakr 2002, 821). Inadequate access to prominent positions in the press restricts the potential to eliminate negative stereotypes while perpetuating the old ones, which disadvantages women in their daily lives (821). In January 2000, Egypt's parliament approved the practice of *Kula*, which gives women the right to divorce their husbands without the customary prolonged court procedures or the need to prove that there is "just cause" for their divorce, such as psychological or physical abuse (836). However, *Kula* also dictates that women seeking divorce must return their dowry and relinquish their

husband's financial support (836). With a few exceptions, Egyptian media did not depict the introduction of *Kula* as an issue concerning women who are dealing with injustice and oppression (838). Many of the political papers in Egypt had headlines such as "Men's era is over," reinforcing the view that this new law is a victory of women over men (837). Despite having an overwhelming number of male employees for the influential roles in the company, Al Jazeera became an exception in the portrayal of how *Kula* may affect the lives of many women by producing programs that offered balanced yet diverse expert opinions on women's issues (837). In the segment "Al-Iltijah al-Mu'akis" ("The Opposite Direction"), Farida al-Naqqash, a female head of the Progressive Women Union, presented her views that *Kula* is merciful even if some women were to suffer financial loss and support (838). She argued that the Arab households are incredibly private in their domestic affairs, and not all women can avoid the suffering of persecution, enslavement, and isolation that sometimes occurs (838). Whereas Ibrahim al-Kholy, a male professor from the University of Al-Azhar Cairo, contested her opinions and raised his concerns that *Kula* could escalate marital disputes that judicial procedure could resolve by encouraging the couples to blackmail each other (838). Kholy believes that fixing the judicial system by eliminating corruption and inefficiency, which causes delays in finalizing divorces, would be more beneficial for the married couples (838). Although the heated arguments between Naqqash and Kholy did not come to a resolution, the audience was not told what to think. Instead, the segment provided the audience with various views, which shows the complexity of Arab women's lives.

Nevertheless, producing media pieces with multifaceted views of Arab women's struggles is arduous without women in instrumental positions to influence the media's narrative. One cannot assume that merely increasing the number of women employees will guarantee to address the antecedent misrepresentations or the prospect of creating heterogeneous reports on women's issues. Studies on Lebanese media delineated that without female executives, having numerous female reporters, announcers, production assistants, and presenters alone cannot impact the media companies' contents (Sakr 2002, 833). If a media company can benefit from subjugating women's bargaining rights, it seems highly implausible that such a company will highlight gender inequality (833). Thus, influential roles in the media and accurate representations of women are crucial for accomplishing gender equality. Nothing

about the media itself causes inherently prejudicial ideas or effects, but its vehicles can generate disadvantages for vulnerable groups (822). Although the media's mediums are neutral, its access and implementation are still dependent on the existing systems of power (822). Hence, its integration into the current oppressive systems can erase the initial potential for neutrality in these vehicles.

First, it is essential to understand that while there are many publications about Muslim women, Arabs and Muslims are not of the same category (Kahf 2011, 111). In fact, some Arab feminists consider religion an extension of the oppressive system that enforces social norms and cultural stereotypes (Hasan 2018, 24). For them, religion endorses rigid gender roles that assign women secondary to men and fetter women to the realm of the household (24). These secularist views of feminism contribute to the incompatibility in feminism and religion for Arab women (24). There can be no singular perspective of Arab women or Muslim women (Ghobashy 2011, 102). Reducing the lives of Arab women into a single story in our attempt to liberate them or represent them is to ignore the diverse life experiences that mark them as unique individuals (102). For instance, Muslim women have different ethnicities (Kahf 2011, 111), sociopolitical, linguistic, and economic backgrounds. Therefore, one should be critical of the media representations that generalize and flatten the portrayals of all Arab and Arab Muslim women.

Nonetheless, the discourse in the media about Arab women is mostly about Muslim women, and those publications often illustrate and establish the victim-escapee stereotype in their narratives (Kahf 2011, 112). Kahf identified several elements in the victim-escapee stereotype (116). The first theme is called "Mute Marionette" or "Exceptional Escapee." Under this theme of a "Mute Marionette," the Western writers equip the Muslim women with voices. Yet, the voices are not of the Muslim women themselves. Instead, they are the analytical voices of the authors' interpreting the lives of Muslim women (116). In their attempts to illustrate the "Exceptional Escapee," the pitying narratives of the authors often eliminate the strong bonds between sisters, grandmothers, and friends that empowered those women (117). Eradicating those robust empowering relationships allows some authors to construct another element called "Meek Mothers" that bolsters the victim-escapee stereotype (116). However, ignoring the strong bonds between Muslim women who support and empower each other dismisses their home-grown feminism.

Of course, the role of "Meek

Mothers" goes well with the concept of "Forbidding Fathers." Unlike Muslim fathers, even fictional father characters such as Mr. Bennet, whose patriarchal actions embody his desires for his daughter to conform to social norms, are interpreted charitably as appropriate or ambivalent in the West (Kahf 2011, 117). How is using Elizabeth Bennet's body to quell his social anxiety any different from the motives and behaviors of Muslim fathers? In most stereotypical descriptions, Muslim fathers' intentions are inherently evil or inscrutable. In some circumstances, the father figure's overt caring gestures are extracted. The purposeful extractions of the fathers' support and affection in these Muslim women's stories can leave the reader with a vague impression that Muslim fathers implicitly relinquished their daughters to be raped (117).

Some authors frequently erase the empowerment of Muslim women's faith in their lives while condemning Muslim countries as inherently hateful toward women (Kahf 2011, 117). Undermining the power of the Islamic faith can create ways to reinforce the "Rotten Religion" element in the victim-escapee stereotype. The word "shariah," which means Islamic law, invokes a connotation of misogyny for many Westerners and secular Muslim feminists (117). Then, the "Cruel Country" element depicts how the religiosity of Muslim countries unleashes their unrelenting hatred upon Muslim women (117). The book's title, *Sentenced to Be Raped*, by Nicholas Kristof, provides the reader with an impression that the country itself is the cause of sexual violence in the heroine's rape (117). Kristof's depiction of Mai's journey as a survivor of gang rape in Pakistan left out how the country's judicial system held the men who committed the crime accountable (118). As in other countries with legal protection for their citizens, Pakistan allowed these men their due process of law before sentencings (118). The men were arrested, booked, charged, tried in court, and then sentenced as rapists (118). If such support from the government is obliterated, the "cruel country" element of the victim-escapee can persist.

Another element of the victim-escapee stereotype is "Erased Economics." When Mai recounted her story in an interview, she included her class as an influential variable contributing to her harrowing experience (Kahf 2011, 118). While expressing her bewilderment toward the assumptions that Islam condoned her rape, she explicitly mentions how her socioeconomic background affected the outcome of her journey (118). Mai stated that the police rarely pay attention to the crimes in poor neighborhoods and most villagers were afraid and vulnerable due to

their social statuses (118). Mai said in her interview, “The others were afraid. They would think ‘we’ are poor, and maybe if we stand up, the tribal lords will abuse us in the same way. In their hearts, these people were with us, but they were too scared to show this” (118). Mai’s economic standing in her society negatively disposed her to the prejudices of her government and the police. Class struggle is a universal theme that many can effortlessly identify across the globe. Ordinary American readers would only need to take a quick peek into their own communities to comprehend why Mai’s poverty permeated her misfortune. Hence, expunging the economic landscape from Arab women’s narratives can create a misconception that they live in inexplicably apathetic communities.

Lastly, the “Vile Veil” element in the victim-escapee stereotype mystifies and vilifies the coverings of Muslim women’s faces and bodies as intrinsically oppressive evil devices for subjugation (Kahf 2011, 118). Most women wear clothing such as hats, veils, and facial covering of some kind to express themselves through their fashion. However, when Muslim women wear something that covers certain parts of their bodies, the symbol of wearing some sort of veil becomes a manifestation of subjugation. The word “hijab” is regularly associated with oppression. It is seen to signal some lack of agency in women and the male dominance within her family and the country itself (Hasan 2018, 26). According to Islamic scholars, it is commonly understood that the full veil is not obligatory (29). Some women choose to wear the full veil to express their religious convictions (29). Meanwhile, some verses in the Bible mandate Christian women to veil themselves fully (29). A verse in the Corinthians says, “For if a woman is not veiled, let her also be shorn: but if it is a shame for a woman to be shorn or shaven, let her be veiled” (Corinthians I, 11:6). Christian women do not lack agency, so they choose to ignore or follow this imperative (29). Yet, Muslim women’s conscious choice to wear a hijab is often discarded as a “false consciousness” (31). If a woman is forced to wear a hijab, one should protest the coercion aspect of the affairs without condemning the hijab itself (35).

Any stereotype, either positive or negative, limits one’s personhood. The view of different races possessing distinctive inherent traits still exists in contemporary racial thinking. In the past, most racial thinking stems from the belief that biology determines one’s inherent, mental, and physical traits that are immutable (Blum 2002, 133). Biology was what distinguished one group of people from another. Nowadays, the

aspect of biology is somewhat diminished in our racial beliefs (133). However, misinterpretations of culture can function in the same way as genetic determinism. Assuming that Arab women’s stories are uniquely homogenous because they share the same Arab culture and therefore, the same fate, is no different from biological determinism in its essence. Stereotypes replicate the idea of inherency by claiming that the traits of specific groups are inherent and unchangeable because of who they are as a group of people. Therefore, stereotypes allude to the inevitable “racial fate” (104). If someone is not fulfilling their “racial fate,” she is an exception, and Blum referred to this bizarre logic as “qualified inherentism” (133). The victim trope of Arab women reveals such problematic racial thinking and othering.

Is it plausible to reason that there is some goodwill behind the media’s stereotypical representations of Arab women as oppressed victims? Perhaps that goodwill is the desire to promote gender equality by underscoring injustices that need to be addressed. Even if one is partial to this charitable interpretation, one can still question how the victimhood trope can inspire any woman to redeem her dignity to empower herself. Our racial thinking distorts our views and abilities to understand the complex dynamics of the communities with limited transparency. Because of the antecedent hegemonic structures, Arab women are especially susceptible to systems that restrict their ability to express themselves transparently in a global context. Therefore, it is a form of imperialistic tyranny and racial othering to scrutinize their lives with a rigid Western feminist gaze that reduces them enough to appear suitable for Westerner’s visual consumption.

The media can sway the intentionality of our thoughts, beliefs, desires, and actions. We are liable for the moral harm of stereotypical threats, even if the misrepresentations in the media were unintentional. The involuntary and non-deliberative nature of one’s actions does not exonerate one from the accountability of harmful acts. When we pay attention to the multiplicities in Arab women’s voyages, familiar patterns from our own lives emerge, and seeing Arab women as the “Other” becomes a logical nightmare. Our brains are prone to form the “Us and Them” dichotomies and make snap judgments about a Them based on our unconscious biases (Sapolsky 2018, 387). Since the “Us/Them” dichotomy is psychological, it can occur based on identifying shared traits with another (390). Our minds are prone to make positive associations when we share similarities, even if these shared traits

hold no significance to one’s existence (390). Therefore, resisting monolithic representations and illuminating the plurality of Arab stories can provide the audience with numerous ways to identify themselves with Arab women’s narratives. A sense of kinship in our minds is necessary to engender goodwill that may satisfy our desires to alleviate sexism and other injustices worldwide.

Providing platforms for Arab women to express the diversities of their existence is crucial to eradicating sexism. Actively absorbing the complexities in Arab women’s dialogues might equip all of us to determine and demand the fundamental changes required for women to thrive to their fullest potential. Misrepresentations from the media should be held morally accountable if they perpetuate the images of Arab women as oppressed victims with limited agencies whose inherent nature is to submit to domination. If an Arab girl is forced to wear a hijab but refuses, her rejection is not due to the inherent wickedness of a hijab-wearing culture. The hijab itself is merely a piece of clothing. Within the context of an Arab woman’s life experiences, her decision to wear a hijab might diminish her humanity or empower her with dignity. Without our preconceived notions, the multiplicities in Arab women’s thoughts and behaviors become less of a mystery. When a hijab becomes a beautiful headscarf or a veil, it is not difficult to see why some women feel empowered sexually or spiritually and adorn themselves in it with pride.

Sexual violence or any violence that Arab women experience is not a defiantly and inherently unique element that only exists in Arab women’s stories. Is it beyond the realm of human history or comprehension to grasp that Mai’s account of how the members of her community felt powerless to help her is because of their vulnerable economic background? A sense of apathy and helplessness caused by the hegemonic structures are not solitary manifestations of reactions within the Arab nations. Injustice and oppression tend to assume archetypal roles in the history of human tragedy. Just because the lives of Arab women seem mysterious and difficult to comprehend, we cannot label every foundation of their suffering as culture. If we are exceptionally open-minded about cultural practices, we should be mindful of our dispositions for unusual charitable interpretations. Any reasonable person can acknowledge that the pain from broken ribs is a natural response. If the pain of broken ribs is genuine, then so is Arab women’s anguish. Yet, hospitals do not refuse to treat the pain or the broken ribs because every symptom of pain from those injuries is natural. Like hospitals, the

world cannot ignore the suffering caused by some aspects of culture because it is a natural response to injury. We still should mitigate, heal, and remedy the ills of humanity.

There is no logical coherence to the monolithic media representation of Arab women as submissive and oppressed vessels of victimhood. Any living thing will respond accordingly to its environment based on its capabilities. Every Arab woman is an individual with diverse biological, historical, social, and economic backgrounds. How can it be possible that all the Arab women respond uniformly to their reality without any deviation? Such crucial considerations of different variables can highlight how media's misrepresentations have flattened and compressed peaks and valleys within

Arab women's existential landscape. There is a recurring theme in Arab feminist literature: their inner resentment toward the dialogues that reduce their minds and bodies as oppressed and inert public properties. Nonetheless, one cannot deny that when Arab women assert the grounds of their grievances, opinions, reactions, and desires, they are the embodiment of diverse personal journeys, with varied necessities and demands.

Symptoms of sexism manifest in many ways within humanity. Nonetheless, the debasing and diminishing of human dignity remains the culprit of misogyny. If we were to treat the cause of our sexist symptoms, we must amplify our senses and attention. Arab women's inner struggle and their combative reactions to objectification resemble our universal

human instinct to assert and affirm our sense of self-worth and belongingness in our natural environment. Dismissing their suffering, perpetuating their persecution, and impeding the empowerment of Arab women have malignant ripple effects on humanity.

One way to assuage our potential for harm and heal our previous injuries toward Arab women is to become conscientious consumers of media representations. We must critically scrutinize the contents of the media and refuse to draw impetuous conclusions. In doing so, we may erase the reasons that make the existence of Arab women seem like a dangling modifier in our global sentence to depict gender equality.

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FAUNAL COLONIZATION: A COMPARATIVE ANALYSIS OF TWO 17TH CENTURY CHESAPEAKE SITES

Emilie Ekholm

ABSTRACT

To better understand the animals that were kept and used by European colonists in Somerset County, Maryland, this project analyzes faunal remains from the 17th century site, known as Thornton. The faunal assemblages used within this project were found within an old midden, left behind by the early European colonists. These assemblages were analyzed by performing a hands-on review of the collection within Salisbury University's archaeological lab. The collection was then compared to another 17th century Maryland site located in Calvert County, known as Patuxent Point. After comparing the two sites, it was found that both sites shared many similarities, such as the species of mammals that were commonly used at both sites, as well as some differences between the sites. The research that was performed on the faunal assemblages is vital to understanding European colonists' lives on the Eastern shore. By using the data retrieved from analyzing and comparing the faunal remains, various insights can be provided about the early colonists' diet and the domesticated animals that they brought over from Europe.

The Eastern Shore is known for its colonial history as it was one of the first places the Europeans landed when they sailed across the Atlantic Ocean. It is because so many colonists passed through the Eastern Shore, that the Eastern Shore supports a wealth of colonial archaeological sites including a Somerset County site known as Thornton (18S0206; Fig.1). On this site, an old midden, which is a trash heap, was left behind by early European colonists and was partially excavated revealing many findings, including faunal remains. By analyzing the faunal remains found within the midden material at the Thornton site, more information can be found about the early Eastern Shore of Maryland colonial settlers' cooking styles, food sources, diets, colonization of the land and other resources they utilized. This data can also be compared to the colonial settlers living elsewhere in Maryland such as the Calvert County site called the Patuxent Point (18CV271; Fig.1). A comparison of their data can be used to better understand the lifestyle differences and similarities between the Western Shore and the Eastern Shore of Maryland European settlers. A hands-on review of the Thornton faunal collection aims to identify the faunal bones found within the Thornton midden so that the data can be compared to the Patuxent Point site's faunal data.

BACKGROUND

The Patuxent site is a 100-acre plot, that was first patented in 1651 by John Hodgins. After he died in 1655, his widowed wife sold the plantation to

Captain John Obder in 1658, who lived on the land until 1663. In 1986, the site was rediscovered by Thunderbird Archaeological Associates when surveying the surrounding area and was excavated in 1989. After excavating 1800 square feet it was discovered that the site was

rich in archaeological artifacts and faunal remains, with the faunal remains amounting to an astounding 87,000 fragments (ACIM 2010). Although there is little information on the history of this site, this is still a wealth of information on colonial lifestyles.



Figure 1: Here is a 17th-century map, showing the Eastern Shore, along the Chesapeake Bay. On this map, Patuxent Point's location is marked by an orange dot (ACIM 2010), where Thornton's location is marked by a blue star.

In 1658, William Thorne patented the Thornton site's 600 acres of land. In 1669, Thorne died, leaving his widow as his kin. In 1670, after marrying Thorne's widow, David Browne became the new owner of Thornton. After gaining Thornton, Browne increased the acres of land the estate had, from 600 acres to 980 acres. Thornton remained under Browne's care until he died in 1697 (Ragan and Perrault 2003). His probate inventory was then compiled to see how much wealth Browne had left behind. This probate inventory shows that Browne left behind almost twelve tons of pork, amongst other things, such as tools and 6,000 lbs. of tobacco, that indicated he was very wealthy (Ragan 2009). This suggests that Browne was a merchant selling pork to the West Indies. It was recorded that Browne also had forty-three head of sheep (possibly for textiles) and a milk house (Ragan and Perrault 2003). The faunal analysis performed in this research showed that Browne also had cattle that were probably used for milk as well as meat.

The site was later found by Darrin Lowery who was conducting a survey on the Somerset County agricultural fields in 1996 (Ragan and Perrault 2003). Once this site was found, an archaeological team excavated the site in 2002. Here both the plow zone and under the plow zone was excavated; the largest feature was a midden. The midden contained oyster shells and the bones of animals, mostly cattle and pigs (Ragan and Perrault 2003). However, very little of this site was excavated compared to the Patuxent Point site, as only 250 square feet (10x5x5) of the site was excavated. Due to the project being an exploratory excavation.

METHODS

After the excavation of the Thornton site, the faunal bones were labeled and cataloged; later an undergraduate student, Olivia Hamilton, re-cataloged the faunal remains. After cataloging them, Hamilton analyzed each specimen for human modification, species, and the identification of which bone the specimen was (Hamilton 2018:2). To improve the accuracy of identification of the specimens, I re-analyzed the same faunal collection. Whenever there were discrepancies between Hamilton's data and my data, the data has been marked in red (Fig.2). However, typically Hamilton and I produced similar results with only slight discrepancies.

The teeth found among the remains were described as selenodont, which means having sickle-shaped edges, or bunodont, which means having round or conical crown-like teeth. These were the most common tooth forms, as most of the animal teeth found were either pig

canines, pig molars, or bovine molars. During this research, various resources were used to identify the animal each specimen belonged to, which were terrestrial mammals, a fish, and a few birds. However, often many specimens were unidentifiable due to the bone or tooth being broken into small pieces, burnt or it was simply hard to identify which species of animal it belonged to. Specimens were also identified if they were calcined or if the specimens had nicks (a small cut or notch) or deep or peculiar cut marks, which indicated human modification (Fig. 2).

ID#	Unit	Level	Cat #	Code	Count	Material	Tooth FonTooth	Class	Order	Family	Genus	Species	Mass (g)	Burnt	Marked	
62	S110 W80	Surface	13	F		1 Tooth	Bunodont Molar	Mammali	Artiodacty	Suidae	Sus	S. scrofa	1.6	N	N	
66	S90 W80	Surface	17	F		1 Tooth	Seledont Molar	Mammali	Artiodacty	Bovidae	Bos	B. taurus	7.1	N	N	
74	S120 W20	Surface	24	F		1 Bone	N/A	N/A	Mammali	N/A	N/A	?	0.2	N	Y	
77	S50 W110	Surface	26	F		1 Tooth	N/A	Lower Car	Mammali	Artiodacty	Suidae	Sus	S. scrofa	0.8	N	N
81	S80 W110	Surface	27	F		1 Bone	N/A	N/A	?	N/A	N/A	?	0.4	N	P	

Figure 2: A picture of the Thornton catalog, showing some of the categories used and the discrepancies of data, which are highlighted in red. The level category indicates which level of the surface the remains were excavated from, and the Unit, Cat# and Code (F= Faunal), categories serve to identify which faunal specimen is being used.

In the Thornton collection overall, there were 1,424 faunal specimens. When compared with Hamilton's data, it was found that there were 132 specimen bags out of 512 which had discrepancies in the faunal data. Although there were several discrepancies, it would be best to have an expert in faunal analysis to perform a more professional analysis. However, using the data, the final report and a comparison between the Patuxent Point site and the Thornton site could be performed.

HUMAN MODIFICATIONS

In the archaeological site report for Patuxent Point, there was no mention of human modification on their specimens (ACIM). This is possibly due to the specimens not having been fully analyzed when the data was entered in the report due to the specimen's immense number. However, when analyzing the faunal specimens from Thornton, research showed that 39% of the bones were marked, possibly from a combination of the colonists' cut marks, or damage in the field or lab, and 32% of these specimens were burnt. This means that at least some of the animals found were indeed used for food. During the colonial period, colonists would have butchered the animal themselves, for meat (Greenfield 1989: 58). After butchering the animal, the meat would be divided into portions that would be cooked and then distributed to be eaten. During the butchering process cut marks would be left on the bones of the animal and depending on how the animal was cut during the butchering process there would be different types and sizes of

marks (Greenfield 1989: 58 -59). The most common cut marks that I noticed during my time analyzing Thornton's bones were little nicks, and straight cuts.

After the butchering process, the meat would possibly have been cooked, and the refuse would have sometimes been burned. The bones that tended to be bluish in color, have black dots, or a light orange tinge were burned at a low temperature (Beisaw 2013: 109). Those that were burned at higher temperatures tended to be slightly or completely black in color. However, some of the bones were very burnt making them unidentifiable;

therefore, making it impossible to identify which species they belonged to with the naked eye. Despite some of the bones being hard to identify due to being burnt, I was still able to identify many of the specimen's species, class, or both.

PROPORTIONS OF CLASSES

Once the data for Thornton had been compiled, the largest class that was identified was class Mammalia (Fig. 3). This is unsurprising because Brown's probate listed large numbers of mammals, especially pigs (Ragan 2009). Although the largest class found on the Patuxent Point site was the class Chondrichthyes, the site also had large numbers of mammals. Using this information, we can determine that those on the Eastern Shore relied on mammals as one of their main food sources.

When analyzing the Patuxent Point faunal data, I realized that they did not display the results for all 87,000 faunal remains (possibly due to the substantial number) and instead only displayed the specimens that could be identified by class, which totaled 9,770 faunal remains. When looking at the data that was provided on the site, Patuxent Point differed substantially in the proportions of classes compared to the Thornton site, as most of the faunal remains that were found were fish (Fig.4).

One of the possible reasons for the class Chondrichthyes, being the majority of what those in colonial Calvert County used as a food source, is because Patuxent Point is on the Chesapeake Bay which is a rich estuary. Fish is one of the

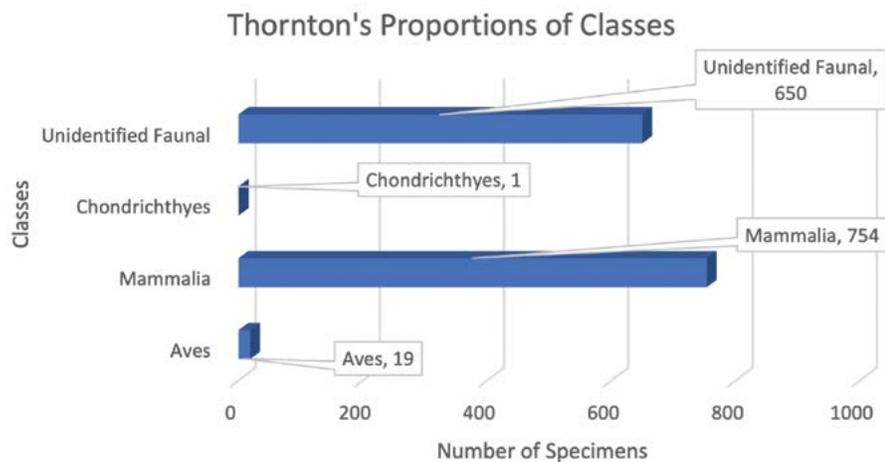


Figure 3: The number of specimens categorized under different classes; with class Mammalia being the majority and the unidentified faunal classes being the second majority. The high number of unidentified faunal is because most of the specimens are unidentifiable.

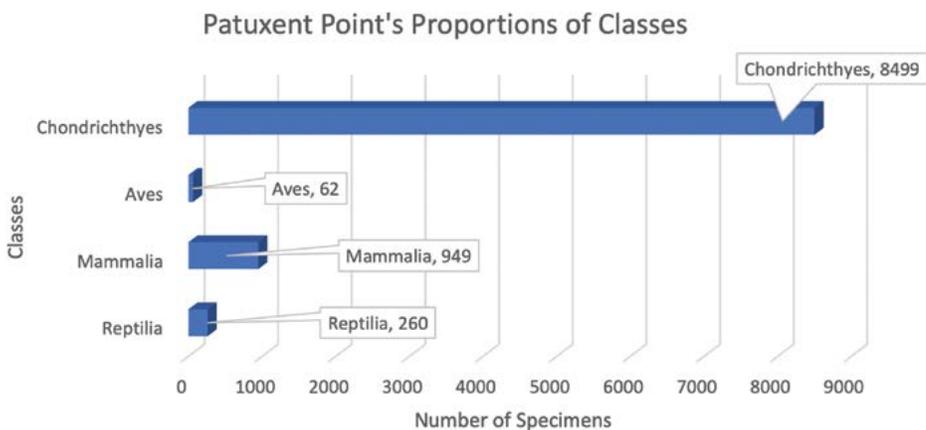


Figure 4: The number of specimens categorized into different classes; with Chondrichthyes being the overwhelming majority. This is mostly due to the number of scales that were found within the faunal remains, which numbered 6,696 (ACIM 2010).

dominant meat sources usually found on a southeastern coastal site, as many of these sites are known to have many aquatic vertebrate specimens (Reitz and Waselkov 2014: 22) that are from the shores of estuaries. This is because the estuaries are abundant in wild resources, that would be easy to access (Reitz and Waselkov 2014: 22).

The Thornton site is located on an aquatic area, the Manokin River, near the Tangier Sound and the Chesapeake Bay. The Manokin River's resources were also utilized by Somerset County colonists, as indicated by the presence of the class Chondrichthyes and numerous oyster shells recorded from the site which were not, however, analyzed as a part of this project (Ragan and Perrault 2003). Although the Thornton site is located close to the Chesapeake Bay, the colonists would have more heavily utilized the Manokin River due to its proximity to the site. The river is a slightly different environment

from the Chesapeake Bay, as the river has a lower salinity of 14 parts per trillion (ppt) (MIORW 2020: 8). The Bay, however, has a higher salinity of 25-30ppt (CBP 2021). This would increase the bay's diversity and provide a wider variety of food resources than the Manokin River, which could explain why more fish remains are found at Patuxent Point than at Thornton. However, it must also be taken into consideration that fish remains are fragile, that only a smaller portion of the Thornton site was excavated, and fish remains do not float so they would not appear in the nearby Manokin river which could also be why Thornton has few fish remains. Despite these differences in the numbers of aquatic animals utilized by the people who lived on these two colonial sites, both shared similarities when it came to the mammals the people were eating.

MAMMALS

Some of the most common meat

resources found on southeastern coastal sites within the U.S are animals such as, cows, deer, and pigs. The mammals played a significant role in the colonists' diets, due to their large quantities of meat and nutrients (Reitz and Waselkov 2014: 33). Thornton and Patuxent Point are no exception to this. On Thornton, most of the mammal specimens identified were pigs, which is to be expected due to the large numbers of pork that was recorded on Browne's probate. The second most common mammal species identified at Thornton was cattle. This could have been because cattle were used for meat and milk, as a milk house was reported on Browne's probate inventory. (Ragan and Perrault 2003). Cattle and pig being the two highest classes of mammals found on the Thornton site is not uncommon since colonists living close to the Chesapeake consumed a yearly average of 200-450 pounds of pork and beef. The colonists also kept many of these animals because they required minimal care and supervision, which meant more time could be given to growing their crops, which were primarily tobacco (Anderson 2004:112).

Since most of the mammal species found are mentioned within Browne's probate inventory, one would expect that the third largest mammals discovered would be sheep as 43 head of sheep were mentioned in the probate (Ragan and Perrault 2003). However, when analyzing the Thornton faunal collection remains, I found only four specimens (as indicated in Figure 5), that seemed to have belonged to sheep. This is possibly because during the early colonial period, sheep could roam through thickets and were considered easy prey for predators, such as wolves (Anderson 2004: 110). It is because they could roam freely, that it is possible many of the sheep could have died off the site, resulting in their small presence on the Thornton site. Their small presence could also be because many of the mammal specimens remain unidentified, as 548 out of the 754 or 73% of the specimens identified as mammal remain unidentified due to many of the specimens being broken into small pieces or burnt to be unidentifiable. Sheep are especially hard to identify because of their similar morphology to other mammals, such as goats. Therefore, there is the possibility of there being more sheep within the Thornton collection. This can be applied to wild mammals as well, as only four wild animal specimens were identified in the collection.

Similarly, the Patuxent Point site also relied on domestic animals as a resource but seemed to also rely on wild animals heavily. According to the faunal specimens found at the site, the main mammals

Mammal Species Found at Thornton

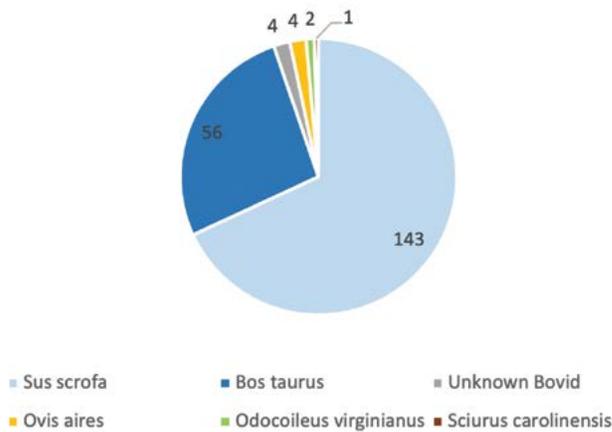


Figure 5: Shows the proportions of mammal species found on the Thornton site, where the Unknown Bovid species represents teeth that were bovine but were either too damaged or burnt to determine which bovine they belonged to.

relied on were cattle and white-tailed deer (ACIM 2010). A possible reason as to why there are more remains of white-tailed deer at Patuxent Point than on the Thornton site is because the settlers had very close trading ties with the Native Americans. It has been suggested that some sites have a larger number of wild animal remains because of the close trading ties (Reitz and Waselkov 2014: 33-35). It was not uncommon for these groups to trade with each other for resources. Interactions with the Native groups would have caused the colonists to use more wild products, some of which would be fish, turtle meat, deer meat, and shellfish (Reitz and Waselkov 2014: 33-35). The possible trade between Native Americans and the colonists would explain Patuxent Point's high usage of deer meat and explain the presence of turtle remains found on the site, (which was the 260 reptilian specimens), and was 3% of the total specimens found (ACIM 2010).

Patuxent Point also had substantial numbers of cattle remains and Thornton had many cattle remains, as well. One of the reasons why cattle were commonly found at these sites is because it was common to have cattle herds due to the large amount of meat they provided. These cattle herds would have been

used to obtain milk, produce hides, and sell meat to the West Indies (Reitz and Waselkov 2014: 36-37), similar to what Browne was suspected of doing with pork (Ragan and Perrault 2003). During the colonial period, cattle could graze freely across the land, except for calves and their mothers which were often kept in pens (Reitz and Waselkov 2014: 36-37). This was because colonists saw that the "untouched" environment would be excellent land to raise cattle and other livestock on. To European colonists, cattle not only provided resources but also signified that the land was becoming a part of civilization. They believed that they ruled over their animals like proper Christians; animals being lower than them because of the order of creation. The colonists also believed their presence and their animal's presence would help make the land Christian and would provide the Native Americans who did not exert their power over animals, a model of what Christianity should look like (Anderson 2004: 76-77).

One interesting commonality both sites shared in their proportions of mammals was the lack of horses. Horses at the time were one of the main cornerstones that helped European civilizations thrive. It could be that the lack of horses found on these sites is

because in the Chesapeake, tobacco was the most common crop that was planted. The owners of Thornton also raised tobacco, as the first owner of Thornton, Thorne, had left behind 34,520 lbs. of tobacco and Browne had left 6,000 lbs. of tobacco to Somerset County (Ragan and Perrault 2003). Colonists cultivated this crop using hoes instead of plough animals like horses and oxen. Therefore, lessening the colonists need, for these animals. Work horses and oxen also require a lot of feed to provide the energy needed to work fields, as a work horse alone needs 20 lbs. of hay and oats a day. To feed these animals, the colonists would have had to clear and cultivate more land; using time and land that could be utilized for cultivating tobacco (Anderson 2004: 111-114). The Chesapeake farmland was usually littered with tree stumps, making it impossible to use horses and other animals to till the land thus causing a lower demand for these animals by European colonists. The colonists also rarely used horses for other work, such as traveling, further lessening the need for these animals on the Chesapeake colonial sites (Anderson 2004: 111).

However, this data could be inaccurate, as many of the mammal specimens found remained unidentified, so there is a possibility of there being more species of mammals that were used. Although, both the Patuxent Point site and the Thornton site share the aspect that they have most of the mammals that are commonly found on southeastern colonial coastal sites. These include the commonly found wild mammals: tree squirrels and white-tailed deer and the domestic mammals: pigs and cattle (Reitz and Waselkov 2014: 22-23), all of which were found on both sites.

From performing a hands-on review of the Thornton collection, many species were able to be identified and recorded. This data was able to provide a deeper understanding of the European Colonists living on the Eastern Shore, especially when compared to the Patuxent Point site. From the data comparison much was learned about the animals utilized by early colonists; such as pigs being a common livestock that was used to provide goods for the colonists. The faunal data also provided more insight into the wild resources the colonists had access to, such as fish from the Manokin River and the Chesapeake Bay. However, much of these sites still have a wealth of colonial information that remains undiscovered because of the unidentifiable faunal remains. It is because the faunal remains provide much insight into early colonial lives, that it is important for both these sites to identify these remains in the future.

Wild Fauna Remains	Deer	Fish	Fox	Squirrels	Turtles
Patuxent Point	59	8,499	1	2	260
Thornton	2	1	0	1	0

Table 1: Displays the proportions of wild faunal remains, identified at both sites. However, there is a possibility that there are more wild faunal remains for both sites that are unidentified (ACIM 2010).

Species of Mammals Found At Patuxent Point

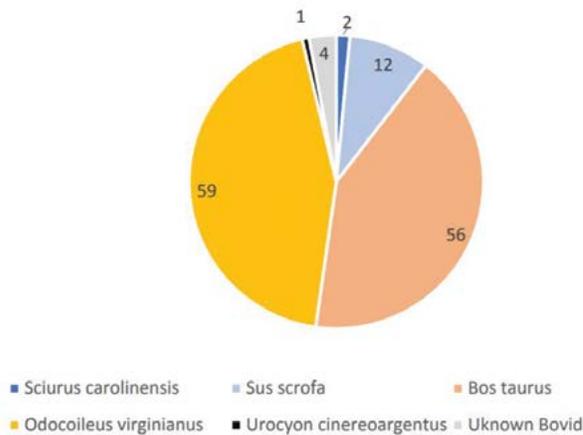


Figure 6: The proportions of mammal species found on the Patuxent Point Site; with *Bos taurus* and *Odocoileus virginianus* being the two primary species (ACIM 2010).

Although, overall, these analyses allowed us to better understand what animal resources the colonists had access to, the types of diets the colonists had, what environments the colonists lived in, and what domesticated animals they utilized.

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SEED-DISPERSING BATS FOR ECOLOGICAL RESTORATION OF SUB-ANDEAN FOREST, UPPER CEIBAS RIVER BASIN

Daniela Baron Cruz & Lizeth Fernanda Hoyos Díaz

ABSTRACT

This study determined the environmental contribution of bats in ecosystem restoration, favoring the connectivity of the sub-Andean Forest in Huila, Colombia. Bats were captured with three mist nets across 24 nights for six months with a sampling effort of 18 hours per net. The fecal samples from each of the bats were collected to perform the sowing of seeds produced by zoochory. Twenty individual bats were identified along with their respective food preferences: eight of the genus *Sturnira* were associated with the consumption of *Piper sp.* and *Saurauia sp.* seeds; seven of the genus *Artibeus* were associated with the consumption of *Retrophyllum rospigliosii* (Pilg.) CN Page – an endemic forest species with the IUCN category of Vulnerable; four of the genus *Micronycteris* associated with fruit pellets and exoskeletons; and an individual of the genus *Eptesicus*, which are considered insectivorous due to evidence of insect exoskeletons in its guano. The genus *Sturnira* had the highest abundance and was the largest consumer of seeds, and the genus *Artibeus* was relevant for its consumption of fruits of *R. rospigliosii*, allowing the colonization and establishment in the restoration area of this forest species as an umbrella species within the forest. This provides evidence that bats, as seed dispersers, can serve as a nature-based solution in the restoration of sub-Andean Forests.

INTRODUCTION

In Colombia, South America, in the department of Huila, is the property of La Colonia. Since 2010, officials of the Corporación Autónoma Regional del Alto Magdalena (CAM) designated an area for ecosystem restoration to establish a natural laboratory and understand the restoration process in the sub-Andean Forest ecosystem. The fast-growing forest species, *Smalanthus pyramidalis*, which reaches the sub-canopy layer to provide structure and vegetation cover, and the endemic forest species, *Retrophyllum rospigliosii* as an umbrella species that contributes to the establishment of other plant species, were found (Corporación Autónoma Regional del Alto Magdalena, 2020).

In the study area, there is evidence of seeds and seedlings present in the substrate, corresponding to dispersed seeds from the parental trees of *R. rospigliosii* that were planted decades ago in this area. This indicates the process of natural dispersal by fauna because some seeds present evidence of bites. Fruit bats are thought to be the culprits due to their morphological adaptations in their teeth that coincide in size and shape with the traces presented on these fruits (Carranza et al., 2020).

This project aims to identify the importance of bat-plant interactions in the natural succession of sub-Andean secondary forests with anthropic disturbance. The project seeks to determine whether these mutualisms contribute to strengthening the supply of ecosystem goods and services and the establishment of connectivity between

forest fragments. Such findings would demonstrate the fundamental conditions needed for supporting biodiversity and a crucial necessity in environmental preservation efforts seeking to promote these collaborations as nature-based solutions (Seddon et al., 2020).

METHODOLOGY

The study area is located on the La Colonia property, in the upper basin of the Ceibas river in the La Plata district of the municipality of Neiva, department of Huila-Colombia. The area is inside of the Siberia Ceibas Regional National Park, at 2100 m, with a sub-Andean Forest ecosystem, averaging an annual rainfall of 1400 to 1600 mm and average temperatures between 12-18 °C (Bourbon et al., 2007).

For the characterization and identification of bats, captures were made with 3, 12 m x 6 m mist nets, opened from 19:00 to 23:59 for two consecutive

nights every two weeks for 6 months. The captures were in perch or resting places, connectivity areas, and restoration zones. Table 1 shows the sampling points together with their description and coordinates.

Each bat captured in the mist net was then kept in a cloth bag for 45 to 60 minutes to complete their digestive process and perform the deposition of the fecal sample (Rios & Pérez, 2015). Before the release of each individual captured in the net, morphometric measurements of forearm length (LA), tail length (LC), leg length (LP), ear length (LO), nose blade length (LHN), femur length (LF), and total length (LT) were taken (Dietz & Von Helversen, 2005). The captured bat individuals were taxonomically categorized to genus level based on taxonomic guides and criteria from two bats experts. Individuals within each genus were then subclassified into morphospecies by taxonomists, based on species-level

Table 1. Areas of capture, nomenclature, and coordinates of the location of fog nets

No.	Type of capture area	Site name	Location of the mist net	Latitude	Longitude
1	Perch areas	P1	Basement 3km from La Colonia	2°46'26"	75°04'43"
2		P2	House 1km from La Colonia	2°45'41"	75°04'28"
3		P3	House La Colonia	2°45'10"	75°04'32"
4		P4	Study Center in La Colonia	2°45'11"	75°04'33"
5	Places of Connectivity	CZR1	Natural corridor, transition between paddock and Sub- Andean Forest	2°45'10"	75°04'36"
6		CZR2	Right margin, entry to La Colonia	2°45'12"	75°04'38"
7		CZR3	Left margin, La Colonia entrance	2°45'11"	75°04'38"
8	Restoration zones - on the ecological trail	ZR1	5 yards from the start of the trail	2°45'19"	75°04'37"
9		ZR2	30 m from the start of the trail	2°45'18"	75°04'37"
10		ZR3	106 m from the start of the trail	2°45'18"	75°04'39"
11		ZR4	2m from the end of the dining area	2°45'14"	75°04'39"

Table 2. Registration of individuals with taxonomic identification found on La Colonia property, la Plata-Neiva vereda.

Family	Genus	Species	# Indiv	Diet	Sites	Relative abundance
Phyllostomidae	Sturnira	sp1	2	Fruit	P2, CZR2	10%
		sp2	3	Fruit	P3, ZR4, CZR1	15%
		sp3	3	Fruit	CZR1	15%
	Artibeus	sp1	5	Fruit	ZR4, CZR2, CZR1	25%
		sp2	1	Fruit	CZR1	5%
		sp3	1	Fruit	CZR2	5%
	Micronycteris	sp1	3	Fruit & Insects	P1	15%
		sp2	1	Fruit & Insects	P1	5%
Vespertilionidae	Eptesicus	sp1	1	Insects	P4	5%
TOTAL			20	100%	11	9

morphological differences.

To identify the feeding preferences of the captured individuals, the fecal samples deposited by the individuals were reviewed under stereoscopy.

Finally, the following ecological diversity indices were used: the Margalef index and Shannon Wiener index for species diversity, and the Jaccard index for similarity in bat feeding preference and overlap at sampling sites (Aguilar et al., 2014).

RESULTS AND DISCUSSION

A total of 20 individual bats belonging to the families *Phyllostomidae* and *Vespertilionidae* were captured over 24 nights (144 hours) of sampling. A capture effort of 18 hours per net and a capture success rate of 1.22 was recorded.

Of the bats captured, eight individuals belonged to the genus *Sturnira*, corresponding to three morphospecies, seven individuals to the genus *Artibeus*, with three morphospecies, four individuals in the genus *Micronycteris*, corresponding to two morphospecies, and one individual from the genus *Eptesicus*, associated with one morphospecies (Table 2).

In terms of feeding preference, there is no niche overlap or competition for resources between genera (Jaccard index of 0), but near-complete overlap between morphospecies within genera (Jaccard index of 100). From the genus *Sturnira*, nine fecal samples were obtained with seeds of primary colonizing shrub species of rapid establishment, *Saurauia sp.* and *Piper sp.*, indicating these bat species play an important role in promoting early successional stages of forest restoration. To the genus *Artibeus*, at the time of capture, the exclusive transport and consumption of fruits of the endemic forest species *R. rospigliosii* were evidenced. This exclusivity is of great relevance for

the ecological restoration of the sub-Andean Forest, since the genus *Artibeus* has morphological adaptations for the frugivory of large fruits of forest species. The forest species *R. rospigliosii* is considered a suitable plant material for restoration, due to its adaptability to compete with angiosperms (Scanlon & Petit, 2015). Its categorization as an umbrella and nucleating species favors the establishment of other plant species, which contributes to the consolidation of the structure and function of the sub-Andean Forest (Cueva et al., 2010; Dumont, 1999; Trujillo, 2013).

The guano of the genus *Micronycteris* contained remains of fruit fibers and insect exoskeletons (Ramos et al., 2017), and the guano from the genus *Eptesicus* contained exoskeletons of insects (Sanchez et al., 2019).

Regarding the ecological index, the Margalef and Shannon Wiener indexes were extremely low in most of the sampling points with values between 0.5 and 0.9 (P2, P3, P4) and null (P1, ZR4). The study area is a secondary sub-Andean Forest with ecosystem fragmentation and lack of connectivity as a consequence of anthropogenic disturbances. Many bat species are associated with the forest interior or buffer zones, so they would not adapt to a high degree of disturbance, or the limited amount and variety of resources in these areas (Casallas et al., 2017; Moreno, 2011). Nevertheless, the Margalef and Shannon Wiener indexes recorded medium diversity in two of three connectivity areas (CZR2, CZR1), possibly due to the presence of mature trees that serve as roosting and feeding areas (Casallas et al., 2017; Moreno, 2011).

The Jaccard index expresses the similarity of the sampling sites and obtained similarity values of 50% between ZR4 and CZR1 with the presence of

Figure 1. The Margalef index determines species richness showing values between 0 to 5. 0 indicates low diversity and 5 indicates high diversity. Only sites with catches are shown in the graph.

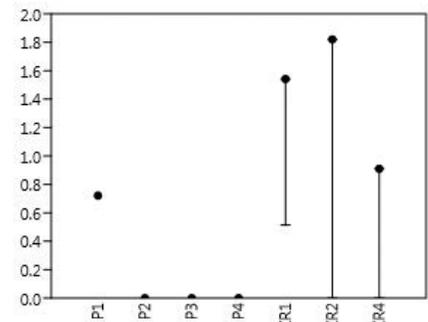
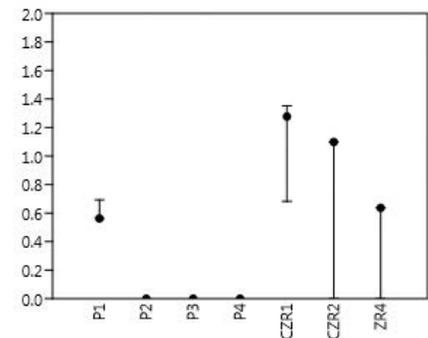


Figure 2. The Shannon Wiener index determines the species equity by obtaining values from 0 to the natural logarithm of S (number of species). 0 indicates low equity and the value of the natural logarithm of S indicates high equity. Only sites with catches are shown in the graph.



Artibeus sp1 and *Sturnira sp2* morphospecies, 50% between ZR4 and P3 with *Sturnira sp2* individuals, 33% between CZR2 and P2 with *Sturnira sp1* individuals, 25% between CZR1 and P3 with *Sturnira sp2* individuals, 25% between ZR4 and CZR2 with *Artibeus sp1* individuals, and 16.67% between CZR2 and CZR1 with *Artibeus sp1* individuals. This represents the proximity between sampling points and similar disturbance conditions.

CONCLUSION

The bats in the study area were taxonomically classified to the genera *Sturnira*, *Artibeus*, *Micronycteris*, or *Eptesicus*.

Sturnira sp. specializes in key pioneer shrub species such as the genera *Saurauia* and *Piper* and has an important role in early successional stages of forest regeneration. *Artibeus sp.* genus has a greater abundance and importance for the dispersal of forest species. Their importance in zoochory is apparent with the great effectiveness established in this study with their dispersal of the forest species *R. rospigliosii*, key for the consolidation of the structure and function

of the sub-Andean Forest ecosystem.

All species captured in this study demonstrated tolerance to anthropic disturbances. This includes the genus *Micronycteris*, which was captured in an abandoned basement and usually inhabits mature forests, suggesting that it can utilize disturbed sites once they are abandoned and allowed to undergo natural shrub succession.

Thus, the bats found in this study are of great importance for the elimination of dispersal barriers in the ecosystem

restoration of sub-Andean forests due to their facilitation of pioneer shrub and endemic forest species dispersal. Additionally, the mutualism documented between bats of the genus *Artibeus* and *R. rospigliosii* is essential to favor the consolidation of the ecosystem structure and functionality, generating ecosystem services and goods, as a nature-based solution for forest restoration.

Recommendations for future studies may include continuing to evaluate changes in the bat dynamics of feeding

preference as fruit diversity increases due to ecosystem consolidation and connectivity of the sub-Andean Forest. Researchers should focus on the particular case of the zoochory of the genus *Artibeus* sp. for the morphological adaptations that allow it to transport large fruits typical of canopy forest trees and use this information in the development of forest regeneration plans and wildlife conservation efforts.

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MASS, ENTROPY, AND ODDS VOL. 2

Joshua Killen

ABSTRACT

The sculpture titled “Mass, Entropy, and Odds” was designed using the virtual reality sculpting programs Gravity Sketch and Medium, printed and assembled using renewable polyactide (PLA) plastic on three-dimensional (3D) printers, and painted with enamel and acrylic paints. 3D printers were intentionally chosen to emphasize using new technology to create physical objects that were designed exclusively in virtual reality. The sculpture takes the form of a book and is the second volume in a collection inspired by the illustrative components found in medieval era illuminated manuscripts. Looking at a book, even in a sculptural form, evokes an expectation that knowledge is stored within. The title and contents of this book are my personal take on unified field theory: a topic of particle physics that yearns to discover the elusive relationship between Einstein’s theory of relativity and quantum physics. In short, the laws of gravity mysteriously do not work the same on the quantum level. The sculpture explores my theory that the gravity discrepancy can be explained by correlating the universe’s mass with the external forces of entropy coupled with the odds of probability. The unique interface of virtual reality gave me the ability to play with my ideas regarding the theory of relativity in a simulated quantum environment. For example, a page would start as a simple thin rectilinear shape (mass), and then using my hands “virtually” I, (entropy) would pull, tear, and twist the shape like the invisible forces of gravity; furthermore, the location and intensity of interactions between mass and entropy were deliberately guided by simple mathematical patterns (odds). The other volumes of this book explore the same concept; however, they are constructed using alternative mediums.

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SURVIVABILITY OF CLINICALLY SIGNIFICANT ORGANISMS ON BLOOD GLUCOSE TEST STRIPS

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ABSTRACT

With the increased prevalence of diabetes, the use of glucose monitoring devices has increased, and with that, the spread of healthcare-acquired infection (HAI). However, there is currently no published research pertaining to organism survivability on glucose test strips. This data, however, is needed in order to evaluate the risk of HAI as vials of glucose test strips are shared between patients. If a contaminant can survive on a glucose test strip for a significant amount of time, it can be introduced directly into the patient's bloodstream. The survivability of *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, and *Candida albicans* were evaluated on glucose test strips to assess their transmission risk of HAI. Glucose test strips were inoculated with a clinically significant dose of organism, and then touched to their designated growth medium each day. Agar plates were evaluated for growth after each 24-hour period to determine the length of time organisms can survive on the glucose test strips. *C. albicans* survived for two days and *A. baumannii* survived for six days. Hourly testing was needed for both *K. pneumoniae*, which lasted twelve hours, and *P. mirabilis*, which lasted one hour. This data suggests that vials of glucose test strips present a great risk of spreading infections when used to test more than one patient as organisms are able to survive numerous hours or days on these strips. Once more research about glucose test strip contamination is completed, steps can be taken to reduce the risk of HAI, such as making improved guidelines for handling or strip packaging.

Even though healthcare-acquired infection (HAI) is theoretically preventable (1-4), it is responsible for an estimated 1.7 million infections and 99,000 deaths yearly in the United States (2, 5-9). HAI refers to any infection that is transmitted in the healthcare setting while receiving treatment; and often arise from the mishandling or lack of proper sanitization of medical devices (2, 3, 7-11). Although significant progress has been made in preventing transmission of infections, namely the mandate of proper hand hygiene for medical staff (3, 4, 12, 13), many causes and factors related to HAIs are still unknown or under-researched (2, 3, 9, 12-16).

HAI spread from the glucose monitoring process is particularly under-researched. Although HAI can occur with any patient, those who frequently test their blood glucose levels are more likely to be affected by cross-transmission due to increased contact to test strips (10-12, 17-21). Diabetics must monitor their blood glucose (sugar) levels using three principal devices in order to provide treatment direction (1, 13-15, 23, 24). The glucose monitoring process utilizes fingerstick lancets (the device that pricks a patient's finger), glucometers (the device that reads the patients' blood glucose levels), and glucose test strips (the device that collects and tests the blood from the patients open wound) (1, 2, 12, 13-15, 22-24). These devices, particularly glucose test strip vials and glucometers, are typically used

by providers to test multiple patients in a short timeframe, a procedure called serial-testing (1, 2, 12-14, 16, 23, 25-27). Therefore, contamination can spread quickly if cleaning procedures are not properly followed (12-15, 23).

Within the last 20 years, studies have researched HAIs caused by this glucose monitoring process (1, 2, 5, 12-16, 23, 26-29). Unlike the glucometer and fingerstick lancet however, there has not been a major call to action regarding the glucose test strip; partly because there has not been as much research conducted on the strips. The available data suggests that these strips may become contaminated when medical personnel retrieve a strip out of the small vial opening with contaminated hands (3, 4, 10, 11). Because these strips are so small, and are primarily held in multi-use vials, when a medical professional intends on removing one strip for testing, they inadvertently touch other strips in the vial (1, 2, 5, 12, 13, 16, 24, 29). The medical professionals' hands can spread contamination if they are not properly sanitized before and after the glucose monitoring test from a single patient or if they come in contact with unsterile equipment or surfaces. (1, 2, 5, 12-16, 23, 24). Glucometers, for example, are also exposed to the patients' blood, so when the medical professional then touches the glucometer, their hands can become contaminated, even if their hands were previously sanitized (1, 12, 13). If their hands are

not disinfected, contamination can spread when they touch the other strips in the vial. Strip-spillage could also occur when medical personnel are trying to remove a single strip. These strips would then be contaminated if medical personnel were to return them back to the vial (13). Although this contamination most likely will not affect the current patient being tested, it has the potential to spread infection to the next patient getting tested. If the pathogens on the glucose test strip from the previous patient can survive long enough, the contaminant could potentially infect the next patient through the open wound created by the fingerstick lancet (1, 2, 5, 12, 13, 16, 24, 29).

Another SU honors student, Meghan Nalesnik (30), previously tested the survivability, or length of life, of the organisms *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), methicillin-resistant *Staphylococcus aureus* (*MRSA*), and vancomycin-resistant *Enterococcus faecalis* (*VRE*). This study presents a continuation of her work by testing the survivability of *Candida albicans* (*C. albicans*), *Acinetobacter baumannii* (*A. baumannii*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Proteus mirabilis* (*P. mirabilis*) which have all caused HAIs in previous research. The organisms in this study were selected because similar pathogenic strains of the same organisms can be the cause of urinary tract infections (UTIs) and other infections (7, 11, 19, 31, 32). UTIs are not only more prevalent in diabetics, but

they account for 32% of all HAIs (6, 7, 9-11, 19, 25, 32-34). Most importantly, the organisms investigated in this study were all found on glucose test strips and/or other medical devices in previous studies (1, 4, 16, 25, 35-37).

In this experiment, these organisms were intended to represent contamination from previous testing events before the user pulled a glucose test strip from the vial. As previously stated, it is very common for diabetics to contract a UTI, so these organisms can very easily be shed from the urinary tract and presented to the environment. Then, when the medical professional is conducting additional tests or cleaning the area adjacent to the urinary tract, their gloves can become contaminated (3, 4, 10, 11). However, even if the medical professional changes their gloves and practices perfect hand hygiene, the new gloves can get re-contaminated if they contact unsterile surfaces or medical equipment that the patient may have introduced the UTI contamination onto (3, 4, 25).

To this date, the survivability of organisms on blood glucose test strips remains unknown. It is vitally important, however, to know the survivability of organisms on these strips to establish if this glucose test strip contamination contributes to the risk of HAI. Theoretically, pathogens adapted to growth in the human body should not be able to survive on dry, non-nutritive glucose test strips (1-4, 9-11, 18). If this is the case, a contaminant should only survive for a negligible time on a glucose test strip and pose no threat to a patient. However, if a contaminant can survive long enough to encounter the next patient, it can spread infection.

MATERIALS AND METHODS

BROTH CULTURE PREPARATION AND SERIAL DILUTIONS

Before experimentation could begin, the organisms needed to be diluted to 100,000 CFU/mL, the minimum organism amount used to diagnose UTI. Because this amount is used to diagnose UTI, it is reasonable to assume that a patient can shed this number of pathogens. Diluting the organisms to the same quantity also ensures that each strip has the same number of viable cells later on. To dilute the organisms the original broth concentration had to first be discovered. Using a sterilized inoculation loop, one colony was collected from a completed streak plate separately for each organism. The colony was then placed into a separate tube of Trypticase Soy Broth (BD BBL™ Becton, Dickinson and Company Sparks, MD. 2 mL) by

inserting the inoculation loop and shaking vigorously. The inoculated broth was then incubated for 24 hours at 37 °C to allow for organism growth. For the remainder of the experiment, the broth was kept at room temperature (25 °C).

With a separate, filtered pipet for each organism and Phosphate buffered saline (PBS), 100 µL of the fresh culture broth was added to 900 µL of the diluent PBS, making a 1:10 dilution. PBS was used as the diluent because it provides no nutrients that would promote organism growth, but it also preserves the organism because it has a neutral pH and a physiologically tolerable salt concentration. Pure water would be more stressful to the organisms and could cause falsely decreased survival times. Then 100 µL was taken from the 1:10 dilution and was added to 900 µL of PBS, creating a 1:100 dilution of the original broth. Following this procedure, serial dilutions of 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000 were produced for each organism. Then, 200 µL of the 1:100,000 dilution was then added to 200 µL of PBS creating a 1:200,000 dilution. This serial dilution method was followed until each organism had dilutions of 1:200,000, 1:400,000, and 1:800,000.

The dilutions of *A. baumannii*, *C. albicans*, and *K. pneumoniae* were all separately inoculated on Mueller Hinton plates, whereas the *P. mirabilis* dilutions were inoculated on MacConkey plates. All inoculated plates were then incubated at 37 °C for 24 hours. The plates with easily countable colonies were then analyzed and counted. Using the colony counts from each serial dilution, the original concentration was calculated, which was used to calculate how much PBS was needed to dilute the organism to 100,000 CFU/mL.

Because the original broth was left at room temperature in between the serial dilutions and colony counts, it is possible that the organisms could have still been growing, albeit at a slower rate, giving it a slightly different concentration. This, in turn, could have made the dilutions slightly more concentrated, potentially adding more organisms onto each strip. If the organisms were held at 4 °C to prevent further growth, however, organism death could have occurred due to the cold, giving falsely decreased survival times.

INOCULATION AND OBSERVATION

Once all the organisms were diluted properly, each organism was inoculated onto 50 separate glucose test strips (the total found in one vial) each. Ten µL of inoculum were added to two points on each glucose test strip, the blood collection site and the non-electrical end, for a total of 2,000 CFU/mL per strip (Figure 1). This was the chosen inoculation volume

because it is a relatively small volume but can be easily transferred onto the blood glucose test strip by the medical professional's hand. Larger volumes would be less realistic. The blood collection site is where the patient's open wound comes directly into contact with the strip, whereas the non-electrical end is where the medical personnel directly touches the strip to take it out of the vial. The blood collection site and the non-electrical end are very close together, so it is reasonable that the zones can overlap in a real-world scenario. The inoculation points are made of different plastics, but no differences in growth between the two areas were observed. The strips were left in room temperature under a biological safety cabinet for the remainder of the experiment until use.



Figure 1: Glucose Test Strip. A 10µL volume of inoculum was placed in the red circles labeled A and C. Point A is the blood collection point. Point C is used by the medical professional to remove the strip from the vial. Point B goes into the glucometer.

Starting on day zero when the strip inoculation took place, a strip, one for each organism, was pressed into its corresponding designated plate for 30 seconds with sterilized tweezers once the inoculum had dried. Thirty seconds mimics the time the strip could be exposed to the patient. *C. albicans* was plated on the same CHROMagar™ Candida plate for four days in different quadrants to conserve materials. This plate is specifically made for yeasts, and contains chloramphenicol, which would inhibit most bacteria contamination if it were present. *A. baumannii* and *K. pneumoniae* were plated on the same CHROMagar™ MH plate each day to conserve materials. To differentiate between the two organisms other than labeling the plate, the *K. pneumoniae* grew

blue on a CHROMagar™ MH plate, whereas the *A. baumannii* had tan growth. CHROMagar™ MH does not have any inhibitors. *P. mirabilis* was originally plated with the *A. baumannii* and *K. pneumoniae*. However, *P. mirabilis* swarmed, meaning it covered the other two organisms, which gave inconclusive results for all organisms involved. Instead, *P. mirabilis* was plated on MacConkey agar to limit its growth. After the 30 seconds, the strip was then discarded. Each day, a new glucose test strip for each organism was tested following this procedure until growth was absent for three days consecutively, making the last day of growth its survivability time. Three days were given to make sure the organisms were truly nonviable since growth can become erratic as organisms are dying. If an organism did not grow after Day 0, strips were tested every hour for 24 hours following the same procedure. The hourly procedures were the same as the normal procedure. However, instead of testing every day, the Hours 13-24 were tested first, as to not spend 24 consecutive hours in the lab. If growth was not present during any of these hours, the Hours 0-12 were tested.

Each plate was also inoculated with the corresponding original broth – acting as a positive control – and the uninoculated, sterilized PBS – the negative control. The original broth ensures that the organism is still viable while the PBS ensures there is no contamination. The plates were then incubated at 37 °C for 24 hours after inoculation.

RESULTS

C. albicans had many colonies from the Day Zero plating (right after inoculation), but by the Day Two sample, there were only a few remaining *Candida* colonies. Growth did not continue after Day Two (Table 1).

Table 1: Survivability

Organism	Survival Time
<i>C. albicans</i>	Two days
<i>A. baumannii</i>	Six days
<i>K. pneumoniae</i>	Twelve hours
<i>P. mirabilis</i>	One hour

Because *P. mirabilis* was able to swarm on the CHROMagar™ MH plate, the experiment was restarted for *A. baumannii*, *K. pneumoniae*, and *P. mirabilis*. After serial dilutions, colony counts and dilutions for these three organisms, 50 new glucose test strips each were inoculated. Because the last attempt failed, the glucose test strips inoculated with *P. mirabilis* were plated on MacConkey agar which

limits growth enough to ensure that the organism was still viable on the glucose test strips. However, because MacConkey agar limited *P. mirabilis*' growth, it is possible that it could have created a falsely decreased survival time. Without the MacConkey, however, there would be no way of knowing if the growth was from the strip or the positive control.

During the second attempt, however, *P. mirabilis* was still contaminating the CHROMagar™ plate. This contamination was most likely spread by the unfiltered pipette tips during the serial dilution and dilutions. The experiment was again restarted. During this attempt, each organism had its own designated pipette and filtered pipette tips were used to ensure no cross-contamination could occur. PBS also had its own pipette. After serial dilutions, colony counts, dilutions, and inoculation, *A. baumannii* grew successfully without contamination; but, because *P. mirabilis* and *K. pneumoniae* did not show growth on Day 1, hourly testing was needed. *A. baumannii* had inconsistent growth. One colony grew from the first day sample and the second day sample. On the third day sample and fifth day sample there was no *A. baumannii* growth, but it resumed on the fourth- and sixth-day samples. But even though growth was inconsistent from Day Three to Day Six, it can be established that *A. baumannii* can survive up to six days, since it did not grow after the Day Six growth (Table 1).

As previously stated, *K. pneumoniae* did not grow past Day Zero, so hourly testing was needed. The *K. pneumoniae* colonies were too numerous to count during the first two hours, however growth soon started to decrease. By the Hour Seven sample, growth sharply decreased until there was one colony during the Hours Eleven and Twelve samples. There was no growth after Hour Twelve (Table 1).

P. mirabilis also needed hourly testing. There was sufficient growth for the Hour Zero sample, but only one colony was present at the Hour One sample. Growth did not continue past Hour One (Table 1).

DISCUSSION

In contrast to this studies results, Nalesnik's experiment demonstrated much longer survival times with her tested organisms (Table 2) (30). Particularly MRSA and VRE survived throughout the duration of the experiment, meaning that these organisms can potentially survive for more than fifty days (Table 2) (30). Because there were fifty test strips in a vial, she was unable to test past 50 days, meaning it was impossible for her to determine how long these organisms could survive. However, the results demonstrate that these organisms can survive long enough to be a potential risk for HAI.

Table 2: Nalesnik Survivability

Organism	Survival Time
<i>E. coli</i> O157:H7	45 days
VREF	>50 days
MRSA	>50 days
<i>P. aeruginosa</i>	11 days

With our data combined, and because of the variability of the tested organisms together, there should be enough data to show a sufficient representation of the average survival rate of common pathogenic organisms on glucose test strips. Even though glucose test strips are plastic and theoretically cannot support life, these organisms still have a very high probability of infecting the next patient because they somehow manage to survive for a significant timeframe (Table 1, 2). Because vials of glucose test strips are shared between patients, this data shows that this could be a cause of HAI for those who frequently use the glucose monitoring process, such as those with diabetes. When glucose strips are held against an open wound to collect blood, the viable organisms could be introduced directly to the bloodstream, which could cause a variety of complications, including sepsis. These data can add to the body of knowledge and help determine if contaminated glucose strips could truly be a cause of HAI. Once enough research about glucose test strips contamination is conducted, steps can be taken to reduce the risk of transmission, such as making improved guidelines for handling or strip packaging (2, 5, 16, 24).

GLOVE CHANGING AND HAND HYGIENE BETWEEN TESTING

Medical personnel do not always change gloves or wash hands in between testing and other medical procedures for a single patient, despite the current protocol (4, 11-13, 27, 28). This lack of hand hygiene is most likely because they may not realize the risk involved. Because a patient cannot infect themselves, many medical professionals may believe that if hand hygiene is followed in between patients, cross contamination cannot spread. It also may be challenging for the medical professionals to take the contamination possibilities into consideration when feeling the pressure of having to time-manage many different patients at once (12, 13). However, because the glucose test strips can spread infection, proper sanitation must be utilized in between testing to avoid strip contamination (1, 3-5, 11-13, 16, 25, 27, 28).

INDIVIDUALLY PACKAGED STRIPS

The most efficient way to make sure the glucose test strips cannot get contaminated, in addition to following hand hygiene protocol, is to individually package every strip, instead of holding them in 25-50 strip vials (1, 2, 5, 12, 13, 16, 24, 29). If they are individually packaged, contamination is less likely to occur. The only way that contamination could occur is if it happens at the factory where they were packaged, or if the packaging is opened or damaged during transit. Studies suggest contamination rate of glucose test strips in multi-use vials range from 25-83% (1, 2, 5, 16, 29, 35). But in the individually packaged vials, the contamination rate is from 0-7%, which is a huge improvement (1, 2, 5)

Originally, glucose test strips were individually packaged, but multi-use vials were sold to reduce the financial and environmental cost (22). Individually packaged strips are much more expensive for the healthcare facilities to purchase (38, 39), and have more material waste; fifty individual packages are discarded, instead of one vial. Even with the research showing the risks involved with using strips in multi-use vials, many places are still hesitant to change protocols. A few hospitals have been designating one vial per patient to try to reduce HAI risk. However, this creates even more financial loss and environmental waste than the individually packaged strips would, making it harder for other hospitals to follow their lead (38, 39). A study by Saborido and Rodrigues demonstrated

that in comparison to the multi-use vial wastage from designating one vial per patient, switching over to individually packaged strips would save \$1,406 per patient (38). In addition, the use of individually packaged strips would save the hospital the most money by reducing the incidence of HAI. HAI is thought to lengthen the hospital stay by 19 days and increase costs by approximately \$43,000 per hospitalization due to medication increase, isolation, and other diagnostic tests the patient may need (1, 5). Using individually packaged strips would save money and limit, if not eliminate, the risk of HAI spread by glucose test strips. (1, 2, 5, 12, 13, 16, 24, 29).

LIMITATIONS

This experiment was intended to discover how long organisms could survive on glucose test strips. However, there are many factors that determine whether the contamination induced to the blood stream could be a detriment to the patient. The first few factors have to do with the organism itself. Placing more organism into the blood, for example, would have more impact on the patient (3, 4, 25). The amount of organism added can change based on when the glucose test strip was contaminated and each organism's survivability. If the strip was contaminated seconds before, a bigger portion of organism would be viable and able to affect the patient. If an organism can better survive on a glucose test strip, the quantity of organism would be present for a longer duration. Another factor that

could affect the contamination impact on the patient is the virulence of the organism. An extremely virulent organism would have more of an impact than an organism with low virulence. Frequency of organism contact can also impact the patient (3, 5, 25). If someone is frequently exposed to a pathogen often, they are more at risk (3, 5, 25). Diabetics are often in this category because they may need testing every 4 to 6 hours depending on the patient (17, 15, 40, 41).

The final factor that could impact a patient's wellbeing is the patient's immune system. If someone who is immunocompromised, like someone with diabetes, gets exposed, they could be more at risk than a healthy individual (9-11, 18, 20, 21, 31, 42). Immune function can be affected by age, diet, sleep, stressors, medications, vitamin D levels, and chronic illness (9, 20, 42). The immune system function also differs from person to person, due to the adaptive immunity from previous possible encounters with the organism (21). If someone had encountered the organism before, it is possible that their body knows how to defend itself against the said pathogen. It is impossible to say how much organism needs to be exposed to a person to cause harm because every immune system and every organism have a different interaction. Since these organisms can survive on blood glucose test strips, they have the potential to cause harm to patients.

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THE IMPACT OF HYPERGLYCEMIA ON INTESTINAL MOTILITY IN *DANIO RERIO* (ZEBRAFISH)

Jack Lenox

ABSTRACT

The enteric nervous system (ENS) modulates gastrointestinal behaviors such as peristalsis and gastric emptying. Diabetes mellitus is a chronic metabolic condition that often leads to gastrointestinal dysfunction. The mechanisms underlying this pathology are not well understood, thus therapies are largely directed at symptom management rather than curative treatment. In the Clark Lab, we have previously shown that induction of hyperglycemia negatively affects the somatic peripheral nervous system. Here, we were interested in determining if this degenerative phenotype was also influencing the nerves of the ENS. Five days post-fertilization (dpf) larval zebrafish were incubated for seven days in 120mM glucose solution and *in vivo* imaged to create time lapse videos. Results indicated that hyperglycemia significantly decreased overall intestinal motility. Segmentation significantly decreased overall (**p=0.0001). Additionally, total peristaltic frequency and wave distance decreased significantly (**p=0.0316 and ***p=0.0002, respectively). Intestinal fillage differentially impacted motility. The most significant decreases in segmentation and peristalsis occurred in fish with medium intestinal fillage (***p<0.0001 and ***p<0.0001, respectively). Observational results report decreased contractile coordination and increased intestinal inflammation. Future experiments should consider investigating the neural circuitry of the ENS to possibly elucidate the underlying pathophysiology of diabetic enteric neuropathy. This research contributes to the use of zebrafish as a model for neurogastroenterology research and may even be employed to explore potential new drug therapies for gastrointestinal disorders present with hyperglycemia.

BACKGROUND

High blood glucose levels, hyperglycemia, is a hallmark of diabetes mellitus (DM), which results from either of a lack of insulin production by the pancreas (Type-1) or a malfunction in insulin receptors resulting in insulin resistance (Type-2). Improper insulin signaling prevents cellular uptake of glucose, resulting in chronically elevated glucose levels in circulating blood¹. This hyperglycemic state in turn contributes to several complications associated with diabetes including increased susceptibility to disease and infection, renal failure, vision loss, gastrointestinal and gastrourinary symptoms, cardiovascular complications, and sexual dysfunction². General intestinal dysmotility, which describes a wide range of symptoms including gastroparesis, diarrhea, constipation, and abdominal pain, is one of the most common side effects in patients with diabetes³. As of now, the mechanisms underlying these digestive disorders in diabetic patients are not well understood, thus therapies are largely directed at symptom management rather than curative treatment.

The enteric nervous system (ENS) is the largest division of the peripheral nervous system and controls intestinal functions and is primarily comprised of enteric neurons and glial cells. It is by far the largest division of the peripheral nervous system⁴; with over 100 million

neurons in the small intestine alone, the ENS is the largest collection of neurons and glial cells outside the brain⁵. Independent of the central nervous system, the ENS is able to locally modulate intestinal behavior, including motility, secretion/uptake of neurotransmitters, blood flow regulation, and interactions with the endocrine and immune systems⁶. Intestinal motility refers to coordinated intestinal activity responsible for the movement of material through the gut. Segmentation describes the coordinated alternated contraction of circular muscle cells and relaxation of longitudinal muscle cells and functions to mix and churn intestinal contents. Peristalsis describes rhythmic ring-like contraction of circular muscle cells and serves to move intestinal contents along the digestive tract⁷.

Damage to ENS control of smooth muscle contractile activity can be life

threatening⁵. Functional segmentation and peristalsis are critical to body function and can have severe impacts on quality of life. In clinical studies, diabetes has been linked with intestinal motility issues such as delayed gastric emptying and gastroparesis⁸. Overall, the effect of diabetes on the function of the small intestine is not well studied. However, electrophysiological studies in humans have suggested a relationship between hyperglycemia, impaired contractile coordination, and peristaltic dysrhythmias⁹. Since the somatic PNS is impacted by hyperglycemia¹¹, it is highly probable that the largest division of the PNS is also impacted by the metabolic state.

Previous literature on the digestive tract and intestinal motility have largely utilized mammalian models, however the inability to directly visualize gut

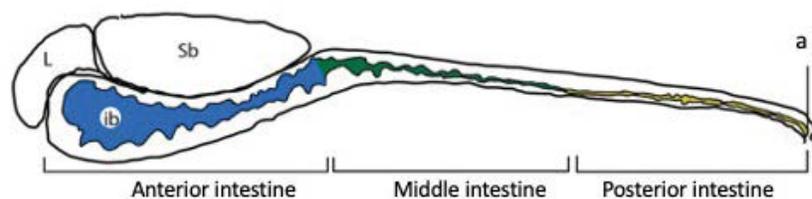


Figure 1. Larval zebrafish digestive anatomy. The different intestinal regions have been color-coded. Blue highlights the intestinal bulb, green highlights the middle intestine, and yellow highlights the posterior intestine. L=liver, sb=swim bladder, ib=intestinal bulb, a=anus.

motility in real time is a disadvantage of these model organisms. Zebrafish (*Danio rerio*) are advantageous as a model for intestinal motility studies due to the optical transparency of their skin in embryonic and larval stages⁹. Utilizing their relatively transparent skin in these stages of growth allows for visualization of the digestive tract *in vivo*; the intestinal tract can be easily observed even at low magnification. Zebrafish are thought to not have true stomachs²⁰, but instead have an intestinal tract that is morphologically divided into an intestinal bulb, middle, and posterior regions (Fig. 1). Much like humans, the zebrafish gastrointestinal tract is a tube extending from mouth to anus. Additionally, zebrafish intestinal anatomy and architecture resemble that of the mammalian small intestine⁷, making zebrafish an ideal model for studying intestinal function and behavior.

Using the advantageous characteristics of zebrafish, the Clark lab has previously developed and characterized a glucose immersion model of diabetic peripheral neuropathy (DPN)¹¹. This model induces acute hyperglycemia in larval zebrafish through incubation in 60mM glucose solution beginning at five days post-fertilization (dpf) for five days¹¹. When immersed in this glucose solution, the zebrafish take up increased levels of glucose due to their transdermal absorptive properties. This results in an acutely hyperglycemic metabolic state¹¹. Recently, the Clark lab has found that immersion in 120mM glucose solution for seven days results in more robust structural changes in the PNS (Voithofer et al., in

prep). For that reason, the 120mM glucose solution was utilized to ensure a more apparent impact to intestinal motility.

Here, we aimed to establish a protocol to investigate the effects of hyperglycemia on intestinal motility, while remaining cost, time, and resource efficient. Taking into consideration the known impact of diabetes on intestinal motility and the effects of hyperglycemia on the integrity of peripheral nerves^{9,11}, we hypothesized that hyperglycemia in zebrafish would coincide with a significant decrease in (1) segmentation and peristalsis frequency, (2) relative segmentation strength, and (3) relative distance traveled by peristaltic waves. Following the induction of acute hyperglycemia, our results indicate that hyperglycemia decreases the strength and frequency of segmentation activity in zebrafish. The establishment of this degenerative phenotype in the ENS lays the groundwork for future research into potential mechanisms behind intestinal dysmotility and enteric nerve damage.

METHODS

Animal husbandry and hyperglycemia induction

All experiments were carried out in accordance with animal welfare laws, guidelines, and policies, and were approved by the Salisbury University Institutional Animal Care and Use Committee (IACUC). Adult male and female zebrafish were placed in breeding tanks overnight. After breeding, fish were returned to their respective tanks and eggs were collected and rinsed in egg water (reverse osmosis water mixed with Instant Ocean). Fertilized eggs were incubated

in egg water for 5 days at 28.5°C in 100x15mm Falcon petri dishes. At 5dpf, zebrafish were randomly separated into control and experimental groups. The control group was incubated in egg water until 12 dpf in 250mL Erlenmeyer flasks in a 28.5°C water bath. The experimental group was incubated in 120 mM glucose egg water for seven days, until 12 dpf, in 250mL Erlenmeyer flasks in a 28.5°C water bath. Larval zebrafish were fed hatchfry once per day beginning 5 dpf and solutions were changed out daily to prevent bacterial growth.

In vivo imaging

At 12 dpf, larval fish were anesthetized using 0.4% Tricaine Methanesulfonate (MESAB). While anesthetized, fish were oriented in the sagittal plane in 1% low melting point agarose (Fisher) on 33mm petri dishes (Fisher). Once hardened, additional egg water and MESAB were added to the dish to maintain the anesthetized state for the duration of imaging.

Ten-minute time-lapse sequences of intestinal activity were taken on an inverted EVOS FL Auto imaging system microscope at 10x magnification (ThermoFisher). Images were captured at a rate of one frame every four seconds resulting in image sets containing 151 frames. These individual frames were then compiled into a 24-second video using the movie-making function of the EVOS FL microscope.

Videos were used to visually assess intestinal motility. Intestinal motility refers to coordinated intestinal activity responsible for the movement of material

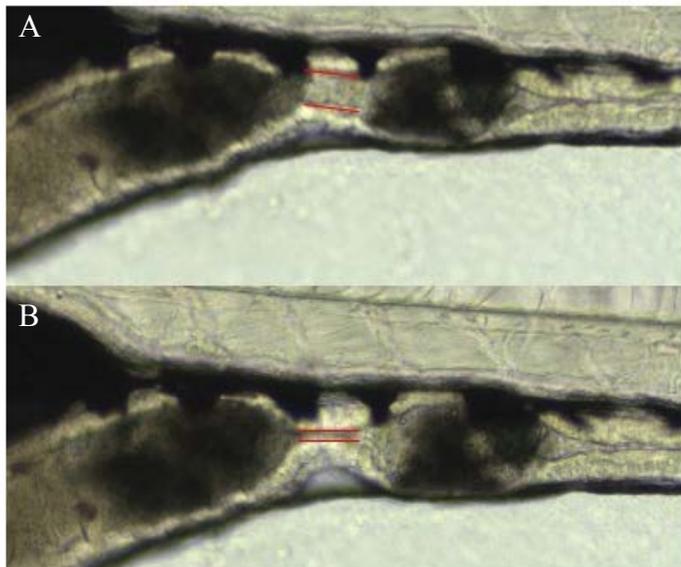


Figure 2. Segmentation contractions. These photos show a control zebrafish before (A) and during (B) segmental contraction. Full contraction of circular muscle is shown in the top image. Notice complete occlusion of the lumen in the bottom image. In control fish, this type of contraction tended to occur in the middle intestine, within somite region 2 or 3.

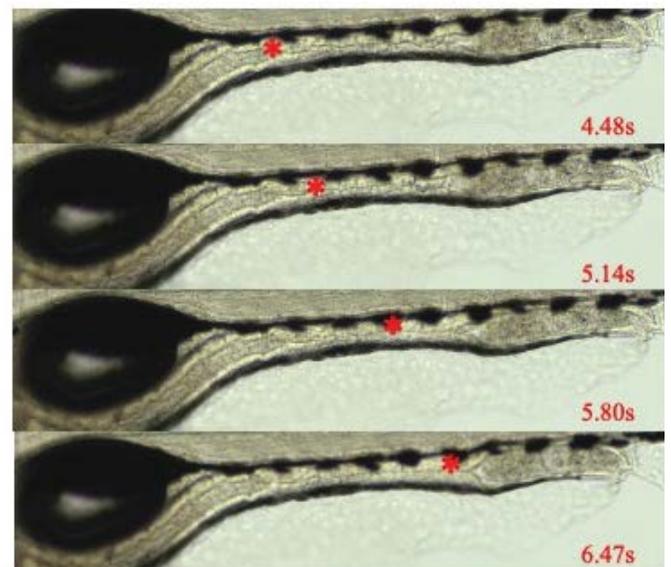


Figure 3. Peristaltic wave movement. This circular muscle contraction can be seen traveling in a wave pattern in a caudal direction along the intestinal tract. Asterisks and timestamps have been used to show the time it takes for peristaltic waves to travel a given distance.

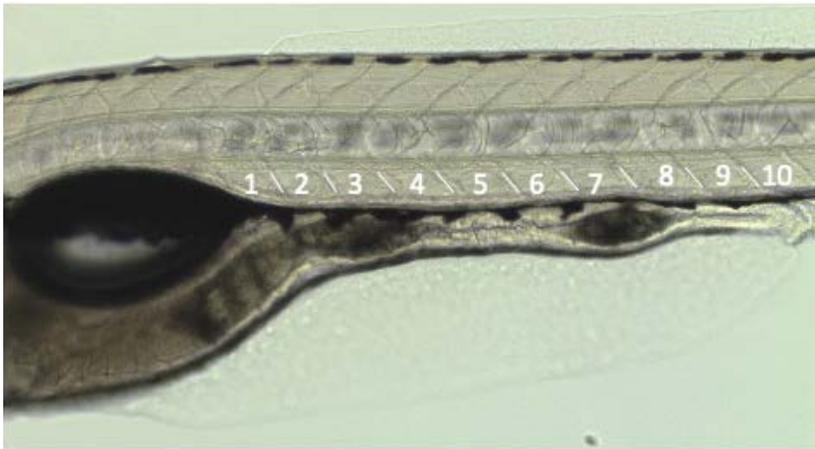


Figure 4. Somite landmarks. Somites are numbered as they were used to approximate gut wave-like motion point of origin and end point. The border between somites has been shown as diagonal white lines. Zebrafish tend to have 10 somites between the swim bladder and anus, making somites a useful way to approximate wave distance.

through the gut. Segmentation describes the coordinated alternated contraction of circular muscle cells and relaxation of longitudinal muscle cells and functions to mix and churn intestinal contents. Peristalsis describes rhythmic ring-like contraction of circular muscle cells and serves to move intestinal contents along the digestive tract⁷.

Frequency and behavior of segmentation and peristalsis were enumerated by eye via frame-by-frame video analysis. In healthy zebrafish, segmentation and peristalsis are rhythmic, separate yet coordinated smooth muscle activity⁷. Here, segmentation is visualized as static contractile activity and appears as the opening and closing (occlusion) of the intestinal lumen. In order to understand if the strength of contractions was impacted by hyperglycemia, segmentation was categorized as either full contractions or partial contractions. Full contractions were defined as a circular muscle contraction that caused complete or near-complete occlusion of the lumen (Fig. 2). Partial contractions were incomplete occlusions of the lumen that were nonetheless more significant than spasmodic behavior. Peristalsis appears in the video data as a rhythmic, wave-like motion. From the contraction point of origin, peristaltic waves travel in a caudal direction along the digestive tract toward the anus (Fig. 3). The range of peristaltic waves was determined using zebrafish somites as landmarks for marking the point of origin and end point of peristaltic waves (Fig. 4). Somites are mesodermic blocks that line either side of neural tubing and are easily visible in larval zebrafish, making them ideal markers of wave distance in developing vertebrate embryo. Within the scope of this paper, intestinal fillage was defined as the relative degree to

which the digestive tract is filled with excrement/waste. To assess intestinal fillage, four control fish were designated as points of reference for standard relative intestinal fillage. Each of the control and treatment fish were analyzed and compared to the reference fish to determine the level of intestinal fillage (Fig. 5). Fish with empty digestive tracts were excluded from intestinal fillage analysis due to an insufficient sample size (n=1).

Data quantification and statistical analysis

Data analysis was conducted using a one-way ANOVA or unpaired t-tests with GraphPad Prism 9.0 (GraphPad Software, San Diego, CA). All values are mean \pm SEM.

RESULTS

Frequency and strength of segmentation activity is decreased by hyperglycemia

To characterize the impact of hyperglycemia on intestinal motility, two paramount digestive behaviors were investigated: segmentation and peristalsis. The average frequency of segmentation contractions throughout the 10-minute time-lapse sequences was significantly lower in hyperglycemic fish (2.667 ± 1.497 , n=39 fish) than in control fish (9.444 ± 1.497 , n=18 fish, $***p=0.0001$; Fig. 6A). Quantification and characterization of full and partial segmentation contractions determined that the occurrence of full contractions in hyperglycemic fish (0.8974 ± 1.014 , n=39 fish) was significantly lower than in control fish (8.056 ± 1.014 , n=18 fish, $****p<0.0001$; Fig. 6B). There was no significant difference in partial contractions between control (1.389 ± 1.014 , n=18 fish) and hyperglycemic groups (1.769 ± 1.014 , n=39 fish).

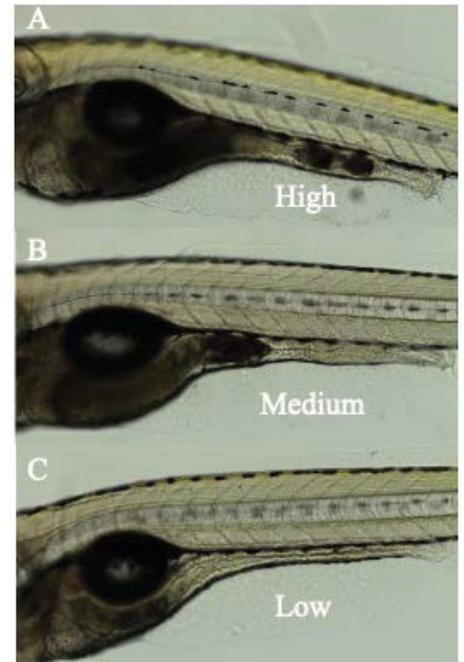


Figure 5. Reference fish used to gauge individual intestinal fillage. Here I am comparing the horizontal percentage of the intestine that is filled. (A) High fillage refers to fish with most to all of their digestive tracts filled with excrement, roughly 75-100% of gut filled. (B) Medium fillage refers to fish with roughly half to 75% of digestive tract filled. (C) Low fillage refers to fish with less than half their digestive tracts filled.

In line with the segmentation findings, the frequency of peristalsis in hyperglycemic fish (3.564 ± 1.474 , n=39 fish) was significantly lower than in control fish (9.056 ± 1.474 , n=18 fish, $**p=0.0306$; Fig. 7A). Range of peristalsis was quantified to assess the relative distance traveled by peristaltic wave impulses. Range of peristaltic waves were also significantly lower in hyperglycemic fish (5.292 ± 0.562 somites, n=24 fish) than control fish (7.467 ± 0.562 somites, n=15 fish, $p=***0.0002$; Fig. 7B).

Intestinal fillage differentially decreases segmentation and peristalsis

The results of intestinal fillage on motility are reported in Table 1. In summary, the frequency of both segmentation and peristalsis were significantly lower in hyperglycemic fish than control for fish with high and medium degrees of intestinal fillage. There was no significant difference in segmentation or peristalsis for fish with low fillage.

Interestingly, in fish with digestive tracts distended by particularly large bodies of waste, the peristaltic waves seemed to terminate at the distended/blocked regions. In control fish, these waves continue through distended regions of the intestinal tract. Additionally, there

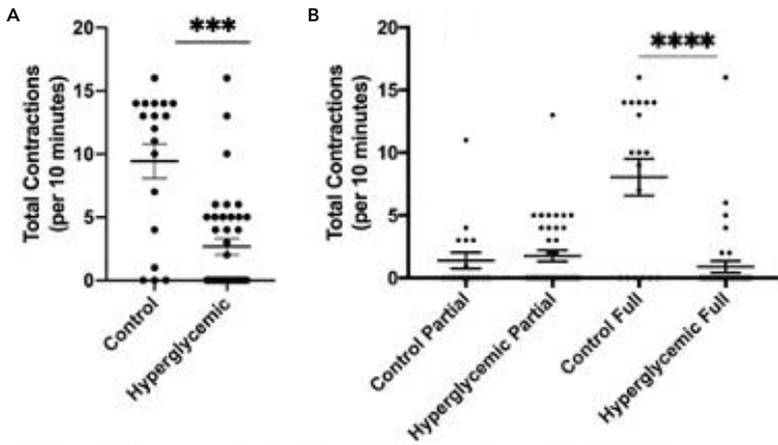


Figure 6. Segmentation is affected by hyperglycemia. (A) Total segmentation contractions through 10-minute time lapse. (B) Frequency of full and partial segmentation contractions is significantly lower in hyperglycemic fish than in control fish. Partial contractions were more common in hyperglycemic fish. All error bars are mean ± SEM.

were numerous instances of a fish with an extremely filled digestive tract (nearly 100%) that seemed unable to release excrement due to a tightly shut anus. One fish seemed to clearly display intestinal inflammation (Fig. 9) as the intestinal wall seemed to swell or balloon as the video progressed.

DISCUSSION

As of 2015, the global rate of diabetes prevalence, including both Type-1 and Type-2 diabetes, reached 8.8% of the world's population²⁸. The global prevalence of diabetes is predicted to rise to 10.4% by the year 2040²⁸, however with each passing year, these numbers are subject to increase as the actual prevalence of diabetes has outpaced former projections by hundreds of thousands of cases²⁹. Naturally, as cases of diabetes increase worldwide, so do cases of associated comorbidities such as intestinal motility disorders. Gastrointestinal manifestations of diabetes are a source of significant discomfort and can be debilitating for those experiencing them. For example, delayed gastric emptying, or gastroparesis, is one of the most common

motility changes related to peripheral neuropathy³⁰. Gastric motility issues are complex and present themselves in numerous ways, which has made it difficult to elucidate what changes at the cellular level cause the morphological presentation of diabetes mellitus. There is a clear need to understand the underlying mechanisms at work in the development of diabetic enteric neuropathies to provide better therapies for these common problems. In the current work, we utilized our 120mM glucose immersion model to (1) characterize the impact of hyperglycemia on zebrafish intestinal motility, and (2) lay the groundwork for future use of Clark lab zebrafish models in ENS and intestinal motility studies. Hyperglycemia resulted in significant alterations to intestinal activity including decreased frequency of segmentation and peristalsis (Figs. 4, 6). The degree of intestinal fillage also appears to be related to decreased segmentation and peristaltic activity. Overall, these characteristics indicate a marked decrease in intestinal motility in hyperglycemic zebrafish.

The precise coordination of smooth muscle activity is necessary for regular,

healthy intestinal function. Interstitial cells of Cajal (ICC) are responsible for generating the electrical impulses which result in rhythmic, coordinated peristaltic intestinal movement^{9,31}. The decrease in frequency of segmentation and altered number of full and partial contractions indicate that in addition to affecting contraction frequency, hyperglycemia may impact contractile strength as well. Additionally, while not represented in the graphs presented here, the recorded time lapses contain evidence of disrupted intestinal muscle contractile coordination. Contractions occur out of sync, sometimes sporadically and seemingly spasmodic. Altered intestinal motility has been shown to correlate with the degree of enteric neuron loss²⁴. Given what is known about the role of ICCs in intestinal motility, our results suggest that hyperglycemia may be damaging ICCs which in turn leads to the observed decrease in segmentation and peristalsis, as well as potential decreased cellular communication and coordination.

There are two different smooth muscle layers that make up gastrointestinal tract tissue; circular muscle and longitudinal muscle^{5,32}. In addition to

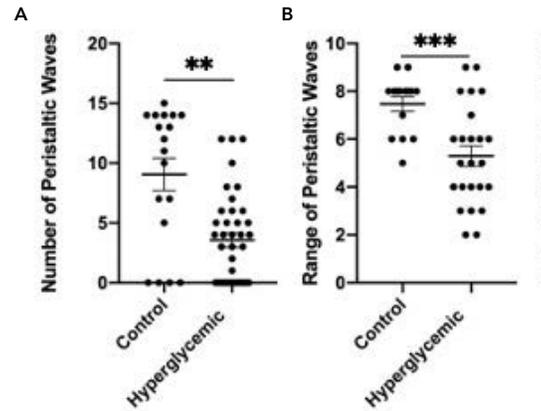


Figure 7. Control fish have significantly more intestinal peristaltic activity than hyperglycemic fish. (A) Total peristaltic wave count in 10-minute times lapse (**p=0.0306). (B) Range of (distance traveled by) peristaltic waves was significantly lower in hyperglycemic fish compared to control fish (**p=0.0002). Range was calculated using zebrafish somites as a measure of relative distance. All error bars are mean ± SEM.

	Gut Fillage					
	High		Medium		Low	
	Control	Hyperglycemic	Control	Hyperglycemic	Control	Hyperglycemic
<i>n</i>	4	9	4	10	4	12
Segmentation (Mean)	14.00	4.556	12.80	2.200	4.400	2.333
±SEM	±2.292		±1.076		±1.999	
<i>p</i>	**p=0.0023		****p<0.0001		ns	
Peristalsis	13.25	5.440	12.60	2.200	3.800	4.083
±SEM	±1.782		±0.9563		±2.067	
<i>p</i>	**p=0.0013		****p<0.0001		ns	

Table 1. Intestinal fillage results. Segmentation and peristalsis are reported as the mean number of occurrences per fish throughout time-lapses sequences.

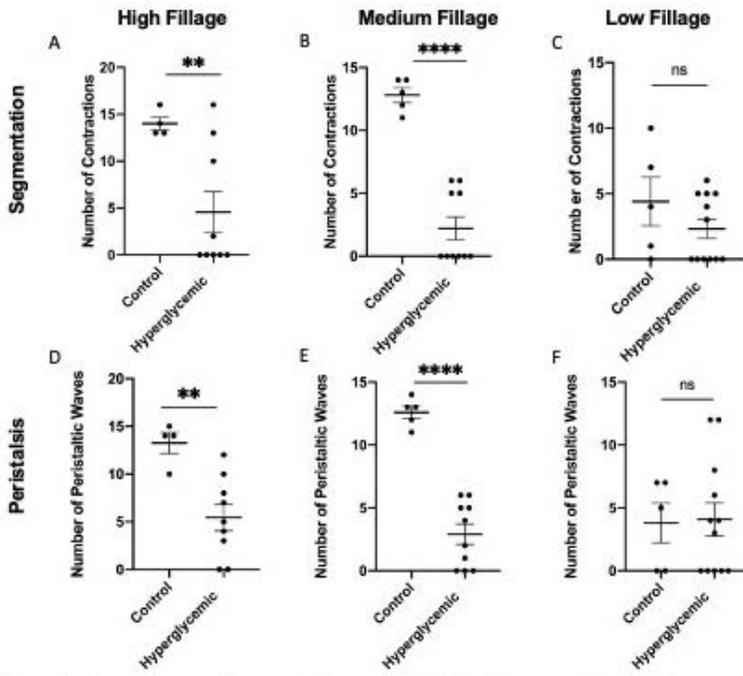


Figure 8. Comparisons of segmentation and peristalsis between control and hyperglycemic groups with varying degrees of gut fillage. Total number of segmental contractions in fish with (A) high intestinal fillage, (B) medium intestinal fillage, and (C) low intestinal fillage. Total number of peristaltic waves in fish with (D) high intestinal fillage, (E) medium intestinal fillage, and (F) low intestinal fillage.

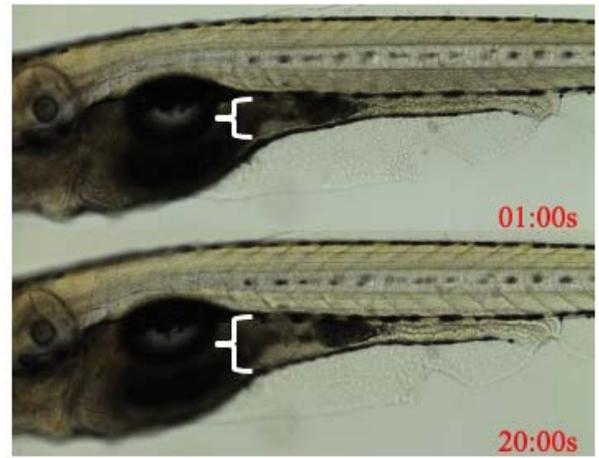


Figure 9. Possible gut inflammation. Shown above are two frames from a time-lapse sequence of a hyperglycemic zebrafish. To show the progression of inflammation, frames were selected at 1 and 20 seconds and white brackets were added to visualize the increased vertical expansion of the intestinal lumen.

possibly affecting ICCs, we believe that hyperglycemia does not impact circular and longitudinal muscle cells equally. In several treatment fish analyzed, peristalsis occurred in the absence of segmentation activity, but segmentation did not occur in the absence of peristalsis. These differences may be due in part to differential glucose uptake in circular and longitudinal muscle cells.

Zebrafish uptake glucose through transdermal absorption as well as through the intestinal lumen, meaning the zebrafish enteric (and thus peripheral) nervous system may be dealing with hyperglycemic damage from two fronts, a possibility not considered in past experiments^{11,12}. Glucose uptake via the intestinal lumen may then be affecting circular muscle more severely than longitudinal muscle due to the proximity of circular muscle to the intestinal lumen. This possible differential glucose concentration between the two layers may be a reason for seemingly uncoordinated segmentation and peristalsis in hyperglycemic fish.

It has been reported that in the zebrafish posterior intestine, intestinal motility patterns are not significantly affected by feeding⁷. Nevertheless, how the presence of material throughout the digestive tract may impact contraction frequency, strength, and distance is unknown. Our results indicated a significant difference in the frequency of

segmentation and peristaltic waves in both fish with high and medium intestinal fillage between treatment and control groups. A number of studies have shown the presence of spontaneous contractions in the zebrafish digestive tract as early as 3 dpf^{7,22,27}. It has been shown that feeding increases intestinal activity in the anterior intestine, but zebrafish gastrointestinal motility patterns in the posterior intestine do not seem to be affected after feeding⁷. The results of this study serve as further evidence that the presence of material in the anterior intestine affects intestinal motility. However, the results show motility patterns in the middle and posterior intestine are affected by intestinal distension. This distention was present for several hours after feeding, which may indicate that hyperglycemia directly affects posterior intestinal motility patterns, contrary to the current body of literature.

Video analysis showed that peristaltic waves were obstructed by distended regions of the digestive tract. It is possible that this is a visualization of disrupted neuronal communication, which may result from inflammation or distension. Inflammation has been previously implicated to play a role in nerve damage, specifically in the pathogenesis of diabetic neuropathy^{33,34}. This suggests there may be a relationship between distended regions of the intestines filled with food and intestinal inflammation. These distended and obstructed regions of the

digestive tract may be creating a feedback loop in which, due to hyperglycemic nerve damage, the intestines cannot move material properly. This may be leading to increased intestinal pressure and inflammation, which in turn feed back into nerve damage and increased constipation. However, the relationship between inflammation and intestinal motility is highly complex and not well understood^{24,35}. While this study does not serve to understand the role of inflammation in diabetic gastric dysmotility, our observations led to speculation about the role of inflammation in ENS damage and the potential for inflammation to be a symptom or proprietor of digestive disorders. The fish examined in this study showed variation in thickness of intestinal tissue. While some variation can be attributed to morphological and developmental differences, inflammation should not be ruled out as a possible causative factor for these intestinal behaviors and morphologies.

The Migrating Motor Complex (MCC) is a cyclic motility pattern present in both humans and zebrafish that occurs during a fasted state and is interrupted by feeding³⁶. The MMC describes passive, self-regulated “housekeeping” intestinal movements and impulses, which differentiates this system from peristalsis and segmentation³⁷. While the

physiological role of the MMC remains unclear, its absence has been associated with intestinal pseudo-obstruction³⁶. Some treatment fish exhibited pseudo-obstruction in the posterior intestine that seemed to prevent the movement of material through the digestive tract, resulting in severe constipation. Due to the observed movement patterns and complexity of intestinal regulation, our findings may indicate that hyperglycemia is inducing damage in the MMC, which in turn may contribute to intestinal dysmotility observed in fish with high and medium intestinal fillage. However,

the role of the MMC has not been described in zebrafish and is not currently understood³⁷, as zebrafish are a stomachless organism and the MMC primarily exhibits control through the stomach in humans³⁶. Thus, there is a need to further investigate intestinal motility behavior in zebrafish to expand its applicability as a model system for digestive disorders.

In the current work, we saw decreased segmentation, decreased peristalsis, and differential effects based on intestinal fillage. To study the effect of hyperglycemia on particular enteric neural cell types relevant to intestinal motility

such as ICCs, future studies should consider the use of fluorescent biomarkers to visualize the integrity of ICCs or nerves innervating the intestine. Additionally, future work should investigate differentially glucose uptake in circular and longitudinal muscle to investigate what role that may play in the occurrence of segmentation and peristalsis. In continuation of this work, the mechanisms behind digestive disorders associated with diabetes may be elucidated, leading to the discovery of more effective therapies for intestinal motility disorders.

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TACKLING THE TRASH PILE: UNDERSTANDING L-ASCORBATE METABOLISM OF *RALSTONIA EUTROPHA* H16 TO ENHANCE BIOPLASTIC PRODUCTION

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ABSTRACT

L-ascorbate (Vitamin C) is a ubiquitous carbon-containing compound present in nearly all plants, food derived from plants, and any environment that contains plants. Being ubiquitous, l-ascorbate is a carbon-containing nutrient for many soil microorganisms. *Ralstonia eutropha* H16 is a common soil bacterium that can produce biodegradable plastic. We previously characterized a novel L-ascorbate metabolic pathway in *R. eutropha*. Manipulation of this L-ascorbate pathway in *R. eutropha* might enhance the production of bioplastics. In this study, we deleted genes hypothesized to encode a regulatory protein (*b1220*) and transporters (*a3010*, *b1218*, and *b0215*) in this L-ascorbate pathway in *R. eutropha*. Strains of *R. eutropha* with deletion of *b1220* (ReDB1220) grew differently and had a lower total amount of cells than the wild-type. Strains with deletion of *b1218* (ReDB1218) had the lowest growth and total amount compared to the two other potential transporter genes we deleted when the strains only had access to L-ascorbate. Overall, our studies provide insight into the function of a putative regulator and transporter genes of this novel L-ascorbate degradative pathway. Investigating and fully understanding the L-ascorbate pathway in *R. eutropha* allows for future methods to manipulate this organism to make biodegradable plastics at an industrially relevant level.

INTRODUCTION

All life requires metabolism, the conversion of external material to energy and biomass, for cellular growth and function. Metabolic pathways are a set of enzyme-catalyzed chemical reactions that transform external material for the accumulation of cellular material. Bacteria are metabolically versatile organisms and are easier to genetically modify relative to other organisms. Specific genetic modification of bacteria can also improve metabolic product yield and purity while reducing toxic byproducts. *Ralstonia eutropha* H16, a Gram-negative soil bacterium, and a metabolically versatile microbes used as a model organism for studies of the production of biodegradable plastics such as poly-hydroxybutyrate (PHB) (1, 2). Most plastic products are derived from synthetic petrochemical polymers, and they do not undergo biodegradation. Bioplastics are an attractive research topic, as they are made up of biomass such as fats, carbohydrates, and proteins, instead of traditional fossil fuel-sourced chemicals and can also be designed to be biodegradable (3).

In 2015, approximately 37,500,000 tons of food waste was produced in the United States (4). Most of the waste is plant material, which contains a compound called L-ascorbate, also known as Vitamin C (5, 6). L-ascorbate is a ubiquitous organic molecule found

in nature and synthesized by many eukaryotes (7). L-ascorbate serves predominantly as a protective molecule in plants and animals (5,8,9). In animals, L-ascorbate is synthesized in the liver and acts as a free radical scavenger, participating in the synthesis of collagen, catecholamine, and carnitine (9). In plants, L-ascorbate serves as a major antioxidant that reduces free radical production which could otherwise damage the cell and acts as a helper molecule for several key metabolic pathways (5, 8). Enteric bacteria, such as *Escherichia coli*, can ferment L-ascorbate in the absence of oxygen but literature on L-ascorbate utilization is limited (10). Recently, we proposed and characterized a novel L-ascorbate degradative pathway in *R. eutropha* (11). Since *R. eutropha* can degrade a variety of carbon-containing compounds to produce bioplastics, manipulation of this novel catabolic pathway may enhance PHB production using L-ascorbate-containing feedstock (6,11,12).

The genes of the characterized

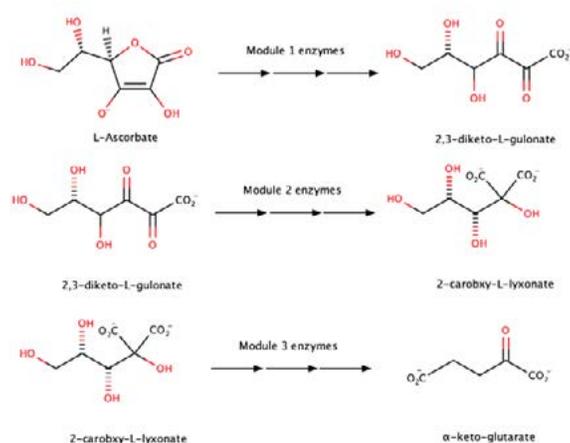


Figure 1. Novel l-ascorbate metabolic pathway in *R. eutropha* L-Ascorbate is converted to α -keto-glutarate in a series of chemical reactions catalyzed by enzymes. L-Ascorbate is a ubiquitous compound and α -keto-glutarate is an intermediate of another central metabolic pathway. The genes that encode enzymes for the conversion of l-ascorbate to α -keto-glutarate are separated into three gene modules. Genes in each module are responsible for converting an intermediate of the l-ascorbate pathway into another intermediate of the l-ascorbate pathway.

pathway are proposed to be separated into three modules (Fig. 1). The first module is associated with the conversion of L-ascorbate into 2,3-diketo-l-gulonate.

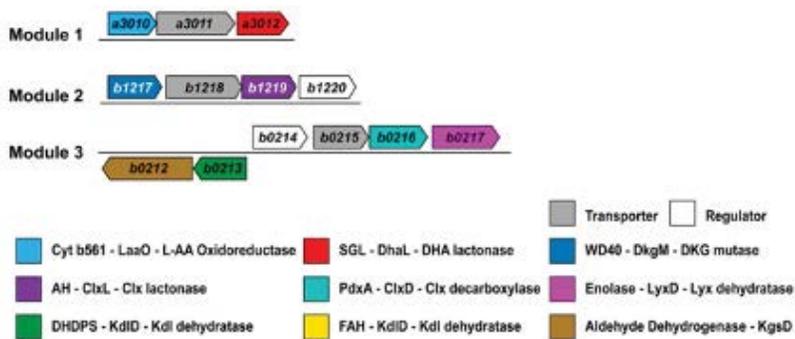


Figure 2. The gene neighborhood diagram of the l-ascorbate catabolic pathway in *R. eutropha* (11). The genes of the novel metabolic pathway are separated into three putative operons. Genes encoding catabolic enzymes are represented as colored arrows corresponding to the color scheme used in gene diagrams: genes encoding transporters are represented in grey, and genes encoding regulators are represented in white. In this study, growth studies of mutant strains with deletions of transporter genes (*a3011*, *b1218*, and *b021*) and a regulator gene (*b1220*) were conducted.

The second module is associated with the conversion of 2,3-diketo-L-gulonate to 2-carboxyl-L-lyxonate. The third module is associated with the conversion of 2-carboxyl-L-lyxonate to α -ketoglutarate. L-ascorbate also naturally oxidizes to dehydro-L-ascorbate (DHA), an intermediate formed in module 1 reactions (13). It is proposed that the novel L-ascorbate pathway also consisted of genes that encode enzymes to catalyze the reactions of L-ascorbate to DHA instead of relying on this natural process (11). PHB in *R. eutropha* is synthesized from acetyl-CoA, a key reactant of a central metabolic cycle, TCA cycle (14). Since α -ketoglutarate, the final product of the pathway, is also an intermediate of the TCA cycle, exploration of this L-ascorbate pathway may give us insight into the future industrial-level production of PHB.

Maintaining and expressing genes for a metabolic pathway requires resources, but in the environment, resources are limited. Thus, it is reasonable that genes for the L-ascorbate metabolic pathway in *R. eutropha* are separated into three gene modules as the organism can encounter different intermediates of the pathway in different environments (Fig 2). Each module was proposed to harbor a gene that encodes a transport protein (transporter) for a different pathway intermediate and a gene whose encoded protein regulates the expression of the genes (regulator) in the respective module (Fig. 2). A common method to observe the function of a gene is to delete a gene and observe the impact on the growth of the organism. While the growth of mutant strains for some of the genes in the pathway has been observed, research is needed on whether the hypothesized genes include a putative regulator gene and putative transporter genes of the pathway (11).

In this study, a putative regulator

gene, *b1220* was deleted in *R. eutropha* and its growth (ReDB1220) was compared to the growth of unmodified, or wild-type, *R. eutropha* in an environment with different carbon sources. In addition, growth studies of mutant strains of transporter gene

deletions (*a3011*, *b1218*, and *b0214*) are also reported. In summary, deletion of the regulator *b1220* gene yielded a slower growth rate and lower yield in mutant strains grown with L-ascorbate media, while the deletion of the transporter gene *b1218* (ReDB1218) affected the growth rate and yield more than the deletion of the two remaining transporter genes *b0215* and *a3011* did (ReDB0215 and ReDA3011). Through further study of L-ascorbate metabolism of *R. eutropha*, including transport of substrates and regulation of expression, the goal is to be able to manipulate *R. eutropha* to optimize generating PHB from L-ascorbate containing feedstocks.

RESULTS

Rate of growth and final density for transporter deletion strains

Transporters are proteins involved in the movement of chemical compounds across a biological membrane. Transporters are vital because they provide a passage for external resources to become accessible for metabolic enzymes inside of cells to generate biomass and

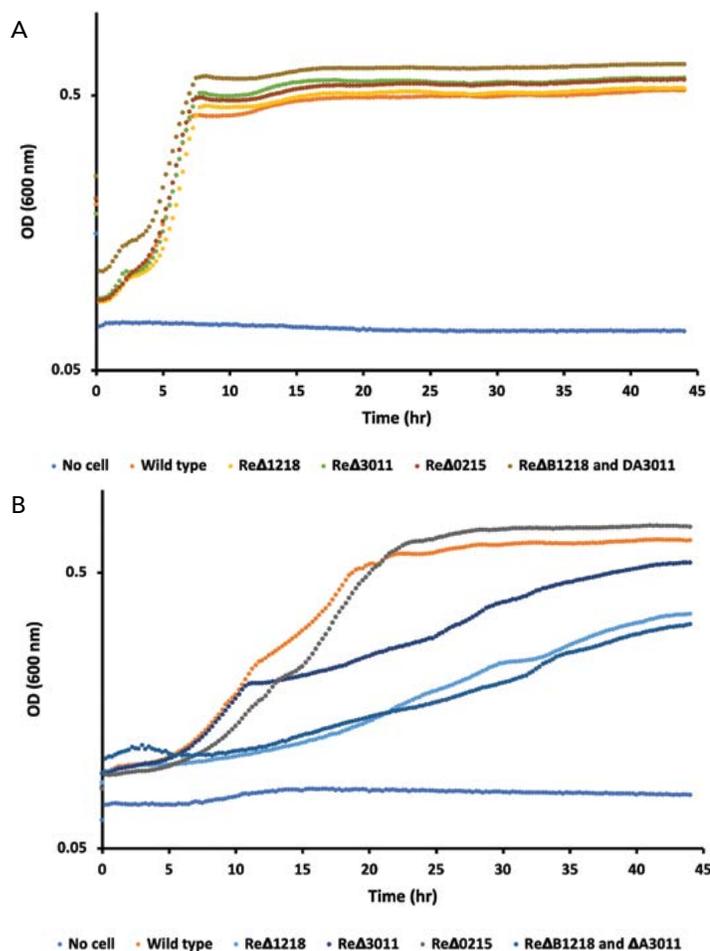


Figure 3. Growth of wild-type *R. eutropha* and mutant strains with deletion of transporter genes in minimal media with different carbon sources. [A] Growth of mutant strains and wild-type on minimal media with succinate. [B] Growth of mutant strains and wild-type on minimal media with l-ascorbate.

energy. Mutants with the deletion of the putative transporter genes *a3011*, *b1218*, and *b0215* were generated through plasmid preparation, molecular cloning, transformation, and conjugation. Strains missing the transporter genes grew with succinate almost identically to the wild-type strain. Cultures reached the exponential phase (the growth phase where bacteria grow at an exponential rate) within 5 hours of incubation time and reached the stationary phase (the growth phase where bacteria cease dividing but remain metabolically active) in ~8 hours (Fig. 3A). *R. eutropha* was grown in minimal media with succinate as a sole carbon source for growth as a control. This was to confirm the difference in growth between succinate and L-ascorbate media was because of the available carbon source. When the mutant strains were grown with L-ascorbate, the mutants ReDB1218, ReDA3011, and mutant with combined deletion of *b1218* and *a3011* all entered the exponential phase. However, for each mutant strain, the exponential phase was extended relative to the wild-type (Fig. 3B). ReDA3011 had a similar growth rate to the wild-type until 12 hours of incubation before its growth slowed (Fig. 3B). ReDB1218 grew more slowly and to a lower final level than the wild-type strain (Fig. 3B). ReDB0215 had a similar growth rate as the wild-type (Fig. 3B). Strains with the combined deletion of *b1218* and *a3011* had the same growth defects as ReDB1218. This suggests that the transporter genes *a3011* and *b1218* are required for optimal growth in an environment that contains L-ascorbate as a sole carbon source.

Rate of growth and final density for regulator deletion strains

Deletion of regulator gene *b1220* was carried out in a series of genetic modifications involving plasmid preparation, molecular cloning, transformation, and conjugation (Fig. 4 A-C, Materials and Methods). Deletion of *b1220* was verified by gel electrophoresis of *R. eutropha* colonies, with the wild-type *b1220* having ~3000 base pairs and mutant *b1220* having ~2500 base pairs (Fig. 4D). Growth studies of mutant strains revealed that in the environment with L-ascorbate as a sole carbon source, deletion of *b1220* resulted in a slower growth rate and yield in mutant strains compared to the wild-type (Fig. 5B). While the wild type *R. eutropha* H16 reached the exponential phase at ~ 8 hours and the stationary phase at ~ 32 hours, all mutant strains entered the exponential phase, only at 20 hours, and the exponential phase

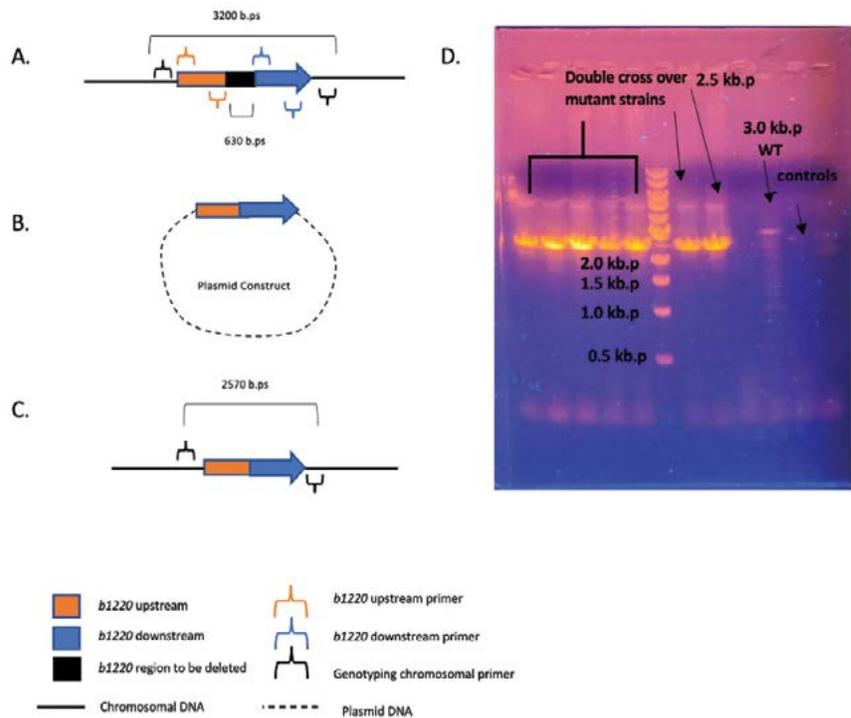


Figure 4. *R. eutropha* *b1220* chromosomal and plasmid models, and agarose gel of $\Delta b1220$ mutant strains. [A] Wild-type chromosomal *b1220* model shows the relative annealing positions of upstream, downstream, and genotyping primers for polymerase chain reaction (PCR). Based on primer design, wild-type (WT) *b1220* was calculated to be 3200 base pairs (b.ps) and the region to be deleted was calculated to be 630 b.ps. [B] Constructed plasmid obtained from Gibson Assembly with a plasmid backbone, and PCR amplified upstream and downstream fragments. [C] Chromosomal *b1220* model after genetic modification and double cross-over events (Materials and Methods). Note the missing black region and a shortened dysfunctional gene region (2570 b.ps). [D] Gel electrophoresis of mutant *R. eutropha* strains. Seven total mutant strains were isolated and gel electrophoresis using chromosomal primers revealed that all samples had fragment sizes of ~2500 b.ps. Wild-type *R. eutropha* *b1220* was found to have an expected size of ~3000 b.ps. No bands were detected in negative control lanes.

continued until 70 hours of incubation. However, all strains including the wild-type had similar growth rates and yield in an environment with succinate. This indicated that deletion of *b1220* was affecting the L-ascorbate metabolism of *R. eutropha* and was the cause for the growth defects of mutant ReDB1220 strains (Fig. 5A). Absorbance values of the media with mutant strains or no cells increased gradually after 20 hours.

DISCUSSION

The accumulation of plastic products in the environment endangers wildlife, humans, and their habitats. Despite the durability, flexibility for different purposes, and inexpensiveness to manufacture, the chemical structure of plastics provides resistance to environmental degradation. Thus, there is a need for alternative plastic-like compounds that serve similar purposes and are biodegradable. Polyhydroxybutyrate (PHB) is a bioplastic molecule of interest that is derived from biomass, is biodegradable, and is produced by microorganisms such as *Ralstonia eutropha*. L-Ascorbate is a

common carbon compound in the environment and can act as a carbon source for *R. eutropha* to produce biodegradable plastic. Understanding the novel L-ascorbate metabolism of *R. eutropha* may give insight into the efficient production of bioplastics such as PHB using L-ascorbate containing feedstock.

In this study, we conducted growth studies of mutant *R. eutropha* strains with the deletion of a regulator gene *b1220* of a novel L-ascorbate degradative pathway. When L-ascorbate was the sole carbon source, *b1220* deletion strains had a severe defect on growth rate and yield compared to wild-type (Fig. 5B). The growth defect of strains with the deletion of *b1220* suggests that the gene might encode a regulator that is an activator. The slight decrease of the optical density (OD) in the wild-type and the increase of OD in mutant strains around 20 hours of incubation time is of particular interest. The negative control also had a slight gradual increase over time. The negative controls were also found to be visibly yellow while other cultures were

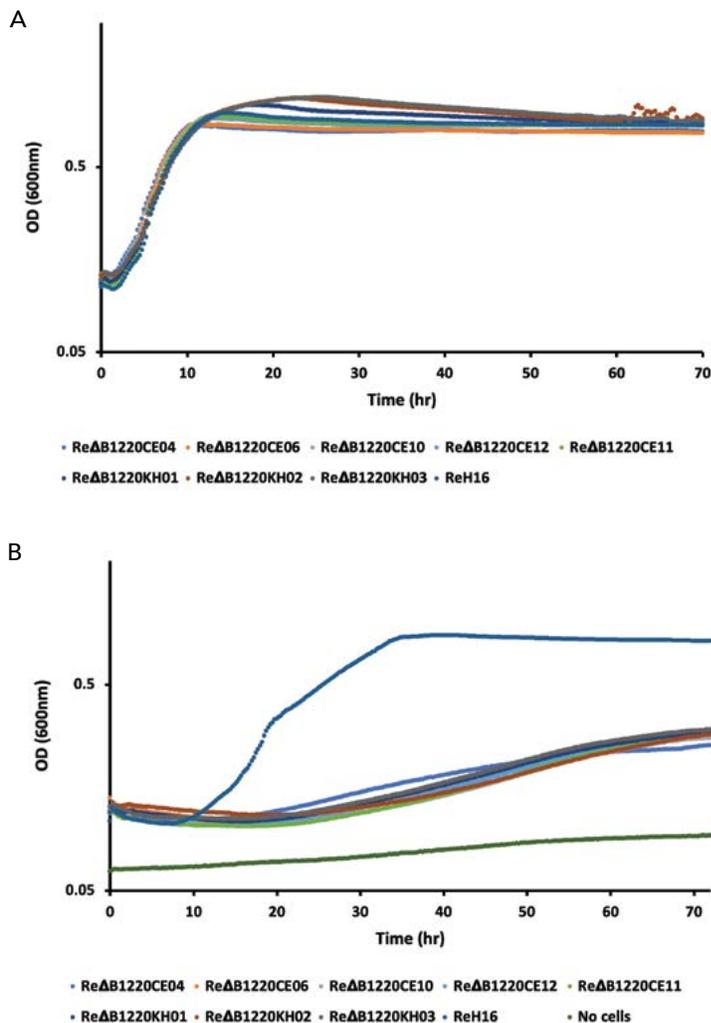


Figure 5. Growth curve of wild-type *R. eutropha* and mutant strains with deletion of regulator gene *b1220* in minimal media with different carbon sources. [A] Growth curve of mutant strains and wild-type on minimal media with succinate. [B] Growth curve of mutant strains and wild-type on minimal media with L-ascorbate.

colorless. This can be interpreted as a normal slow increase in OD due to the spontaneous breakdown of L-ascorbate to dehydro-L-ascorbate (DHA), a colored product. This spontaneous breakdown of L-ascorbate to DHA would mean that mutant strains that lacked *b1220* could be affected by the accumulating DHA level around the time of interest (at 20 hours of incubation).

The transporter deletion data highlighted the impact of deletion of different transporter genes on growth with L-ascorbate (Fig. 3B). Strains with deletion of *b1218* had the lowest growth rate and growth yield compared to the deletion of the other two transporters, *a3011*, and *b0215*. The reason for the impaired growth of the deleted *b1218* strain might signify the presence of certain carbon compounds outside the cell that were unable to be accessed by the cell. Since B1218 is a putative transporter for the second module, it may transport the end product of the first module,

2,3-diketo-l-gulonate. Therefore, when all L-ascorbate was depleted, the cell relied on the second transporter, B1218, to help continue bringing in carbon for growth. The deletion of *b0215* did not affect the growth, likely because cells were able to use transporters for earlier intermediates in spontaneous degradation of L-ascorbate. Further studies should conduct in vivo experiments to test which compounds the transporters are transporting.

A series of genetic modification techniques were used to further understand the metabolism of *R. eutropha*, specifically looking at the role of putative transporters and a regulator in a novel L-ascorbate pathway. Some transporters were found to be required for optimal growth with L-ascorbate, but the removal of the transporters did not abolish growth. The regulator B1220 was also shown to be required for optimal growth with L-ascorbate because when *b1220* was removed, growth rate and yield were impaired. Future

studies should explore which compounds the transporters are responsible for transporting and measure transcript levels of *b1220* using quantitative real time-PCR (qRT-PCR). Measuring transcript levels will quantify the number and sequences of the transcripts present and increase our understanding of this regulator gene. Novel metabolic pathways such as L-ascorbate metabolism by *R. eutropha* should be investigated to produce more efficient methods of PHB production using L-ascorbate containing feedstock. Production of novel types of biodegradable and non-fossil-fuel based plastics is needed for a sustainable future. Novel metabolic pathways in microbes such as *R. eutropha* can be exploited and utilized to generate biodegradable plastics. Our study of microbial metabolic flux and how microbial metabolic pathways are controlled brings us one step closer to our goal of the production of sustainable plastics.

MATERIALS AND METHODS

Process of Genetic Modification

There are few methods to alter the bacterial genetic composition, whether naturally or artificially. Figure 6 highlights the general procedure used to delete the genes of interest in this study. The first method is molecular cloning where regions of interest in a gene are replicated in high abundance and modified to the purposes of the research. The replicated genetic information is then put onto another fragment of DNA which is then introduced to other organisms through methods of transformation and conjugation. Transformation is the incorporation of genetic material from the environment and conjugation is the exchange of genetic information, usually plasmids, between two species of bacteria. A plasmid containing the deleted gene was prepared and was used to transform *E. coli* cells. Then the plasmid was conjugated from *E. coli* to *R. eutropha*. The final mutant strain of *R. eutropha* had the gene deleted because it lacks an in-frame portion of the gene of interest. Deletion is conducted by relying on the recombination mechanism between the conjugated plasmid and the chromosomal DNA of *R. eutropha*. Recombination is the exchange of identical genetic material between two DNA molecules, and the chromosomal gene will be swapped out with the plasmid gene, resulting in a strain lacking an internal region of the entire gene of interest.

Preparation of Plasmid for Deletion

All primer stocks were prepared at 100 mM with TE buffer. All working primer stocks were prepared to 10mM. PCR reactions were 55 M water, 5x

buffer, 0.2 mM dNTPs, 0.5 mM forward and reverse primers (Table 1), 10 ng of template, and 1mL enzyme. All primers were ordered from Integrated DNA Technologies (IDT, IA). The primers were designed so the amplified products incorporated extensions that would overlap with the extension from the other amplified fragments. The overlapping extensions of the amplified PCR products allow for a Gibson Assembly reaction with the plasmid backbone pK18mobsacB, which contains an antibiotic-resistant gene *nptIII* and levansucrase gene sacB. The PCR tubes were loaded into the ThermoCycler and cycled 40 times through from 96°C for 30 s, to 64°C for 15 s, to 72°C for 1.5 mins. Electrophoresis gels were prepared with 0.8% agarose, in 100 µL TAE buffer with 10 µL of ethidium bromide, and then were run for 35 minutes at 150 V. The DNA fragments in each microcentrifuge tube were purified according to Omega E.Z.N.A Bacterial DNA Kit instructions (Omega Bio-Tek, GA). Volumes of upstream and downstream fragments were assembled with linearized pK18mobsacB and Gibson MasterMix.

Transformation of NEB5α *E. coli* cells and plasmid isolation

After Gibson Assembly, NEB5α *E. coli* cells were transformed using the obtained plasmid. To select the *E. coli* colony with correctly assembled Gibson products, we conducted a conventional X-Gal/IPTG blue-white assay. Correctly transformed *E. coli* NEB5α cells were verified by the presence of white colonies on kanamycin (50µg/µL) LB plates treated with X-Gal and IPTG. The plasmid backbone has a multiple antibiotic-resistant gene *nptIII* and the desired correct assembly of the plasmid of interest requires a plasmid with PCR amplified regions inserted within the *lacZ* gene. IPTG induces expression of *lacZ* and X-gal acts as a substrate for LacZ which releases a blue color when broken down. Cells that have the desired Gibson product would be resistant to kanamycin and have white color from the disruption of the *LacZ* gene in the plasmid. We conducted the plasmid isolation from such white colonies and further verified the assembled plasmid by digesting it with the *KpnI* enzyme and gel electrophoresis.

Transformation of S17 *E. coli* cells and conjugation with *R. eutropha* H16

We then transformed S17 *E. coli* cells using the isolated correctly assembled plasmid. The transformation was also verified by the blue-white screening method. To obtain the *R. eutropha* of interest, we conjugated the transformed *E. coli* S17 with *R. eutropha* H16. Successful conjugation was verified by the presence

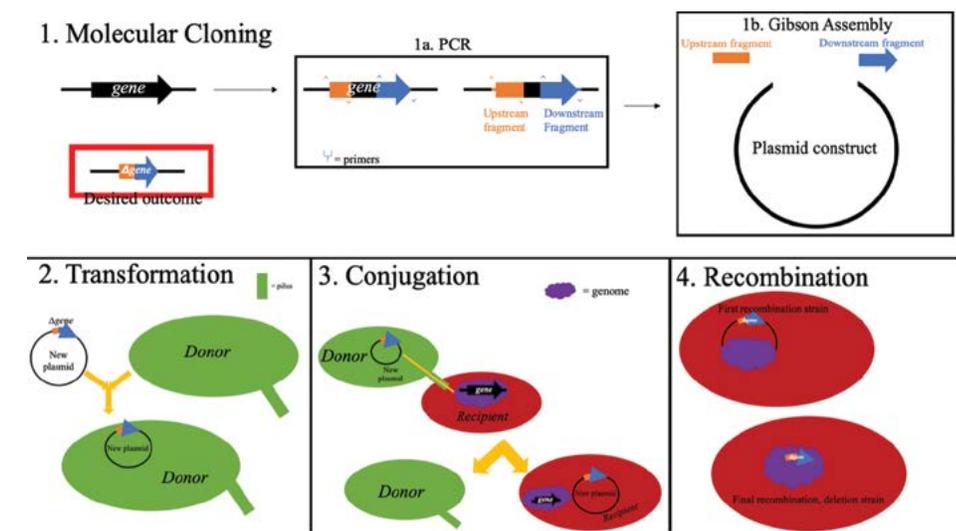


Figure 6. Process of Genetic Modification. [1a] Polymerase chain reaction (PCR) was used to amplify regions of the gene of interest, for example, *b1220*. [1b] The fragments of the amplified regions were assembled with a plasmid construct in Gibson Assembly to form the desired plasmid. [2, 3] *Escherichia coli* strains were transformed with the plasmid to then conjugate with *R. eutropha*. [4] After conjugation, cellular mechanisms of recombination were relied on for crossing over events between the modified and unmodified gene regions. Cells were selected for two crossover events that yielded mutants with only the desired outcome, the deletion of *b1220*. Generated mutants were used for the growth study.

Table 1. Nucleotide sequences of primers used in the study.

Primer Name	Primer Sequence
Cloning Primers	
DB1220upF1	TGCATGCCTGCAGGTCGACTCTAGATGCTGGCCACTGTGCACGTAGAG
DB1220UpR1	CGCCTGCGGAGCGGCGATGGCGGCAAATTCATCGTG
DB1220DnF1	GCCGCCATCGCCGCTCCGCGAGGCGATGACTGAATTG
DB1220DnR1	GAATTCGAGCTCGGTACCCGGGGATCCACCTGCGCTACCGCTTCTGAC
DB1218UpR1	GTAGACACCGGCACCGGTGAGTGGTGTGCGCATC
DB1218UpF1	CTTGATGCCTGCAGGTCGACTCTAGAAGTTGCTGACTATCCGCATGAATTCG
DB1218DnR1	GAATTCGAGCTCGGTACCCGGGGATCCCGGCTTGAACATTGATCCGATAGAAC
DB1218DnF1	CCACTCACCGGTGCCGGTGTCTACCTGTGTGGTC
DA3011UpF1	GCATGCCTGCAGGTCGACTCTAGACCGCTATCCACCCTGTGCCATTG
DA3011UpR1	GATCAGGCCAAGCGCACCGCGAATATCAGTATTGCC
DA3011DnF1	TTCGGCGGTGCGCTTGGCTGATCCTCTTGCTGTGG
DA3011DnR1	GAATTCGAGCTCGGTACCCGGGGATCCCGTGGAGTTCGTCGAAACGCGCTAAG
DB0215UpR1	CTTGCCATAGCGCGCCAGGCATTGCCCGTTAC
DB0215UpF1	CTTGATGCCTGCAGGTCGACTCTAGAGGAGCTGGAAGGCATGCTGGAAG
DB0215DnR1	GAATTCGAGCTCGGTACCCGGGGATCCGCCCTGCTGCGTGATGTCGAAC
DB0215DnF1	AATGCCTGGGCGCGCTATGGCAAGCTGATCGCTGAACTG
Genotyping Primers	
KHDB1220GenUpF1	CGTGATTAATGCGCTGGGCAACCTGGG
KHDB1220GenDnR1	CACACCTCGGCCAACCACGACATTCCAC

of colonies on kanamycin minimal media plates. *E. coli* S17 colonies were auxotrophic and therefore could not grow well on minimal media. The

conjugated *R. eutropha* cells were grown on kanamycin (300 µg/µL) LB plates for a single crossover event to occur. Single crossing over event would initiate

the exchange of the already-present chromosomal gene in *R. eutropha* with the now conjugated plasmid containing an in-frame portion lacking the gene. We verified the *R. eutropha* cells with a single crossover by gel electrophoresis of PCR amplified products. The combined upstream and downstream regions from gel electrophoresis verified the occurrence of a single crossover event.

Isolating mutant double crossover *R. eutropha* strains and growth study of mutant strains

The single crossover strains were incubated in liquid LB media for another crossover event to occur. A double crossover event would lead to the complete swap of the chromosomal gene and the plasmid gene. The cells from the liquid media were then grown on an LB plate supplemented with 10% sucrose. The plasmid contains a levansucrase encoding gene *sacB*. Single crossover strains would not survive on a sucrose plate, as levansucrase converts sucrose to D-glucose, a harmful chemical to *R. eutropha* and other Gram-negative bacteria. Double crossover

events would ensure the viable colonies on the sucrose plates were the desired mutant deletion strain or a wild-type revertant strain. In addition, the plasmid is a suicide vector in *R. eutropha*, meaning no further replication of the plasmid in subsequent double crossover *R. eutropha* strains. Viable *sacB* mutants, which could lead to false double crossover strain selection, were counter-selected using an additional kanamycin LB plate before conducting colony PCR and gel electrophoresis. Using genotyping primers KHDB1220GenUpF1 and KHDB1220GenDnR1, the PCR products had ~2500 base pairs and were shorter than the original wild-type ~3200 base pairs, verifying the successful deletion of the gene.

Growth Study using L-ascorbate

The overnight cultures of deletion strains and wild-type were grown with LB. The OD at 600 nm was measured of the overnight cultures and the concentration (equal to the OD) was used in the equation $M_1V_1=M_2V_2$ to then calculate the volume of minimal media needed to be added for a final concentration of 0.524. Minimal

media used in the study contained only the essential vitamins, trace elements, salts, phosphate buffers, and no essential amino acids to be metabolized to ensure the growth rate and yield of the samples were only due to the supplemented carbon source. Then 0.5 mL of overnight culture was placed in a sterile microcentrifuge tube and washed twice with 0.5 mL of minimal media. The calculated volume of minimal media to be added to the washed cells was added to each respective tube and aspirated to mix. The final concentrated cells of each tube were used to inoculate the 96-well plate. Each well was filled with 300 μ L of media of either LB, L-ascorbate supplemented minimal media, succinate supplemented minimal media, or minimal media with no carbon source. There were 2 replicate rows of each media type. Mutant strains, wild-type, and no cells were the different strains used in different columns. Once loaded, the 96-well plate was put in the spectrophotometer and the OD at 600 nm was measured for 72 hours.

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SILVER IONS AND THE STABILITY OF HISTONE-LIKE NUCLEOID-STRUCTURING PROTEIN FROM *E. COLI*

Bailey Didriksen

ABSTRACT

Silver (Ag^+) ions have been used as antimicrobials since ancient history, though the antimicrobial mechanism has not been fully understood. Previous research suggests that one source of silver's antimicrobial activity lies in the interaction between Ag^+ , a bacterium's DNA, and its histone-like nucleoid-structuring (H-NS) proteins. It is thought that Ag^+ induce the dehybridization of DNA into single strands, causing the H-NS protein to detach from the DNA. Once the proteins are detached, the nucleoid of the bacteria loses some of its complex structure, altering all gene expression influenced by H-NS proteins. This project further investigated the interaction between H-NS proteins, Ag^+ , and DNA. H-NS proteins were recombinantly expressed and purified. To assess the denaturation of the H-NS protein in the presence of Ag^+ , the intrinsic fluorescence of H-NS proteins was investigated in the presence and absence of Ag^+ . We found that the presence of the Ag^+ led to a decrease in intrinsic fluorescence suggesting a destabilization of the proteins. Additionally, the intrinsic fluorescence of H-NS proteins was measured to investigate the interaction between H-NS and DNA. Since H-NS proteins regulate over 200 genes in the bacterial genome, manipulation of the H-NS protein will be advantageous in the fight against antibiotic resistance. Ag^+ were found to disrupt the interaction between H-NS and DNA and destabilize H-NS protein. Further understanding the damage that Ag^+ can cause to the bacterial genome adds to the toolkit in the fight against antibiotic resistance.

BACKGROUND

An arms race has raged between bacteria and antibiotics since 1942 when *Staphylococcus aureus* was found to be resistant to penicillin, which had become publicly available a year earlier.¹ *Streptococcus pneumoniae* became resistant to penicillin in 1967 and *Neisseria gonorrhoeae* in 1976.¹ Antibiotic resistance continues to be a threat today, as certain bacterial strains lead to thousands of deaths a year. For example, antibiotic resistant *Clostridium difficile* causes life threatening diarrhea and inflammation of the colon and led to 12,800 deaths in 2019.² *C. difficile* is the leading health care-associated infection in the United States and is the most frequent hospital-care intestinal infection in Europe.³ Usual treatment of *C. difficile* consists of antibiotics such as metronidazole, vancomycin, and fidaxomicin. However, *C. difficile* is a spore-forming bacterium, which allows the spores to survive antimicrobial therapy and can lead to recurrence of the infection in patients. Antibiotic resistant *C. difficile* rates have been constantly rising. Clindamycin resistance ranged from 15% to 97% and moxifloxacin resistance ranged from 2% to 87% in the past 15 years.⁴ *C. difficile* is only one of many bacteria strains that are resistant to multiple antibiotics, making antibiotic resistance a rising concern. More than 2.8 million antibiotic-resistant infections occur in the U.S. each year leading to more than 35,000 deaths.² With antibiotic resistance on the rise, researchers are working to find new

weapons to fight this crisis. While building our arsenal to combat antibiotic resistance, one old, reliable antimicrobial came to mind: antimicrobial metals. Antimicrobial metals have been used to fight this battle against bacteria since before bacteria was even discovered.

Metals including copper (Cu), mercury (Hg), and silver (Ag) have been used as antimicrobials since ancient history dating back to the time of the Persian kings.^{5,6} Agriculture has been using silver compounds for a wide array of applications, such as antimycobacterial agents (to defend against Mycobacteria infections) nano-pesticides, as well as fungicides.⁷

Although metals assist in the fight against bacteria, they have their downsides. Mercury exposure has been linked to liver and kidney damage, reproductive and fetal effects, permanent central nervous system damage, fatigue, weight loss, tremors, personality change, inflammation of the mouth and gums, excessive salivation, and loosening of the teeth.⁸ Similar to mercury, copper also can lead to detrimental health effects, such as dermatitis, liver and kidney damage, and lung damage.^{9,10} Due to the adverse health effects of mercury and copper, silver compounds are more viable options for fighting against antibiotic resistant bacteria. Although silver has some side effects, silver compounds in any form are not seen as toxic to the immune, cardiovascular, nervous, or reproductive system as copper or mercury.¹¹ The

antimicrobial metal chosen for this study was silver.

Elemental silver has been utilized since ancient times, and silver nanoparticles are being investigated for their antimicrobial activity today. However, Ag^+ currently have more modern uses. From invasive surgical tools to topical treatments, Ag^+ are used as an antimicrobial coating.^{4,12} Interestingly enough, a new patch has been designed that slowly releases Ag^+ into wounds as the use of silver treatment on wounds leads to reduced scarring.¹²

Although the antimicrobial effects of silver compounds are known, the mechanism of how silver is toxic to bacteria is not fully understood. Ag^+ are known to damage the cell membrane by increasing the permeability of the membrane. This increase in permeability deters the cell from properly regulating transport, as well as allow for the release of lipopolysaccharides (LPS) and membrane proteins.¹² The loss of these essential characteristics ultimately leads to cell death. Additionally, Ag^+ are known to weaken DNA replication and inactivate functional proteins. Ag^+ interact with electron-rich functional groups such as oxygens, nitrogen, and sulfurs leading to the denaturation of the proteins.¹³ Due to silver's ability to damage a broad range of targets within the cell, resistance to silver is unlikely as several simultaneous mutations would be required to avoid cell death.¹⁴

New evidence suggests that Ag^+ might also cause damage to the bacterial DNA.

The genetic information of bacteria is highly organized into a complex structure known as the nucleoid.¹⁵ The complex structure of the nucleoid is maintained by DNA supercoiling, macromolecular crowding, and DNA-binding proteins. The proteins bound to the nucleoid, nucleoid-associated proteins (NAPs), drastically change in abundance depending on the surrounding environment.¹⁵ The change in abundance of NAPs leads to the alteration of the overall shape of the nucleoid and mediates gene expression. One NAP in particular, histone-like nucleoid-associating (H-NS) protein, regulates ~5% of the bacterial genome, using mostly negative regulation. Several of the genes regulated by H-NS proteins are attributed to the virulence of bacterial strains.¹⁶ The family of histone-like nucleoid-structuring (H-NS) proteins have several roles within bacteria cells. H-NS protein are found in *E. coli*, *Salmonella enterica*, and other Gram-negative bacteria. H-NS protein have two main functions: they regulate expression of over 200 target genes and contribute to the scaffolding of the nucleoid to create chromosomal microdomains.¹⁷ H-NS protein regulate gene expression using different mechanisms, though are primarily known as transcriptional repressors.¹⁹ Transcription may be repressed either by inhibiting the RNA polymerase from binding to the promoter region or preventing the RNA polymerase from traveling down the DNA.¹⁵ Additionally, H-NS protein can indirectly regulate gene expression by manipulating the shape of the nucleoid. With scaffolding, DNA is physically organized by H-NS protein into various conformations such as large extended filaments, hairpin-like large DNA bridges, and higher-order DNA condensation.¹⁵ Previous research has shown that H-NS stiffens DNA upon binding.¹⁶

The H-NS protein is relatively small, having only 137 total amino acids, with a molecular weight of 15.54 kDa. H-NS protein quaternary structure contains two domains: The N-terminal oligomerization domain and the C-terminal DNA-binding domain (Fig. 1). The oligomerization domain contains four alpha helices comprising two dimerization sites. Because H-NS protein directly interact with the nucleoid of the bacteria to bend, coil, or turn the DNA, H-NS has the ability to form higher-order homomeric or heteromeric complexes.¹⁶ When forming a complex, the dimerization sites bind H-NS proteins to other H-NS proteins. The oligomerization domain is connected to the DNA binding domain via a small, unstructured, flexible linker (Fig. 1). The DNA-binding domain binds to the double-stranded DNA. H-NS generally binds to unspecific regions of DNA but

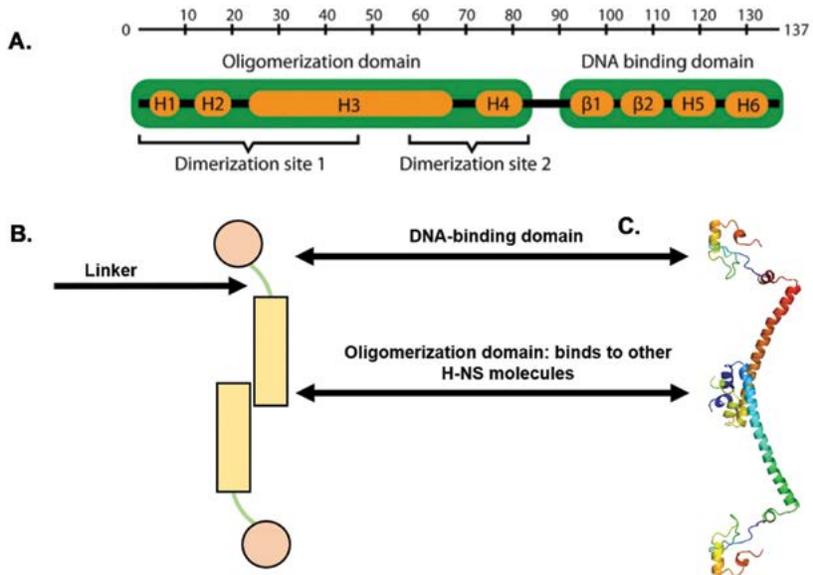


Figure 1. General structure of H-NS protein. (A) Domain organization of H-NS protein. H-NS protein have 137 amino acids with two defined domains. H represents the α -helices and β represents the β -sheets. Figure taken from Winardhi et al. (B) Schematic structure of H-NS protein. The yellow box represents the oligomerization domain, and the orange circle represents the DNA-binding domain, the green line represents the linker that links the two domains together. The specific domains are indicated by the arrows. (C) Crystal structure of H-NS protein dimer from Protein Data Bank ID 1HNR & 3NR7. The domains are indicated by the arrows.²⁰

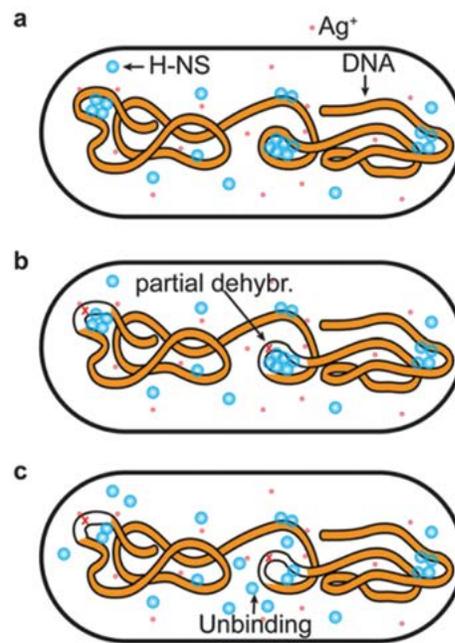


Figure 2. Hypothesized mechanism of silver ions' effect on the diffusive dynamics of H-NS proteins in *E. coli*. Figure taken from Sadoon et al., 2020. (A) Silver ions are introduced to the bacterium. (B) Silver ions then interact with the double-stranded DNA leading to partial dehybridization of the DNA into two single strands. (C) As H-NS protein only bind to double-stranded DNA, H-NS protein fall off the DNA.

has a higher affinity for AT-rich sequences. AT-rich regions are known to be correlated with DNA that has been acquired by horizontal gene transfer.¹⁵

Due to the importance of H-NS proteins, the interaction between DNA and H-NS protein is starting to be investigated. In 2020, Sadoon et al. found that that Ag^+

directly interact with DNA by appearing to dehybridize double-stranded DNA, causing it to dissociate into two single-strands of DNA (Fig. 2B).¹⁸ When the double-stranded DNA dissociated, the H-NS proteins appeared to fall off the DNA, as H-NS proteins solely bind to double-stranded DNA (Fig. 2C).

This study, however, did not look at any direct interactions between the Ag⁺ and the H-NS proteins themselves. Because Ag⁺ are known to bind to oxygens, nitrogens, and sulfurs in protein, an interaction between Ag⁺ and the H-NS proteins seems likely.¹³ Specifically, an H-NS monomer contains one cysteine within the oligomerization domain of the protein, and this cysteine may lead to the formation of an Ag-thiol interaction that may affect the binding affinity of H-NS to DNA.¹⁸ Two possible mechanisms are that the Ag-thiol interaction may cause a change in the protein allosterically, affecting the binding site, or that the Ag-thiol interaction affects the H-NS proteins by impacting the oligomerization domain or by causing the denaturing or misfolding of the proteins.¹⁸ Both possibilities could reduce the binding affinity between H-NS and DNA.

Here, we aim to examine the binding and stability of H-NS proteins, DNA, and Ag⁺ by assessing the denaturation of H-NS proteins with and without Ag⁺ and evaluating the interaction between DNA and H-NS proteins in the presence of Ag⁺. This work contributes to understanding the dynamic relationship between Ag⁺ and the destruction of the bacterial nucleoid building the toolkit in the fight against antibiotic resistance. The dynamic relationship will be analyzed utilizing the natural intrinsic fluorescence of H-NS protein due to the one Trp residue.

Trp is the most abundant aromatic amino acid in proteins and the most influential on intrinsic fluorescence. Since H-NS protein have one Trp residue, H-NS has intrinsic fluorescence. We are able to utilize this to study the effects of Ag⁺ on H-NS proteins. If, as hypothesized, Ag⁺ destabilize the H-NS protein, then, samples containing both protein and H-NS will denature at a lower concentration of urea and with a smaller change in free energy. This analysis can be used for a variety of applications in protein research, such as thermodynamic, cellular, and evolutionary principles and mechanisms of protein stability.¹⁹ Urea-mediated protein denaturation and tryptophan fluorescence were used to evaluate the change in protein stability with and without the presence of Ag⁺.

H-NS proteins are major contributors to gene expression and regulation due to their interaction with double-stranded DNA. Unraveling how this interaction is affected by the presence of Ag⁺ is important to understanding the antimicrobial properties of the ions. Recent studies suggest that Ag⁺ interact with the DNA, leading to the dissociation of H-NS proteins.¹⁸

MATERIALS AND METHODS

Transformation of B121(DE3) cells with pHisHNS.

The pHisHNS plasmid, which encodes 6×His-tagged-HNS proteins was generously gifted from the Sadoon Lab, University of Arkansas, Fayetteville, AR, USA. The plasmid (1 μL) was added to 1 mL of B121(DE3) *E. coli* cells (Novagen) and then moved to ice bath for 20 min. The *E. coli* cells were then transformed by heat shock at 42 °C for 35 s and then returned to the ice bath. 250 μL of S.O.C. medium (Novagen) was added to the culture and incubated shaking at 37 °C and 6 x g for 1 hr. After incubation, 40 μL of the transformation mix was spread plated onto an LB agar plate containing ampicillin (100 mg/mL) and incubated at 37 °C overnight.

Expression of the *hns* gene.

A single transformed B121(DE3) colony was used to inoculate a 3 mL culture of LB medium (Fisher Scientific) with ampicillin (100 mg/mL). After overnight incubation, the culture was transferred to 400 mL of fresh LB-Amp media. When the optical density at 600nm was 0.4, gene expression was induced with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 30 °C for 5 h. After induction, the cell suspension was centrifuged at 2,500 x g at 4 °C for 30 min. The cell pellets were stored at -20 °C.

Purification of H-NS protein by IMAC and Gel Filtration.

The cell pellets were first frozen and thawed to promote cell lysis. Then the cell pellets were resuspended in 25 mL lysis buffer (50 mM Tris, 150 mM NaCl, 2 mM MgCl₂, 0.5 mg/mL lysozyme (Thermo Fisher), 0.04 μL/mL nuclease (Thermo Fisher)) and incubated for 30 min. The resulting suspension was centrifuged at 10,000 x g for 20 min. The supernatant was applied to an immobilized metal affinity chromatography (IMAC) column with 2 mL of HisPur™ Cobalt Resin (Thermo Scientific) washed with 10 mL wash buffer (50 mM Tris, 150 mM NaCl, 10 mM imidazole). After the wash, the His-tagged H-NS proteins were eluted from the column using 5 mL of elution buffer (50 mM Tris, 150 mM NaCl, 680 mM imidazole) twice. The eluted proteins were concentrated to 3 mL using an Amicon® Ultra-15 centrifugal filter (Millipore Sigma). Purification was assessed via SDS-PAGE.

A 3 mL solution of the concentrated proteins were loaded onto a Sephadex G-100 column for further purification by gel filtration chromatography. The column was equilibrated and eluted with 30 mM MOPS (pH 7) by gravity alone. Five 1 mL fractions were collected followed by 35 0.5 mL fractions. The fractions were

analyzed at 280 nm to determine which fractions to be analyzed by SDS-PAGE. Using a molar coefficient of 9,970 M⁻¹ cm⁻¹, determined by primary sequence, the final concentration of H-NS protein was 886 μg of protein per L of original culture. Fractions containing the H-NS proteins were combined and stored at -20 °C. Purification was assessed via SDS-PAGE.

Measurement of fluorescence quenching of H-NS protein with dsDNA.

Fluorescence measurements were recorded on a Shimadzu RF-100 spectrophotometer. A quantitative analysis of the potential interaction between dsDNA and H-NS protein was performed by fluorometric titration. A 600 μL solution, containing 5.60 x 10⁻⁵ M H-NS and 30 mM MOPS (pH 7), was titrated by successive additions of 2 μL of λDNA (539 μg/mL) to give a final concentration of 95.5 μM H-NS protein. The fluorescence emission spectra were recorded at 25 °C in a wavelength range of 295-400 nm with exciting wavelength at 280 nm.

Analysis of fluorescence quenching.

Fluorescence quenching is described by Equation 1, the Scatchard Equation.

$$\frac{Q}{[L]} = \frac{n}{K_d} - \frac{Q}{K_d}$$

where Q is the quenching factor, [L] is the concentration of ligand, n is the stoichiometry, and K_d is the dissociation constant. To determine the binding affinity, [Q]/L versus Q is plotted. The quenching factor is calculated using Equation 2.

$$Q = \frac{F_0 - F}{F}$$

Once the linear trend is calculated, the slope of the plot is 1/K_d. To calculate n, the y-intercept of the linear equation from the Scatchard plot is multiplied by the K_d. The fluorescence intensity is calculated using the area under the curve.

Determination of urea denaturation curve for H-NS protein.

Stock solutions of urea "UREA" and buffer "MOPS" were prepared and mixed as in Table 1. To prepare the UREA, 60.0500 g of urea (Sigma Aldrich), 0.649 g MOPS (ThermoFisher), 1.8 mL 1M HCl, and 52 mL of distilled water were added to a weighed beaker at room temperature without heating to make a 9.99 M urea solution. 1 M NaOH was added dropwise to adjust pH to 7.0. To prepare the MOPS, 6.27 g of MOPS was added to 1 L solution and adjusted to a pH 7.0 using 1

M NaOH. The H-NS solution contained 56 μ M protein in 30 mM MOPS. Samples were equilibrated for 6 hours at room temperature before measuring the intrinsic fluorescence using Shimadzu RF-100 spectrophotometer at an excitation of 278 nm and emission scan from 295 to 400 nm. For tests involving Ag^+ , 1 mM AgNO_3 in MOPS was added to each sample. Once the denaturation curve is determined, the free energy change (ΔG_U) of the folding/unfolding reaction is calculated. A denaturation curve utilizes a two-state mechanism for the folding/unfolding reaction. This assumption allows for the use of Equation 3.²⁰

$$f_F + f_U = 1$$

where f_F is the fraction of folded protein and f_U is the fraction of unfolded protein. The observed fraction value can be defined using Equation 4.²⁰

$$y = y_F f_F + y_U f_U$$

where y is any observable parameter chosen to investigate denaturation of a protein, and y_F and y_U are the characteristic of the folded and unfolded protein. The least squares analysis can be then used to obtain the Equation 5.²⁰

$$f_U = (y_F - y)/(y_F - y_U)$$

Once Equation 5 is formulated, the equilibrium constant, K_U , and the free energy change, ΔG_U , can be calculated using Equation 6.²⁰

$$K_U = f_U(1 - f_U) = f_U/f_F = (y_F - y)/(y - y_U)$$

and Equation 7.

$$\Delta G_U = -RT \ln K_U = -RT \ln [(y_F - y)/(y - y_U)]$$

Where R is the gas constant and T is the temperature in Kelvin. K_U is found to be most accurate when calculated in the midpoint of the denaturation curve.²⁰

RESULTS

H-NS protein may have leaky or native expression.

The confirmation of successful protein expression was observed on an SDS-PAGE gel. It is expected for there to be a large band after induction to indicate

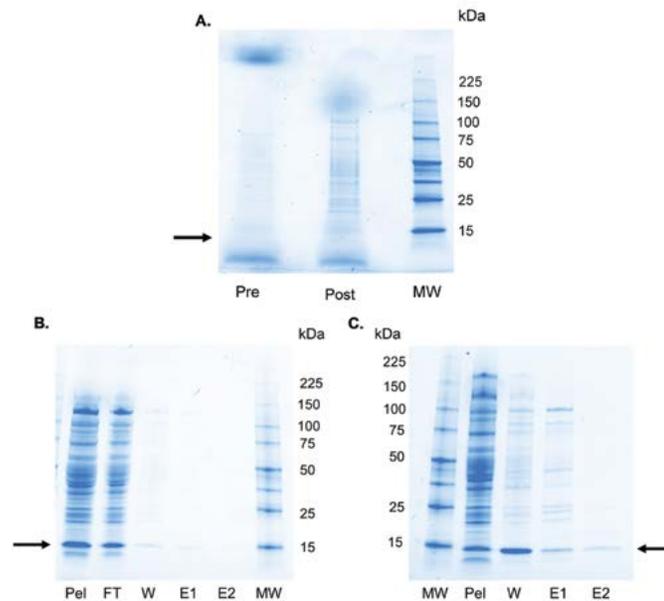


Figure 3. Expression of the *hns* gene and purification of H-NS protein. (A) Expression was induced by IPTG. SDS-PAGE analysis of recombinant protein expression. Lanes: Pre, lysate of cells prior to induction; Post, induced lysate of cells; MW, broad range protein molecular weight marker. The arrow indicates the molecular weight of H-NS protein (15.5 kDa). (B) SDS-PAGE analysis of purification by IMAC. Lanes: Pel, cell lysate prior to IMAC; FT, flowthrough; W, wash with 10 mM imidazole; E2 and E1, fractions corresponding to elution one and elution two; MW, broad range protein molecular marker. The arrow indicates the molecular weight of H-NS protein (15.5 kDa). (C) SDS-PAGE analysis of purification by refined IMAC. Lanes: MW, broad range protein molecular weight marker; Pel, cell lysate prior to IMAC; W, wash with 10 mM imidazole; E1 and E2, fractions corresponding to the first elution and second elution. The arrow indicates the molecular weight of H-NS protein (15.5 kDa).

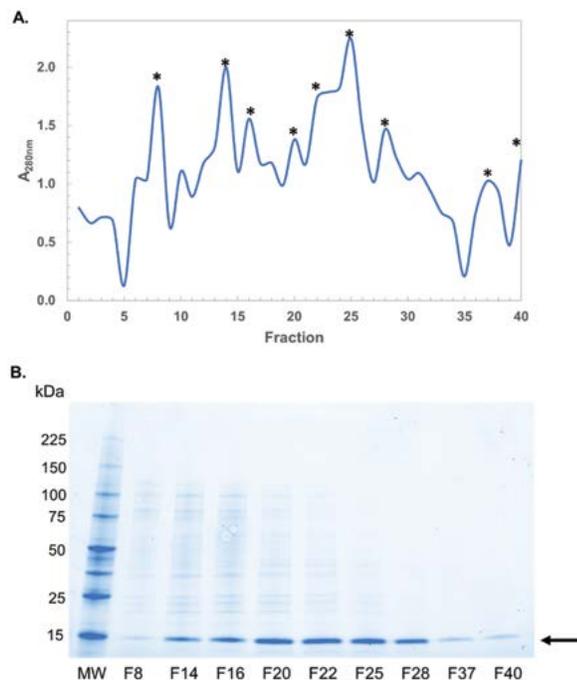


Figure 4. Final purification of H-NS protein by gel filtration chromatography. (A) Gel filtration chromatograph of protein fractions on a Sephadex G-100 column. Fractions were eluted with 10 mM Tris (pH 7.5), 50 mM NaCl. Fractions 1-5 were 1.0 mL while 6-40 were 0.50 mL fractions. The asterisks indicate which fractions were collected for SDS-PAGE. (B) SDS-PAGE analysis of gel filtration. Lanes: MW, broad range protein molecular weight marker; F8 refers fraction 8 from (A), F14 fraction 14, F16 fraction 16, F20 fraction 20, F22 fraction 22, F25 fraction 25, F28 fraction 28, F37 fraction 37, F40 fraction 40 were fractions containing high concentrations of protein. The arrow indicates the successful purification of H-NS protein in fractions F20 – F28.

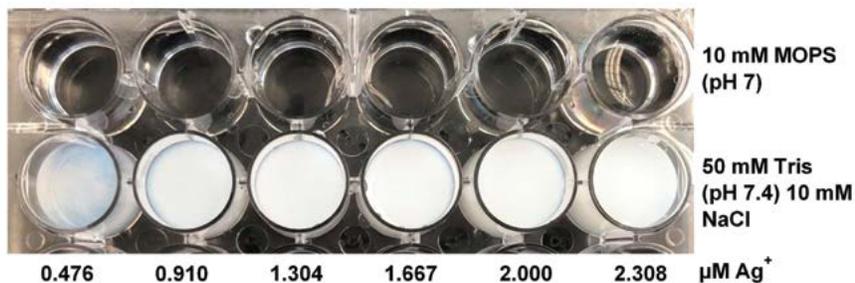


Figure 5. Solubility of AgNO_3 in varying buffer. 2.000 mL of each buffer were titrated with varying concentrations of AgNO_3 from 0.476 to 2.308 μM .

No interaction between MOPS and silver.

To determine if there is a potential interaction between H-NS protein and Ag^+ , the intrinsic fluorescence was observed. Initially, the intrinsic fluorescence of H-NS protein was observed with and without the presence of Ag^+ , in a solution containing Tris buffer, which had been used in previously published protocols.¹⁸ During this observation the solution became cloudy with a white milky precipitate (Fig. 5). Despite its reported use with silver and H-NS¹⁷, tris(hydroxymethyl)-aminomethane (Tris) interacts with Ag^+ to produce a white precipitate.²¹ It was unclear how this worked in the published experiment.¹⁸ As the original buffer housing the H-NS proteins contained Tris, all solutions became cloudy as Tris and the silver ion were interacting. To eliminate this interaction, new buffers were chosen: MES or MOPS. MOPS was selected as the buffer for further experimentation, as MOPS has a pKa closer to 7.

H-NS protein binds to dsDNA.

To analyze the binding of H-NS protein binds to μDNA , the intrinsic fluorescence of the H-NS protein was measured as successive additions of dsDNA was added to solution (Fig. 6). Increments of dsDNA were added until a concentration of 95.8 μM was reached and the intrinsic fluorescence change became stagnant (Fig. 6A). The fluorescence decreased in proportion to the amount of dsDNA added to solution, suggesting the binding of H-NS to dsDNA.

Addition of Ag^+ decreases binding affinity of H-NS to dsDNA.

The experiment mentioned previously was repeated in the presence of 1 mM of Ag^+ . The intrinsic fluorescence drastically decreased when Ag^+ was present in solution (Fig. 6B). The fluorescence intensity and fluorescence maximum were calculated to show the difference in intrinsic fluorescence with Ag^+ in solution (Figs. 6C,6D). This confirms there is an interaction between H-NS and Ag^+ . To calculate the dissociation constant of H-NS protein with dsDNA, the maximum fluorescence was used to calculate the quenching constant. The K_d of 74.62 μM was calculated using the slope of the Scatchard Plot as $1/K_d$ (Fig. 7), and the stoichiometry was found to be $n = 2.5$ using $n = y\text{-intercept} \times K_d$. The low binding affinity confirms the strong interaction between Ag^+ and H-NS protein.

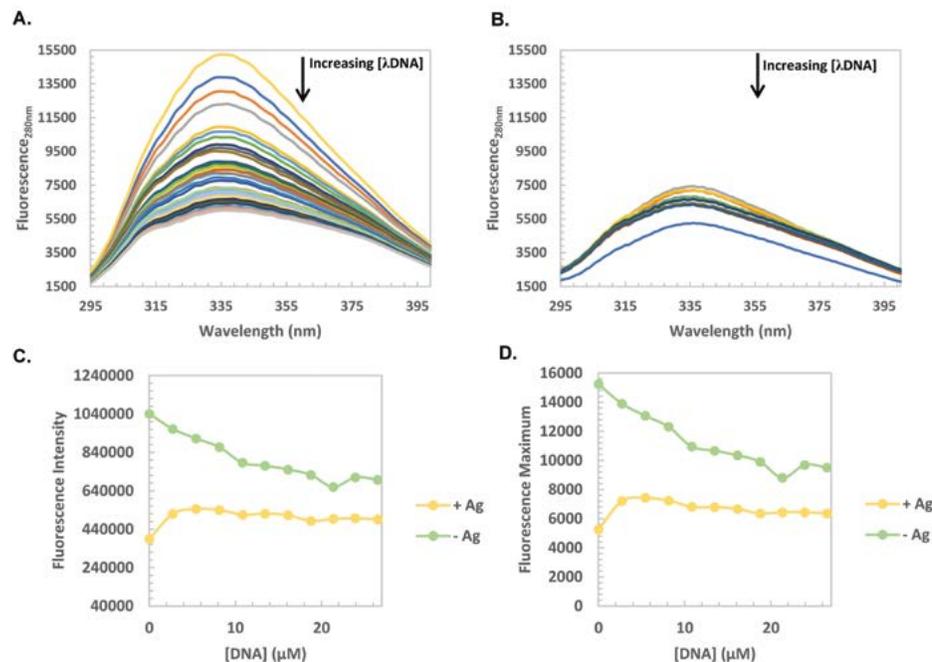


Figure 6. Quenching of tryptophan fluorescence of H-NS protein with and without the presence of Ag^+ . (A) Quenching of tryptophan fluorescence of H-NS protein in MOPS (pH 7) buffer at 25 °C on progressive titration with increasing lambda DNA concentration (0 – 26.5 μM) with and without the presence of Ag^+ . (B) Quenching of tryptophan fluorescence of H-NS proteins in MOPS (pH 7) in the presence of Ag^+ . (C) Fluorescence intensity of H-NS proteins with and without the presence of Ag^+ . The fluorescence intensity was calculated by calculating the area under each curve. (D) Fluorescence maximum of H-NS protein with and without the presence of Ag^+ .

the expression of the H-NS protein, but the large band was observed in both the pre-induction and post-induction (Fig. 3A). The presence of the H-NS protein before induction suggests that H-NS protein have a potential for leaky or native expression. In the case of leaky expression, the expression of the H-NS protein fused with a His-tag was not regulated properly. Additionally, native expression of H-NS protein occurs naturally in *E. coli*, the competent cell type being used, so the shown protein (Figs. 3B,3C) may not have a His-tag and be incapable of purification by IMAC.

To further purify the H-NS protein, a size exclusion gel filtration column

was used as the H-NS protein are much smaller than the other proteins in the fractions (Fig. 4A). After the fractions were collected, the fractions were analyzed at an absorbance wavelength of 280 nm to find out which fractions contained the highest concentrations of protein (Fig. 4A). The fractions that contained the highest concentration of protein were analyzed via SDS-PAGE to find which fractions contained the desired H-NS protein (Fig. 4B). The final concentration of H-NS protein was 0.886 mg per liter of bacterial culture to be used for further study.

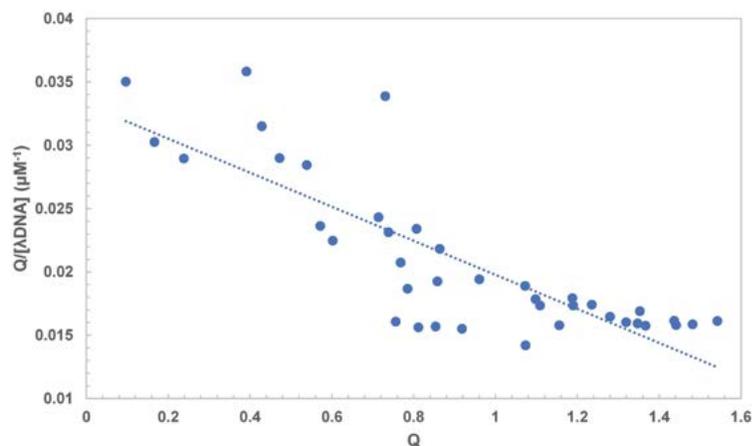


Figure 7. Scatchard plot for titration of H-NS protein with increasing concentrations of lambda DNA describing the binding of lambda DNA to H-NS protein. The H-NS protein was titrated with concentrations of 0 μM to 95.5 μM . The titrations were performed in 10 mM Tris (pH 7.4) 50 mM NaCl at room temperature. The H-NS protein was excited at 280 nm and the emission was measured from 295-400 nm. The maximum value was used to calculate Q . The trendline that represents the data is $y = -0.0134(1/\mu\text{M})x + 0.0332$ with an R^2 value of 0.680. The estimated K_d was 74.62 μM and the stoichiometry $n = 2.5$.

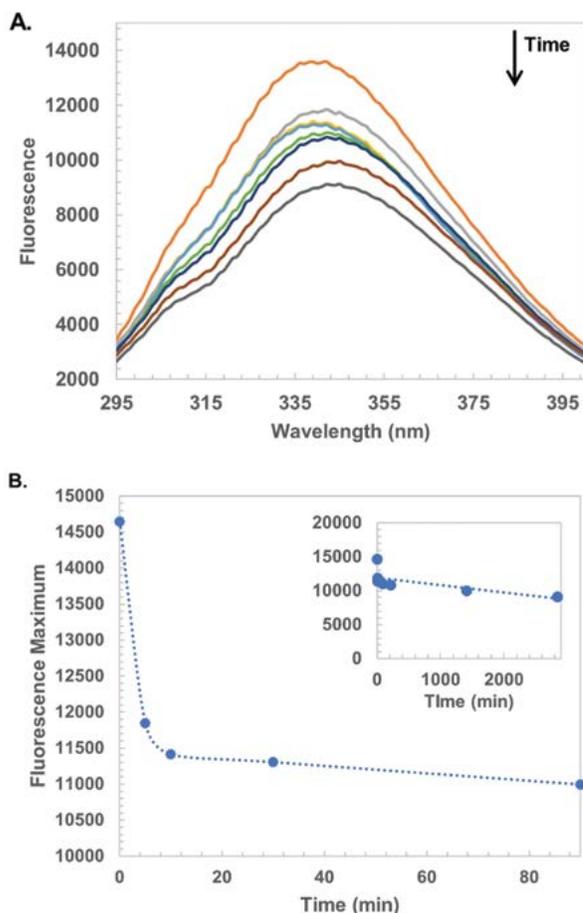


Figure 8. Intrinsic fluorescence emission spectra upon excitation at 278 nm with an emission range of 295 – 400 nm. (A) The intrinsic fluorescence of H-NS protein was measured at varying time points (0 min, 5 min, 10 min, 30 min, 90 min, 3.5 hr, 23.5 hr, 48 hr) in solution with 8.42 M urea. (B) The fluorescence maximum was calculated and plotted against time. The inset graph shows the decrease in fluorescence over a period of 48 hr. The significant decrease in fluorescence was seen from 0 to 20 min.

Denaturation of H-NS proteins by urea.

The native confirmation of soluble protein folding leads to the hydrophobic amino acids being buried inside the protein to form a hydrophobic core and shield the nonpolar residues from water.^{22,23} Hydrophilic amino acids remain towards the outside of the protein, as they can form hydrogen bonds, dipole-dipole, or charge-dipole interactions with water.²⁴ To unfold a protein, the hydrophobic residues must be exposed. To determine the time frame for the urea-mediated protein stability experiment, the highest concentration of urea was introduced to solution containing H-NS protein. Protein intrinsic fluorescence is sensitive to the environment and therefore can be used to analyze protein structure.²⁵ To analyze the interaction between H-NS protein and DNA, the intrinsic fluorescence was measured with the addition of DNA into solution. As the DNA binds to H-NS protein, the intrinsic fluorescence is quenched. This is due to the decrease in quantum yield of fluorescence of the tryptophan.²⁶ When introducing DNA to H-NS in solution, the change in emission can be used to analyze the binding between the DNA and H-NS protein. The intrinsic fluorescence was observed at varying time points to confirm that urea would denature H-NS protein (Fig. 8A). The negative slope shows that the fluorescence decreased over the 48 hours, with the sharpest decrease occurring in the initial 20 minutes (Fig. 8B). Notably, the fluorescence did not flatten as fully as would be expected for complete denaturation.

The denaturation curve did not follow the continuous downward slope predicted by theory. All samples were heated to 37 °C for 3 hours, to overcome the activation energy to attempt to promote unfolding. Fluorescence was measured as before (Fig. 9). Fluorescence intensities of the silver samples were plotted on a single plot to compare before and after heating (Fig. 10).

CONCLUSION

Here, we analyzed the interaction between H-NS protein, DNA, and Ag^+ by: (1) evaluating the interaction between DNA and H-NS protein in the presence of Ag^+ (2) determine if Ag^+ led to the denaturation of H-NS protein. In this study, Ag^+ were found to affect the interaction between H-NS protein and double-stranded DNA. Before experimentation, H-NS proteins were recombinantly expressed, purified, and then optimized.

Initial preparation for analysis of the interaction between H-NS proteins and DNA revealed an unfavorable interaction

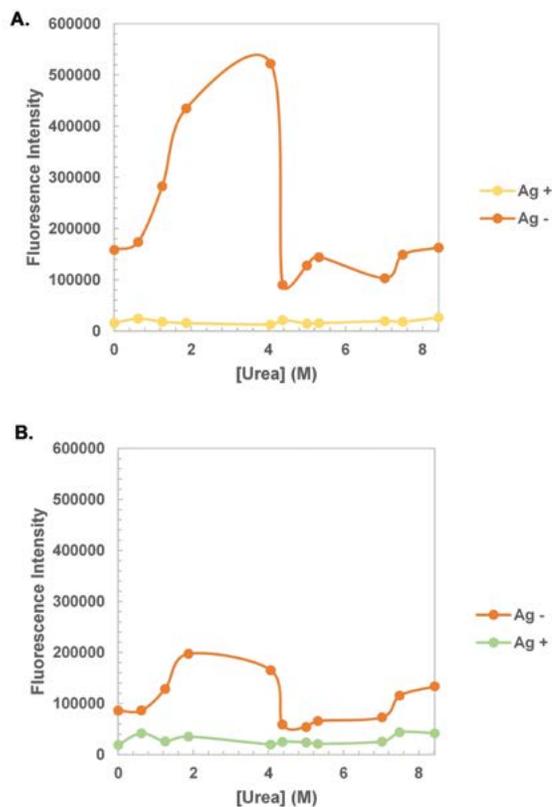


Figure 9. The analysis of a urea denaturation curve of H-NS protein (30 mM MOPS, pH 7.0) with and without the presence of Ag⁺. (A) The fluorescence intensity from 295 to 400 nm was measured after excitation at 278 nm. The varying concentrations of urea was from 0 M to 8.42 M. Orange line represents the denaturation curve generated without silver in solution. Yellow line represents the denaturation curve generated with silver ions in solution. (B) Analysis of a urea denaturation curve of H-NS protein (30 mM MOPS, pH 7.0) with and without the presence of Ag⁺ at 37 °C. The fluorescence intensity from 295 to 400 nm was measured after excitation at 278 nm. The varying concentrations of urea was from 0 M to 8.42 M. Orange line represents the denaturation curve generated without silver in solution. Green line represents the denaturation curve generated with silver ions in solution.

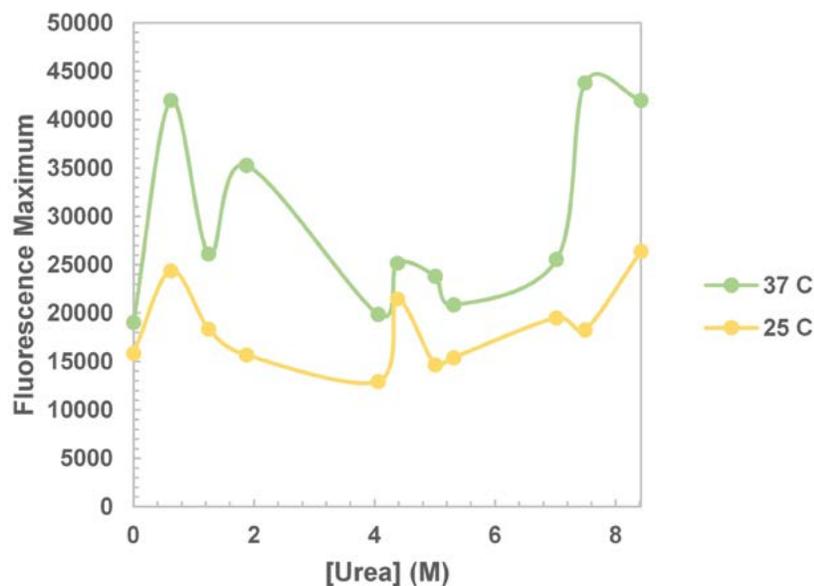


Figure 10. Analysis of the urea denaturation curve of H-NS protein (30 mM MOPS, pH 7.0) with the presence of Ag⁺ at 25 °C and 37 °C. The fluorescence intensity from 295 to 400 nm was measured after excitation at 278 nm. The varying concentrations of urea was from 0 M to 8.42 M. Green line represents the denaturation curve generated with silver at 37 °C. Yellow line represents the denaturation curve generated with silver at 25 °C.

between the initial buffer (10 mM Tris (pH 7.4) 50 mM NaCl) and Ag⁺. This interaction would lead to complications in analyzing the binding between H-NS protein and dsDNA. After eliminating the Tris from experimentation, MOPS was used, as MOPS did not interact with silver (Fig. 5).

The intrinsic fluorescence of H-NS protein confirmed the binding of H-NS protein to dsDNA, as the fluorescence decreased in proportion to the amount of dsDNA added to solution (Fig. 6). When adding Ag⁺ to solution, the intrinsic fluorescence drastically decreased (Fig. 6). The decrease in fluorescence confirms that Ag⁺ decrease the binding affinity between dsDNA and H-NS protein. As the H-NS protein was in buffer containing Tris, an improvement would be to no longer use Tris as a component in the buffer. When protein purification was repeated, MOPS buffer was used to ensure there is no interaction between any components of the buffer and Ag⁺.

As the intrinsic fluorescence decreased when Ag⁺ were added to solution, there is confirmed interaction between H-NS and Ag⁺. As Ag⁺ are known to bind to oxygens, nitrogens, and sulfurs in protein, an interaction between Ag⁺ and the H-NS proteins seems likely.¹³ Specifically, an H-NS monomer contains one cysteine within the oligomerization domain of the protein, and this cysteine may lead to the formation of an Ag-thiol interaction that may affect the binding affinity of H-NS to DNA.¹⁸ Two possible mechanisms are that the Ag-thiol interaction may cause a change in the protein allosterically, affecting the binding site, or that the Ag-thiol interaction affects the H-NS proteins by impacting the oligomerization domain or by causing the denaturing or misfolding of the proteins.¹⁸ Both possibilities could reduce the binding affinity between H-NS and DNA.

The confirmed denaturing of H-NS proteins will be one of many steps to understand the dynamic relationship of how Ag⁺ interact and manipulate the structure of the nucleoid. As H-NS has several roles within the cell, such as negative regulation of genes, compacts and helps synthesize DNA, and reconstructs the structure of the nucleoid. The ability to manipulate the H-NS protein is detrimental to bacteria cells. H-NS proteins control expression over 200 genes in the nucleoid of Gram-negative bacteria.¹⁸ Losing the ability to regulate over 200 genes would ultimately lead to cell death. As Ag⁺ are harmful to both extracellular and intracellular components of bacteria cells, the use of Ag⁺ to combat antibiotic resistance can be monumental.

A theoretical plot of intrinsic fluorescence against urea concentration

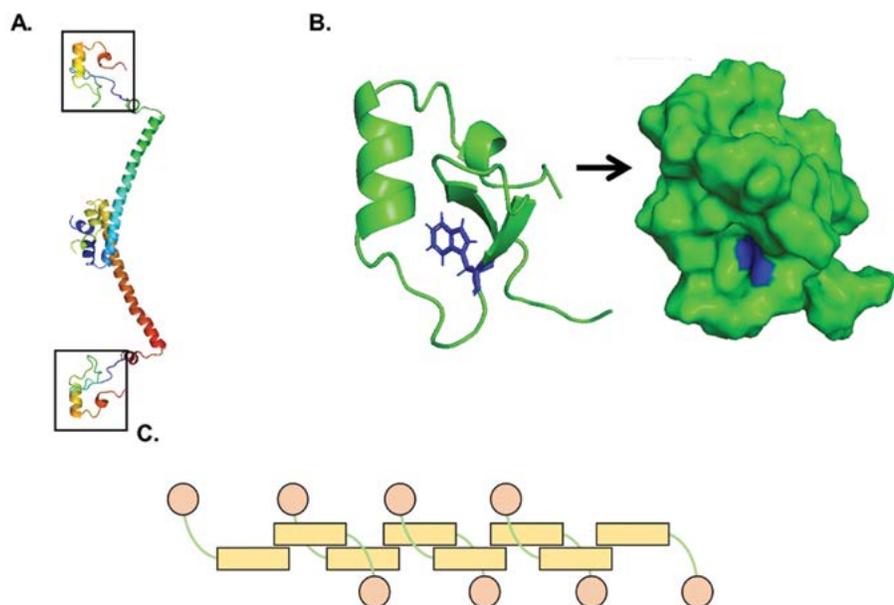


Figure 11. Crystal structure of H-NS DNA binding domain with tryptophan location indicated in purple. (A) Crystal structure of H-NS dimer indicating the location of the DNA binding domains shown in black boxes. (B) Secondary structure representation showing the location of the Trp residue in the color blue and the surface representation showing the location of the Trp residue indicated in the color blue. (C) Visual representation of the oligomerization of H-NS proteins. The yellow box represents the oligomerization domain, and the orange circle represents the DNA-binding domain, the green line represents the flexible linker that links the two domains together.

would result in continuous downward slope if urea induced protein unfolding. However, in the absence of silver, the intrinsic fluorescence of H-NS protein increased rapidly at 4 M urea, then quickly fell back down. Aggregation in the oligomerization domain precluded proper folding in the DNA-binding domain where Trp was present. This might be explained by the known propensity of H-NS proteins to oligomerize.¹⁵ We hypothesize that 4 M urea may be needed to break apart H-NS multimers (Figs. 9). H-NS protein contains a Trp residue near the dimerization domain and within the hydrophobic region of the protein (Fig. 11). Trp fluorescence is highly sensitive to local environment.³⁸ As the Trp is in close proximity to the dimerization domain, the H-NS protein in close proximity to the Trp may lead to a change in the residue's local environment, leading to a decrease in fluorescence intensity. Urea concentrations

up to 4 M may induce the dissociation of H-NS oligomers, leading to an increase in fluorescence. As the urea concentration continues to increase from 4 M, the fluorescence drastically decreases, as the protein denatures. The slight increase after 4 M urea may be due to pipetting error as small volumes of each solution component were used. At all urea concentrations, fluorescence in samples containing Ag^+ was severely reduced (Fig. 9). This suggests that the proteins are destabilized relative to those without the silver, further supporting silver-induced denaturing of H-NS protein.

The free energy of unfolding was not able to be calculated due to the abnormalities in the denaturation curve. One potential problem that caused the abnormal curve is the lack of complete folding of the H-NS protein. To optimize the complete and reversible folding, adjustments in temperature, time, and

urea concentration should be made. Second, the tendency of H-NS proteins to oligomerize may have affected the ability of urea to denature the protein in a straight-forward manner. High-performance liquid chromatography (HPLC) might be used with known protein standards to determine the size of H-NS protein aggregates. If HPLC was run with different low urea concentrations, it may be possible to determine how the oligomerization was urea dependent. Protein concentration might also be optimized to change or reduce the oligomerization. These adjustments would allow for determination of the ΔG_U for free H-NS. For the introduction of silver, the Ag^+ appeared to destabilize the protein so significantly that there was barely a denaturation curve. The experiment could be repeated with a lower concentration of silver to see if there is a formation of a curve.

Overall, the experimental data supports H-NS protein denaturation in the presence of Ag^+ . Further experimental optimization should allow for the calculation of change in ΔG as silver destabilizes H-NS protein. Broadly, the introduction of Ag^+ into solution lead to denaturation of H-NS protein. This suggests an additional mechanism for silver ion bacterial toxicity, and further strengthens the idea of using Ag^+ as a weapon to fight against antibiotic resistance. As past research has shown that Ag^+ damage the cell membrane, weaken DNA replication, and inactivate functional proteins.¹² H-NS protein have a vital function within Gram-negative bacteria. Inducing damage of these proteins leads to loss of function of several genes that contribute to bacteria virulence.¹⁶ The ability to weaken the virulence of the bacteria as well as promote cell death would drastically decrease the threat of antibiotic resistant. Future directions for this study may include the confirmation of the Ag-thiol bond as the detection of the mechanism of destabilization by Ag^+ . As Ag^+ were shown to both disrupt the interaction between H-NS and DNA and destabilize H-NS protein, it can be predicted that Ag^+ will drastically affect the viability of the bacterium cell as H-NS proteins are involved in regulation of over 200 genes in the cell.¹⁸

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CAST METAL MENAGERIE

Jeri Alexander, Philip Croft, Hannah Gordon, Joshua Killen, & James Turner

ABSTRACT

The “Cast Metal Menagerie” features work by students in Professor Bill Wolff’s Beginning Sculpture and Figure Sculpture courses. According to Wolff, the sculptures are all made of bronze using a 6,000-year-old lost wax process. This process involves creating a model from wax or another material, building a system of “plumbing” (gating and vents) around it, encasing the structure in plaster (investment), placing it in the kiln to melt the wax, pouring the bronze, and removing the investment mold. The resulting structure is then cleaned and treated with chemicals to alter the color (patination) and is waxed. For the majority of students, this was their first experience with this process. The work was installed at the Salisbury Zoo June 3-August 20, and then at the Ocean City Art X Festival August 21-22.



The Hands of Mother Nature by Hannah Gordon



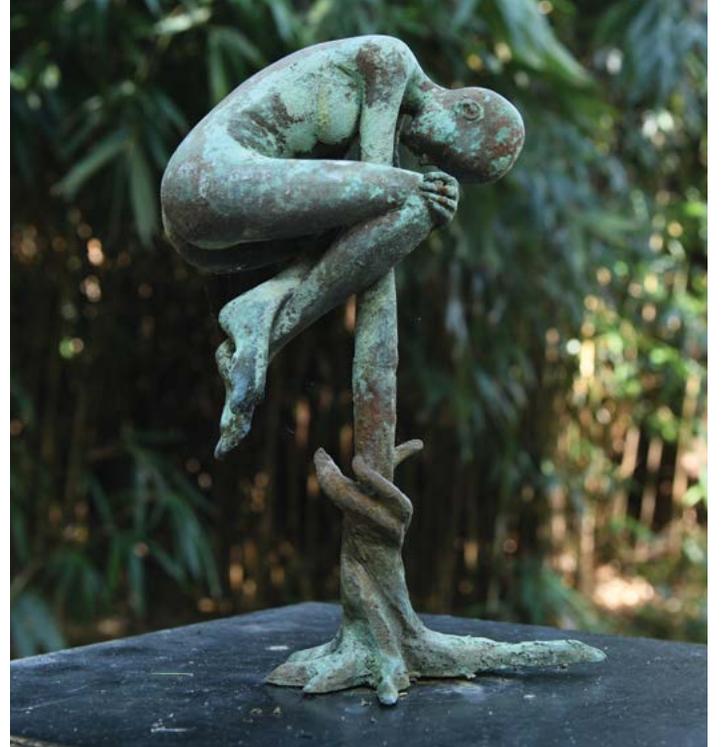
Arachnivision by James Turner



Do Not Touch Me by Jeri Alexander



The Green Woman by Joshua Killen



Treembryo by Philip Croft



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