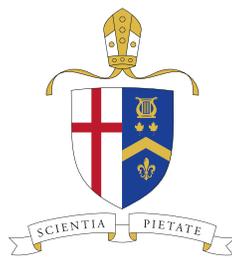


ISSN 2560-9815

Volume 2 | Issue 1 | August 2018

The Young Researcher



RSGC
Royal St. George's College

The Young Researcher

SUMMER 2018 VOLUME 2, NUMBER 1

**A journal dedicated to the publication of original research
from secondary school students**

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ISSN 2560-9815 (Print)
ISSN 2560-9823 (Online)

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Editorial

The truth is hard to find.

We live in a world with so much information that it is difficult to tell what is right and what is wrong. Opinions can become fact, with little research done to support them. *The Young Researcher* is published with the goal of dispelling this notion among youth, providing a forum for reliable and truthful research, and inspiring future young people to become more involved in academia. We believe this can be achieved no matter a person's age or location. Anyone who is willing to work hard can add to our collective knowledge.

Every paper published in this journal is devoted to truth. The authors featured left no stone unturned in their quest for new and meaningful information. The papers represent a range of topics and represent the broad range of research necessary to find truth. The papers analyzed pertinent topics in several important fields.

To ensure the journal did not fall to publication bias, any result could be published regardless of its findings: whether it established a landmark conclusion or concluded that the original hypothesis was incorrect. *The Young Researcher* prides itself on utilizing a blind peer-reviewed process. In this manner, it ensures that the published research is of a high quality, and the research conducted is valid and truthful.

We hope you enjoy this collection of studies and that it gives you a new understanding about the world.

Welcome to *The Young Researcher*.

The Editors

Arshia Amirzadeh
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On Fire For God: Exploring Adolescent Religious Development

Ella F. Moxley

High school students are navigating new social relationships and milestones; in this process, they may be forced to reevaluate their own religious identity. This research study asked the question, “How do adolescents ages 16-18 view their own religious development in a time when they have gained greater autonomy from their parents, and what factors do they see as most important in forming their own religious identity?” Through a series of half-hour phenomenological interviews with six carefully selected students, this study seeks a better understanding of those factors influencing the religious identity of adolescents. Interview questions were aimed at finding shifts in the participants’ religious identity in adolescence. The study is a critical investigation as it allows for a better understanding of what kind of events, people, or institutions are most influential in a young person’s life. Data analysis revealed four distinct themes: community, personal relationships, education, and morality. I examine those themes and show how they reveal that late adolescence is a time of complex identity development. My principal conclusion is that adolescents are active agents in their development of religious identity. They use the messages they receive from outside sources to inform their own choices about their religious identity and the role that religion plays in their lives.

Keywords: Religious development, adolescents, socialization, autonomy

Introduction

Religious activities are an integral part of many American teenagers’ lives, yet for many young people, their religious identity exceeds the ascribed beliefs in which they were raised. Throughout adolescence, exploration, fueled in large part by doubt, can help young people better define their religious identities (Baltazar & Coffin, 2011, p. 188). Smith (2011) holds that identity for any group is made up of their membership, position, and status within a social group (p. 217). Nelson (2010) describes religious identity as composed of three significant elements: belief, action, and membership (p. 337). As young people age, their personal beliefs, as well as memberships to certain groups, may shift in response to outside factors.

Another aspect of religious identity relevant to understanding how religion factors into a teen’s view of oneself is religious salience, which James, Lester, and Brooks (2014) describe as how one sees the impor-

tance of religion within their overall identity (p. 879). Religious identity can or perhaps should be viewed as involving multiple factors, in which the family is a compelling factor. Regnerus, Smith, and Smith (2004) recognize how religion is inherently practiced in the company of others (p. 27), which is why Zhai, Ellison, Stokes, and Glenn (2008) make the claim that family can provide a socializing pull for the development of a religious identity (p. 380). This means that in order to understand a young person’s religious identity, it is critical to also understand how they are influenced by their parents.

This paper aims for a better understanding of how complex social interactions, as well as important moments in adolescents’ lives, can shape their religious identities. The principal research question for this study asks “How do adolescents ages 16-18 view their own religious identity development in a time when they have gained greater autonomy from their parents, and what factors do they see as most important

in forming their own religious identities?” This question illuminates the lives of young adults and facilitates an understanding of those factors holding importance for a young person’s growth into an adult.

Literature Review

High School Student’s Religious Experience

Throughout the last few decades, researchers focusing on religious identity have illuminated the religious lives of adults by focusing on college-aged young adults (18-22 year olds). This has left a considerable gap in understanding younger age groups, which led Bebiroglu, van der Noll, and Roskama (2017) to focus on the “need to move beyond examining adult conversations to study children and understand their perspectives” (p. 290). The body of literature that does focus on a younger age group is clear that the experiences of high school students are critical to the field due to the fact that young adults are biologically primed to begin thinking more deeply about abstract religious concepts (Barry, Nelson, Davarya, & Urry, 2010, p. 312).

Understanding how American teenagers develop their religious identity is especially relevant in the digital age. According to Bobkowski and Pearce (2011), social media forces students to consider how they present their own identities in the face of decreasing privacy and increasingly complex social norms within a matrix of rich social interactions (p. 744). Gaining insight into the religious identity development process from the perspective of high school aged young adults is a vital yet understudied domain of inquiry. As Petts (2009) clearly acknowledges, a better understanding of religious change and stability can help adults understand adolescent development as a whole (p. 552).

Early Adolescent Religious Development

In a qualitative study of Muslim students, Peek (2005) concludes that for many young people, their Muslim identity went unquestioned throughout their early childhood since it was just a routine feature of their daily lives (p. 224). This is consistent with observations from Hoffman (2012) who notes that

young people’s orientation towards religion is often more rigid and concrete during early stages of the life course in which parental stipulation is likely a potent factor (p. 1027). These observations reveal that early stages of religious identity are often one dimensional due to the fact that young children have not yet critically examined the religious identity ascribed to them by their parents.

As adolescents move further into biological and social development they not only gain new cognitive abilities, they also go through what Kox, Meeus, and Hart (1991) would call “turning points,” where changes in life correlate with shifts in how adolescents think about their religious identity (p. 234). These changes are immensely important because Armet (2009) finds that such changes can culminate in the development of a young person’s identity, which is “one of the most important tasks of adolescence” (p. 279). As children begin to mature, the way they relate to their own religious identity may shift. To become an active agent in defining one’s own identity is a critical transition into more autonomous functioning and higher level thinking.

Older Adolescence:

Watson, Howard, Hood, and Morris’s (1988) research reveals that religious orientation often becomes more exploratory in the adolescent years which they attribute to youth experimenting with the freedom they have to develop their own identities (p. 276). Different researchers have concluded that this kind of exploration can result in a number of different outcomes. For Twenge (2017), data, collected in a national survey, suggests that as adolescents gain more autonomy, and start to question the status quo, they are more likely to leave their religion (p. 122).

However, Pearce and Denton (2011) contradict this through a series of qualitative interviews from which they conclude that increased autonomy results in youth “[having] matured to the point that their faith is their own and that makes it deeper and stronger” (p. 3). Although these theories seem contradictory to each other, they both rely on the assumption that as youth age, they may be inclined to take control of their identities.

Other researchers have found conclusions similar to those of Rew, Wong, Torres, and Howell (2007),

who break down religious identity to find that although young people's religiosity (religious practice) may decline in adolescence, their subjective beliefs (spiritual beliefs) are constant (p. 57). This implicates the potential for religious shifts that occur at multiple layers of a student's religious identity. It is important to recognize religiosity as a key element of religious identity, but it is not the full story. This is why religious researchers Lopez, Huynh, and Fuligni (2011) indicate that there is potentially no correlation between participation and affiliation (p. 1298). In fact, the decline in participation may be a product of young people's social environments, not changes in identity, according to Desmond, Morgan, and Kikuchi (2010) who note that peer pressure may not always influence the subjective beliefs of adolescents (p. 266). Therefore, the religious lives and identities of adolescents may produce a variety of changes, best understood through a lens that takes into account the multiple ways adolescents view and act on their religious identities. Due to the complex nature of religious identity, a qualitative methodology is used in order to better understand the process from the perspective of participants.

Methods

Qualitative Methodology

As a result of the complex nature of religious development, which goes beyond simple group membership, and the fact that research fails to include adolescents, a methodology that gave a direct voice to young adults' experiences proved essential to addressing the research question. In a similar study, one that examined the religious development of college students, the authors point out that "qualitative studies are thus needed for fuller descriptions" (Rew et al. 2007, p. 57). Qualitative research can be effective as it gives greater meaning to quantitative trends recorded in literature, and it helps illuminate the inner workings of a larger phenomenon.

A phenomenological interview process was determined to be the best method to understand how high school students see their religious development in relation to their life experiences. The interview process was best suited for the research as it allowed the

researcher to deeply understand the experiences of each individual participant.

I designed an interview schedule that was semi-structured, with ten open-ended questions based on the literature review. In a qualitative study done by Ravishankar and Bernstein (2014), the researchers asked people about the impact of religion in lives through "interviews [that] were based on a guide, but themes were freely explored as they arose" (p. 1900). In a similar fashion, I created the interview schedule so it was flexible in order to fully follow up with students' responses.

Recruitment of Participants

Six students from a high school located in a college town (located within a Bible Belt state) between the ages of 16-18 were interviewed for approximately 20-30 minutes. Although I originally intended to perform an hour-long interview, the length had to be revised since the content of the interview was usually sufficiently covered in under 30 minutes. The number of students was chosen based on a number that would provide a saturation of data in the time constraints. Participants were selected through announcements in various school groups (i.e. Speech and Debate, Christians on Campus, Band). My use of announcements to recruit participants was not productive, so I began using a snowball method, where I asked participants to nominate other potential participants who were within their social networks. Participants completed consent forms indicating that they were informed about the potential risks and benefits of their involvement as informants. In order to protect confidentiality, each participant was assigned a pseudonym: John (Baptist), Sadie (Catholic), Melody (Baha'i), Sahana (Hindu), Ceedee (Buddhist), and Nathan (Deist).

Interview Content

During the interview, the researcher asked students questions that illuminated their shifts in religious identity over the course of their lives. One such question is "How do you feel your current religious identity differs or is constant with the way you thought about religion 10 years ago?" The questions that were incorporated into the schedule were also loosely based on important themes in adolescent

religious development that were identified through the literature review. For example, a paper by Vaidyanathan (2011), analyzed how socialization functions with religion, noting that religion and ethnicity are deeply intertwined (p. 384). Therefore participants were asked, “Do you think that your racial or ethnic identity influences your religious identity?” All questions can be found in Appendix A. These questions captured a narrative of adolescents’ religious history that focused on how their religious development shifted or remained constant throughout their adolescence. The responses also gave insight into what factors promoted adolescents’ changes in or continuity of their identities.

Data Analysis Procedure

All responses were recorded and then later transcribed. After transcription, I analyzed the data by first sensitizing myself to the interview transcripts. Then through multiple readings of the transcripts, I became familiar with the raw data and I oriented to prevalent themes that repeated themselves within and across multiple transcripts. I was attentive to shifts in adolescents’ religious lives. A shift was defined as a factor, person, or event that prompted a change in religious identity or otherwise influenced the change.

Important themes were coded and organized like Halama and Halamová (2005) organized their data in a qualitative study of Christian conversions. Themes were placed into categories and subcategories (p. 71). I used such a procedure to help organize the data so I could compare categories across all six participants, and I located four shared categories and eight common subcategories. The common themes across participants were then analyzed using analytical questions developed from this researcher’s original questions.

Results

I identified four major themes and eight sub-themes after comparing codes across all six participants. Overall themes moved from external factors that affect young people to more personal factors. Major themes were community, personal relationships, education, and morality.

Community

Religious Community

All participants talked about the way their religious community had a tremendous effect on their religious development because it created a sense of belonging. For both Sadie and John, their religious community was something they could turn to when they faced bullying in their schools. John was an especially salient example of someone who found deep friendships in his religious community after facing social exclusion from his school peers. Sahana also saw the way that the community she found in religion was helpful as it gave her a break from the external turmoil she faced in her middle school. She reflects that “Sunday was not just like me going to Sunday school it was like our family’s kind of ritual... it was like really solid and all the holidays were extremely fun.” For her and many other students, a religious community was a source of stability within their lives in the midst of external stress.

Summer Camp

For three participants, summer camp was also one of the greatest forms of community that affected their connection to their religious identity. For John, whose family was not very religious, “It was really moving to be there surrounded by so many people that are really religious.” For Nathan, summer camp served to move him further away from the Christian faith in which he was raised when he realized that, “this isn’t spiritual energy this is more of just human energy... even if you were going to a Nirvana concert you would get the same feeling.” Most participants noted that the “energy” these camps offered was critical to either reaffirming their religious identity or forcing them to reevaluate their participation in their religious institution.

Personal Relationships

Role Models.

All students spoke of specific personal relationships that were especially monumental in shifting

how they viewed their religious identities. Many students had role models who affected their identity the most. Some students spoke of peers like Sadie, whose Mormon friend helped reaffirm her Catholic views as a young high school student because, in the words of Sadie, “she gave me a different point of view from someone that’s my age and going through the same thing as me.” Melody also had an older friend that was able to provide her with religious guidance that Melody trusted because of their proximity in age. This kind of empathy was something most students looked for in a religious role model. When unable to connect to his religiously ambivalent parents, John turned to his youth pastor. John reflected on the “surrogate family” he found with his pastor saying that “I feel like it just plugged a missing hole because you want your family to do the things you do.” Role models have great power to shift an adolescent either further into a religious identity or away from it especially if they are able to provide a young person with support and empathy that they might be missing from other figures in their lives.

Parents

The most repetitive relationship seen in all six interviews was the relationship between children and parents. Students repeatedly talked about their parents in the context of freedom, choice, and autonomy. Sadie says that “I didn’t really have strict enforcement of certain things that other Catholic families might. I feel like I wasn’t forced to believe any specific thing.” She sees this freedom as contributing to her growing interest as an adolescent in the Catholic faith.

On the other hand, Nathan had the opposite experience. He describes conversations with his parents saying, “When I talk to my dad about religion he gets furious. It’s an open-minded thing. When you talk to open-minded people you tend to keep an open mind.” Nathan desired to have parents who would listen and support his own independent exploration of his religious identity. He felt like his parents’ unwillingness to hear his own ideas contributed to his shift away from the Catholic faith. Parents can be powerful figures in adolescents’ lives and their response to young people’s growing desire for independence can be critical in shaping youth’s identity development especially in the context of religion.

Education

Learning

For all students, learning in all contexts was critical to their religious development. For Ceedee, schools were a critical component of this. During her earliest years of elementary school, she spent her weekdays at a Christian private school and her weekends taking classes at a Buddhist temple. Despite the confusion about her religious identity this created in her youth, she says that her experience with both means that “I can see the differences and why this is better for me.”

Learning about different religions was important for Melody and Sahana who credit the US History class and its teacher as helping them understand how they wanted to interpret their religious doctrines. Sadie says that learning about other religions through interactions with different people was helpful to her commitment to Catholicism owing to the fact that “When I have exposure to everything I have a reason to keep being who I am.” All students took steps to critically interpret the religious messages around them to make an informed decision about their religious identities, which seemed to shift over their lifetime.

Teaching

Teaching others helped participants reaffirm their own identities. Most taught younger students at their religious institution. Ceedee and John both participated in youth groups where they took on leadership roles. Others, like Melody, felt that they had a duty to inform the others around them about their religious identities, especially if they were from a religious minority. Melody used social media to create awareness of her Baha’i Faith. Nathan sought to create religious awareness among peers. He used Instagram to create a podcast in order to create an open dialogue about religion with his friends. Social media for both Melody and Nathan, was a key platform used to inform users of different religious identities about their own beliefs. They felt that these differences made it all the more important that they educate others about their beliefs.

Morality

Moral Code

Especially important for participants was the adoption and application of a moral code embedded and taught by the religions with which they identified. John saw others who acted with what he viewed as Christian morals, and this made him more interested in connecting to his Baptist identity. Soon he became concerned about also demonstrating these morals. On the other hand, Sahana left her religious identity due to its moral code. In middle school, she felt she could not reconcile her sexual identity with her religious identity as a Hindu. When she entered high school, she associated with a new friendship group which is why she said, "I realized that I could be a good religious person and not be the exact definition of what some super old scriptures say you should be" and this helped her reconnect with her Hindu identity. This reinterpretation of one's religious doctrine was very common for the participants. When one aspect of their religion did not work well with another aspect of their identity, they reinterpreted their religion and critically evaluated its doctrine. This was done in a way different or even contrary to their parents' interpretation in order to personalize their religion and imbue a religious doctrine with their own personal perspective.

Politics

Politics was also very relevant to most of these adolescents. And, politics influenced their stance on religion. For Sadie, she often felt attacked for her identity as a liberal Catholic. She felt like many people saw the two identities as mutually exclusive. Sadie says "with anything you're defending you should think about why you're defending it." The more she was forced to justify her religious identity to others the more she was able to reinterpret Catholicism to make it fit for her. Melody was also very concerned about politics. After asking questions about her religious doctrine, and realizing it matched many of her progressive stances on social issues, she says, "I was just really proud of my faith in that moment. Like they aren't going to allow something like that and that just made me really happy." Participants

demonstrate that their religious identity did not exist in a vacuum. As they develop religious, moral, and political identities concurrently, they modify all identities in order to fit together with each aspect of their personalities.

Discussion

The central question asked by this study was how do adolescents ages 16-18 view their own religious development in a time when they have gained greater autonomy from their parents, as well as addressed those factors they might see as most important in forming their own religious identities. The coding of various adolescents' descriptions of their religious developmental process reveals young people are very much active agents in determining their own identities. The participants were aware of their parents' influence on their choice, yet they are also conscious of how a variety of other factors shape their choice. This matches Pearce and Denton's (2011) findings that parents can function as "social scaffolding" to influence their children's religious beliefs and practices but do not have the power to completely dictate a young person's religious identity. (p. 9). For most of these students, their religious identity is one that they create for themselves, an identity that may be consistent with or inconsistent with their parents' commitments. The participants reinterpret their religious identities through their experiences with role models or their own reflections on morality and the other identities they hold. A combination of these institutions, people, and their other experiences have led them to integrate their religious doctrine in a way that is unique from their parents or other religious role models.

The data indicate that the participants likely begin to apply their religious knowledge to their everyday life, much like Brown's (1964) description of an intrinsic religious person: "He has made his Church's beliefs his own (although he does not necessarily hold them all strongly), and he carries them into his everyday dealings with others" (p. 94). This kind of description is parallel to the way John emphasized "acting with Christian values" or the way Sahana chose to volunteer in her community in order to satisfy Hinduism's doctrine of Karma. All students emphasized

the relationship between their religious identities and the doctrines they embody and the morality of their actions in a wider world. Ultimately, the process can make them more autonomous in the formation of their religious identities. The data suggest that the participants were functioning as intrinsic religious thinkers.

How has autonomy affected religious development?

For adolescents, a religious summer camp was one of the most obvious examples of the way that the autonomy materialized in their life to give them an experience that helped solidify their religious identity. Desmond et al. (2010) clearly note “parents and peers provide models for adolescents to observe and imitate and positive and negative reinforcement (rewards and punishments) for religious behaviours” (p. 248). This is true for many students until an event, such as summer camp, helps them partially break away from the influence of their parents and peers; those who had previously either closely regulated their religious behaviour or had not participated in religious socialization. The freedom of summer camp allowed them greater agency in interpreting or reinterpreting their religious doctrine in a way that made sense to them, not just to their parents.

Autonomy also surfaces as a critical component of the development of religious identity as youth become leaders and educators. Many students talked about taking on leadership roles within their youth group which matches Ji and Tameifuna’s (2011) research which states that students who are given the chance to have leadership roles will have a higher level of commitment to their religious institution as they will feel more cognitively engaged (p. 309). This is most certainly true of students participating in the study. Ceedee, who taught younger girls at her youth group, felt more engaged with her religion in a leadership role. Whether teaching younger students at their religious institution, their friends of different religions, or other peers, they felt more connected and more confident of their religious identity. Sharing this aspect of themselves with others seemed to solidify the commitment they had made to their religious paths.

What factors have the greatest influence on religious identity?

For most students, understanding their ascribed religious identity on a deeper level was influential to them claiming an identity for themselves. Trusted people, like parents, mentors, and teachers who were willing to logically explain the more abstract aspects of religion became important sources of influence in these young people’s religious development. Even media, such as a textbook or book explaining a religious doctrine, was helpful to these youths as they sought to understand what their religious identity was in the context of the wider world. This trend is supported by Armet’s (2009) observation that “exploration is an important dynamic in the process of forming an intrinsic commitment to an ideology” (p. 281). Once students learned about their religious identity, they felt more empowered to apply it to their everyday life.

This was especially true for students that practiced a minority religion. For many of these students, peer support was lacking as they began to explore their religious identity. This is unsurprising as Bowman and Smith (2010) clearly state that “students from these minority groups are often faced with a lack of support and/ or understanding of their religion” (p. 598). Even so, for some students, teaching those around them about their identity or at times defending their religious choices became easier once they were fully educated about the religious identity they were practicing. Therefore mentorship and education serve as important sources of influence in these young people’s lives.

What has contributed to students’ shifts in religious identity?

High school is a time of increased autonomy but also a time when social pressures are magnified. Youth are influenced by peers especially since peers can often give students experiences with new perspectives and religious identities that can be influential in solidifying their own. Petts (2009) explains that exposure to new religious beliefs can often prompt students to shift their own religious identities to make it better fit their own (p. 556). Even if exposure to peers did not prompt a conversion in religious identity, for many students their interactions with friends

of different religions helped them reinterpret their religious doctrine to make it better suit themselves. This is a phenomenon noted by Shalev, Baum, and Itzhaky (2016) whose study reveals that people can be influenced to make changes in their religious lives and identities through their interactions with people with whom they sustain close relationships (p. 135). Ultimately, as students progress through adolescence, they will begin to develop relationships with people outside of the social circles of their parents. This will prompt exposure to new ideas that can result in shifts in their religious identity. This clearly shows that it is imperative that young people are given safe social environments and positive mentors who help them explore the opportunities they have available to them. This allows adolescents to solidify their identity and develop into well adjusted young adults.

Limitations

Although this study reveals critical knowledge about the way adolescents process the world around them to solidify their religious identities, it is not without flaw. Like any study that uses a qualitative methodology, the information in this study cannot be generalized to a larger population. Compensating for this is that qualitative inquiry can give a more nuanced explanation of a phenomenon. The phenomenological aspect of this study also gives more agency to the participant to tell their own story. The other major flaw in this study is location based. The school in which the study was performed, is located in a college town in the Bible Belt. This location is known to be more liberal relative to other cities in the state. The city itself is also known to have two high schools largely divided by class.

This study was performed at the more affluent and less diverse high school which could contribute to some of the trends seen in the study as most students never had to contend with economic instability. Many of the students interviewed who came from minority religious backgrounds go to school with predominantly Christian peers. This might affect their development of religious identities because of the possible alienation they felt in the school and community in which they were living.

Conclusion

Ultimately, this research shows that high school is a dynamic time for youth. Family changes, peers, a growing sense of autonomy, and leadership contribute to shifts in adolescents' religious identities. This directly contradicts Lopez et al.'s (2011) statement that the "stability of adolescents' social environment across high school results in few challenges to their existing religious identities, with little need to renegotiate the extent of one's identification" (p. 1305).

As students progress through high school they are likely to reevaluate how they relate to their ascribed religious identity or even shift to new ones. Recognizing that young adults are influenced by a myriad of factors around them is critical. Future studies can recognize the agency that younger adolescents have. Students are likely free thinkers who are processing the messages they are receiving around them to make coherent choices about their identities. Future research can look into the way a secular education can impact students' formation of their religious identities. The surprising impact of US History on the religious identities of several young people in this study shows that a secular education can introduce new ideas into a students' consciousness that changes their relationship to their identities. Ultimately, this research reveals that adolescents can display advanced forms of critical thinking about their religious identities that show they have much to add to the field and should not be disregarded as passive or minor actors who simply follow the beliefs their parents ascribe.

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Appendix A

Is there a pseudonym you would like to choose for this research paper to protect your identity?

Warm Up Question: Can you briefly tell me a little bit about your family's religious identity and practices?

What religious identity did your parents instruct you in and how do you identify currently?

If different: Tell me about what prompted this change and when?

How do you feel your current religious identity differs or is constant with the way you thought about religion 10 years ago?

Do you remember the first time you ever thought about your religious identity?

Can you explain what it was like to think about your religious identity for the first time?

Has there ever been a time you doubted your religious identity?

Yes: What factors led to a turning point in your religious identity and contributed

to your doubt? How did you respond to this doubt?

b. No: What factors made you feel more secure in your religious identity?

Can you identify important individuals (peers, mentors, family, religious leaders) that you think have had the most influence on your religious belief?

How do you feel they have influenced your view of religion?

Do you feel as though you've influenced your peers religious development?

Do you express your religious identity online or have friends who express their identity online?

Do you change the way that you express your religiosity in front of their friends.

Do you think that your racial or ethnic identity influences your religious identity?

Do you feel as though the ways that you practice your religion have changed over the last few years?

In the coming years, do you expect your religious identity to shift?

Is there anything else you would like to share with me about your religious development today?

Flight to the Stone Age: Investigating the Impact of FAA Cockpit Regulations on Kidney Stones in American Airline Pilots

Noor Said

Following the incidents of 9/11, the FAA implemented safety regulations that restricted pilots from exiting the cockpit. While pilots and other individuals from the commercial aviation industry speculated that these regulations prevented pilots from using the lavatory, thereby exacerbating dehydration and kidney stone prevalence, the issue remains relatively unexplored. To address this gap, a survey involving 280 American cargo and passenger airline pilots was conducted. Based on the results, passenger pilots intentionally reduced fluid intake in-flight due to FAA protocol, and the rate of nephrolithiasis amongst passenger pilots was higher than that of cargo pilots, who do not have a cockpit door and therefore were representative of a control group. From these findings, it is recommended that further investigation is conducted on this issue and that current FAA protocol be remedied to protect the safety of pilots and passengers.

Keywords: nephrolithiasis, FAA, aerospace medicine, airline pilots, dehydration

Introduction

The most exigent health issues impacting developed countries today are not the plagues which dominate discussions; rather, this age faces the onslaught of chronic disease and a rise in preventable health conditions. Amongst these overlooked issues lies the rise in kidney stones, deposits of minerals and acid salts which crystallize in the kidney and fall into the ureter (Ford-Martin & Cataldo, 2015). In recent years, the United States has faced a steady increase in the prevalence of kidney stones, and a National Health and Nutrition Examination Survey (NHANES) (2012) estimates that the condition will affect approximately one in ten Americans during their lifetime (Scales, et al., 2012). If untreated, kidney stones cause severe physical discomfort and hinder daily activities. It has been confirmed that nephrolithiasis, or kidney stone formation, is influenced by several modifiable lifestyle factors, which may stem from occupation.

Within the realm of exposomics, defined as the cumulative exposures to health issues a person has during their lifetime, individuals are more susceptible

to nephrolithiasis as a result of their work and the behaviour that it requires. According to Dr. Goldfarb (2016), a nephrologist (kidney specialist) at New York University who investigated 2012 NHANES data, workers in the American transportation sector face high exposure to kidney stones, with an average rate of nephrolithiasis that is significantly higher than the general population (Goldfarb, 2016). While several factors render these individuals more susceptible to the condition, the proportion of those afflicted with nephrolithiasis in one particular group has seen a troubling increase within the past two decades: commercial airline pilots.

Defined as aviators who operate aircraft carrying passengers or cargo, commercial pilots are a particularly crucial group to monitor because they may experience renal colic, the sudden onset of pain from nephrolithiasis, while operating a plane. Renal colic's symptoms include nausea, vomiting, a radiating sense of pain, and fainting. Therefore, pilots may become incapacitated, posing danger not only to themselves but also to passengers. A unique and unexplored reason for the suggested high kidney stone rate in pilots may

lie in federal policy. Following the attacks of September 11th, 2001, new safety regulations were implemented by the Federal Aviation Administration (FAA). As a result of this intricate set of rules, a pilot may not exit the cockpit to use the restroom without calling in flight attendants. As described in an FAA handbook (2004), attendants open and lock the cockpit (while one attendant remains inside with the co-pilot), block the aisle as the pilot uses the lavatory, and escort the pilot back into the cockpit (“Crew Resource Management Training,” 2004). Those in the aviation industry have complained about the process and speculated that it may contribute to nephrolithiasis in pilots by preventing them from using the lavatory, though little research has evaluated the validity of such a claim. In a 2015 interview, president of the Aviation Medicine Advisory Service Dr. Snyder disclosed that “more pilots have been calling... about kidney stones in recent years than in the period before September 2001” (Werfelman, 2014). Additionally, Silberman (2016), a former FAA medical certification manager, notes that those who fail to eliminate all traces of kidney stones or have recurrent nephrolithiasis may not be certified for aviation (Silberman, 2016). Therefore, while the issue is overlooked, the consequences of a pilot having kidney stones are rather significant and complex.

In the attempt to explore the cause of nephrolithiasis in American airline pilots, this paper investigates the impact of the aforementioned FAA policies, guided by the question: to what extent has the implementation of post-9/11 cockpit regulations by the FAA influenced kidney stone prevalence in American airline pilots? Extensive research was conducted to arrive at the justification of this question, and in order to fully understand its significance, it is necessary to examine the interconnected perspectives which revolve around it.

Literature Review

The issue of nephrolithiasis in airline pilots was derived from a pre-existing body of literature discussing the rise in nephrolithiasis, FAA policies, and pilot health. This literature review provides a comprehensive understanding of the trends and modifiable risk factors for nephrolithiasis and a range of perspectives on the rise of kidney stones in pilots. In this review, it is important to understand some background circum-

stances. Firstly, it should be established that pilots are put through rigorous testing to receive a medical certification and operate a commercial plane. Additionally, while factors such as heredity, global warming, gender, and location influence nephrolithiasis, as demonstrated by researchers who analyzed 2012 NHANES data at the Cleveland Clinic’s Glickman Urological & Kidney Institution, this paper will only investigate primary modifiable risk factors (Roudakova & Monga, 2014). With regard to gender, only six percent of active commercial pilots are female according to a recent FAA study, although the following studies did not separate pilots by gender (FAA, 2018). Following the notion that overall kidney stone prevalence has been increasing, it is first necessary to understand these factors and how they differ in American pilots.

Obesity

Body mass and dehydration have dominated the discussion surrounding the increase in nephrolithiasis, and there exists a considerable body of evidence showing that pilots have a greater susceptibility to these risk factors, such as high Body Mass Index (BMI). High BMI is associated with intensified excretion of uric acid and oxalate in the urine, which causes nephrolithiasis. Harvard Medical School professors Ferraro, Taylor, and Curhan (2015) worked in collaboration with nephrologists Sorenson and Gambaro in a risk-factor study. After analyzing National Health Surveys I and II and categorizing data by factors including BMI, they concluded that BMI had a strong direct correlation with nephrolithiasis (Ferraro et al., 2015). Supported by extensive evidence, these findings are particularly unsettling when the population is narrowed to U.S. pilots, who have been suggested to have unusually high BMIs.

Bryman and Mills (2007), doctors of Aerospace Medicine, searched FAA medical databases to establish a pilot obesity rate. After analyzing the files of 630,670 pilots, they found that nearly 70% of American pilots were overweight or obese, a rate considerably higher than that of the general population at the time of publishing, which was a possible result of prolonged sitting (Bryman & Mills, 2007). While the study is outdated, it involved a large sample size and was the first to determine a BMI distribution for U.S. pilots. Though American pilots appear to have higher

BMI than non-pilots, this does not hold true globally.

The findings of cardiologists Syburra et al. (2017), who wrote and published an article in the peer-reviewed *European Journal of Cardio-Thoracic Surgery* on European pilot obesity in collaboration with Swiss and British federal aviation branches, differ from those of Bryman and Mills (2007). From searching the MEDLINE database for cardiovascular disease, the researchers found that the average pilot BMI was equal to or lower than that of other Europeans (Syburra et al., 2017). This implies that flying an airplane alone is not associated with high BMI, contrary to the suggestion made by Bryman and Mills (2007). While the suspected American pilot BMI may be a reason for pilot nephrolithiasis, water intake also plays a critical role.

Dehydration

With consistent dehydration, uric acid dilution is inhibited, raising acidity levels in the kidney (Ford-Martin & Cataldo, 2015). This acidic environment is the primary cause of nephrolithiasis. According to an article from *The Gale Encyclopedia of Medicine*, the primary method of kidney stone prevention recommended by health organizations such as the National Kidney Foundation was to simply drink over eight cups of water daily (Ford-Martin & Cataldo, 2015). Yet, the aforementioned article by Goldfarb (2016) found that transportation workers were most likely to develop kidney stones from dehydration (Goldfarb, 2016). Goldfarb (2016), who cited a large, representative survey of Americans (NHANES), has practiced nephrology for decades, and while this may contribute to personal bias, the source offered relevant information on nephrolithic exposomics. Goldfarb's (2016) findings are applicable to the general transportation sector; however, several recent sources note that American pilots have avoided water in-flight.

According to an article by Werfelman (2014), senior editor for the Flight Safety Foundation, American pilots are severely dehydrated and must drink more water. After interviewing Dr. Snyder and evaluating data previously acquired by researchers from the *Aviation Medicine* journal, Werfelman noted that nearly two-thirds of pilots avoided drinking liquids while flying, contributing to fatigue and chronic health issues (Werfelman, 2014). While Werfelman (2014) was heavily reliant on the interview of Snyder

in her article, she included survey data showing that pilots consumed less water than recommended, and disclosed that more research should be done on the subject. Werfelman's perspective gave this research direction, as it was possible that pilots avoided liquids due to cockpit regulations. Despite the idea that pilots are more susceptible to these risk factors, there is inconsistency in establishing a rate for pilot nephrolithiasis.

Rate of Pilot Nephrolithiasis

Due to these factors and their apparent exacerbation the aviation medical community believes that American pilots are highly susceptible to nephrolithiasis. However, there are discrepancies in establishing a stone rate. In Werfelman's (2014) article, data included from the aforementioned NHANES shows that pilots were 6.5 percent more likely to be affected by kidney stones than the general population, which is significant given the non-pilot rate of nine percent; in his interview, Dr. Snyder claims that this rate has been growing in the past decade (Werfelman, 2014). However, a source from the peer-reviewed *Journal of Urology* refutes this claim. In response to airline companies claiming that pilots have had more kidney stones as a result of strict cockpit rules, the FAA funded researchers to investigate the issue. CAMI, the Civil Aerospace Medical Institute, is the research branch for the FAA. Using its data, researchers Hyams, Nelms, and Silberman (2014) found that pilots had a rate of nephrolithiasis which was nearly equivalent to that of other Americans. Furthermore, they claim that the rate has not been rising, refuting airline company complaints (Hyams, Nelms, & Silberman, 2014). However, suspension may arise as a result of nephrolithiasis detection, and this data excluded inactive pilots. In a 2016 article, Silberman, former FAA medical certification manager, disclosed that "many pilots with kidney stones were unable to receive recertification" in past years, meaning FAA data may have been biased in favour of healthier pilots (Silberman, 2016). Therefore, there are discrepancies in the establishment of pilot nephrolithiasis rates, and no research has been published showing changes in this rate over time, further complicating the current status of the issue.

Hypothesis

The holistic review of this literature determines that, while pilots are susceptible to the same risk factors as non-pilots, these factors are likely more prevalent in pilots. Still, the true rate of pilot nephrolithiasis is unclear, and a pilot-specific cause influencing nephrolithiasis and risk factors has yet to be validated. Though pilot nephrolithiasis is a significant issue, current literature on the subject is sparse. Dehydration has not been attributed to a root cause, and only one outdated American pilot BMI analysis was found, meaning these factors should be accurately evaluated. Furthermore, nephrolithiasis and its risk factors have not yet been analyzed to address how different genres of commercial pilots are influenced. Cargo pilots, who lack a cockpit door and may therefore use the lavatory freely, may be influenced differently than passenger pilots, who have a cockpit door and must accordingly adhere to the aforementioned FAA protocol. These issues, combined with anecdotal evidence and complaints from the aviation industry connecting cockpit regulations to nephrolithiasis, render further investigation necessary.

Given that these risk factors are modifiable, identifying a pilot-specific cause may bring forth a viable solution. This research aimed to identify the impact of post-9/11 FAA cockpit protocol on nephrolithiasis in American pilots, by establishing a BMI distribution and addressing how the cockpit rules have influenced dehydration. Furthermore, this study aimed to establish the frequency of nephrolithiasis for American commercial pilots, grouped by their assignment and resulting adherence to the cockpit protocol. Based on existing literature, this research investigates the hypothesis that pilots are more overweight and obese than the general population, and that passenger pilots consume less water than recommended as a result of the FAA rules. Most importantly, passenger pilots were hypothesized to have a higher susceptibility to kidney stones than cargo pilots, as a result of cockpit regulations altering pilot behaviour and exacerbating pre-existing risk factors for the condition.

Method

Study Design

For the purpose of addressing the influence of FAA regulations on pilots, it was necessary to obtain a sample of commercial pilots employed by the United States. There was no control over variables and subjects reported all information, meaning that this study was descriptive in nature (Kobayashi, n.d.). Because this research ultimately explored a relationship dependent on the individual habits of its population of interest, the study included both qualitative and quantitative elements. Experimentation to identify true causation was neither ethical nor plausible, and it was impossible to control nephrolithiasis and pilot assignment. Therefore, a survey was conducted to identify nephrolithic prevalence and address all aspects of the research question.

To establish large, representative samples that could be used for hypothesis testing, probability sampling was used: random selection occurred after collecting all pilot surveys and separating cargo pilots from passenger pilots. The modifiable risk factors addressed in the survey were water intake (additionally measured through lavatory usage), and BMI. In addition to BMI, the survey identified some external factors which could be influencing nephrolithiasis, such as family history of kidney stones, gender, and age. The survey addressed the overall nephrolithiasis rate in American pilots, where cargo pilots were compared to passenger pilots to identify whether significant differences could be found based on FAA rules.

Ethical Considerations

Given the need for many pilots to provide information on a potentially sensitive topic, all measures were taken to keep the research ethical. Pilots could feel uncomfortable answering questions regarding their lavatory usage and kidney stone affliction. To address these components as noninvasively as possible, only a yes/no response to kidney stone history was recorded, no surgical procedures or complications were addressed, and a minimal number of questions addressed lavatory usage. Prior to the administration of the online surveys, all research materials, including

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the survey and an email for participants detailing the purpose of this research, were submitted to the student's high school Institutional Review Board (IRB) for a full review. With the addition of background to the email message, along with minor modifications to the wording of survey questions, the project was approved by the IRB.

The final approved survey (see Appendix A) was anonymous and featured the least invasive questions which aligned with the research question. To preserve respondent confidentiality, the survey was stored on a password-protected account belonging to the researcher. Following the conclusion of the research project, the survey was discarded digitally by erasing the content files on the researcher's computer, ensuring that respondent records were absolutely confidential.

All participants were given contact information for the researcher and advisor, informed of the background and nature of the research project, and allotted a space in the survey for additional questions or concerns. Moreover, subjects were informed that the survey was voluntary and anonymous, no questions were required, the survey could be withdrawn at any time without repercussions, and the electronic records would be safely stored and destroyed at the conclusion of the project by the researcher.

Participants

In accordance with the population of interest, American commercial pilots, the primary requirement for the survey respondents was that they were commercial airline pilots employed by the United States. Retired pilots were removed to avoid potential confounding variables such as old age, which influences likelihood of illness. The study frame included all cargo or passenger pilots over eighteen years of age, with the goal of establishing samples representative of the current pilot population.

These subjects comprised two simple random samples (SRS) of cargo and passenger pilots who provided basic information, which addressed the key components of the study. Because subjects would not be met in-person, it was necessary to verify that only American commercial pilots would complete the survey. For greater variation amongst participants, the survey was both emailed and shared with pilot forum users. Prior to survey administration, a minimum sample size

of 100 pilots was established to yield more accurate results. Emails were acquired from a passenger pilot information list: the Delta, American, and United rosters (which included pilot names, flight hours, and activity status) were searched and compiled into an email list of those who would receive the survey. In addition, an FAA database naming all pilots with certifications was used to add individuals to the email list, for a total of fifty pilot emails. Aside from receiving it by email, pilots could access the survey through the AOPA Hangar, ProPilotWorld.com, and Airline Pilot Central forums. The split-distribution method allowed pilots from numerous locations and airlines to participate, contributing to a representative sample.

Procedure

The independent variable examined was the adherence to the FAA cockpit rules. Commercial cargo pilots, who did not have a cockpit door and could use the restroom freely, were the control group representing all pilots if the post-9/11 FAA rules were not in place. The primary dependent variable was the presence of kidney stones; however, due to the complexity of nephrolithiasis and its reliance on several risk factors, fluid intake and BMI were also addressed. Given its observational nature, there was no control over the other existing variables in my survey. In addition, the year of kidney stone diagnosis, airline, age, sex, and retirement status were recorded to verify subject inclusion. While questions based on pilot judgment regarding the effect of FAA cockpit rules on their behaviour employed a Likert scale, others, such as flight hours, were multiple choice or free response. The primary areas of interest were:

- Airline, to identify cargo pilots from passenger pilots
- Presence of nephrolithiasis and year of diagnosis
- BMI
- Influence of FAA regulations on lavatory use and water consumption

For comparison and hypothesis testing, subjects were categorized into one of two samples based on whether they flew cargo or passengers. After the online *Google Forms* survey was drafted and approved by the IRB, proper contact information and permissions were obtained. Prior to posting the surveys on the aforementioned forums, consent was obtained from

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the AOPA (Aircraft Owners & Pilots Association) Director of Medical Certification and forum administrators. Titled “Research Survey Opportunity for U.S. Pilots,” the survey was posted with a description of the research project. The ProPilotWorld.com survey was posted once by the administrator, while the other sites permitted me to post the survey several times. Because the Airline Pilot Central site was divided into sub-forums, I posted in the popular, yet relevant, “Pilot Health” and “Major Airlines” categories, ensuring that many pilots could access the survey.

To avoid bias towards forum users, fifty Delta, American Airlines, and United Airlines passenger pilots were emailed the survey with a detailed message on the nature of this research. Emails were acquired from the aforementioned FAA “Pilots with Certifications” database and airline rosters. If the email was not immediately located, pilot names and airlines were input through Skrapp.io, a contact finder program for those with business emails. Absolutely no subjects were coerced into participation, and all premises for the research and confidentiality were disclosed.

Once two weeks had passed from the start date of survey collection, the survey link was deactivated for analysis. Cargo pilots were separated from passenger pilots, and a total of ten military pilots, non-American pilots, or individuals who failed to verify their current occupation as a pilot were excluded from the sample. Pilots were not excluded for non-response to certain questions, including height, weight, and geographical residence, as their opinion on FAA protocol remained valuable. After separation, the resulting 146 passenger pilot surveys were assigned numbers 1-146 and the 134 cargo pilots were numbered 1-134. One-hundred pilots of each group were selected for analysis using the random number generator on a TI-Nspire calculator.

A 2-proportion z-test was performed using the random samples to determine whether the passenger pilot population had greater frequencies of nephrolithiasis, and pilot stone diagnoses were graphed by year to observe potential trends. In addition to kidney stone rate differences, risk factors were addressed for each group. The BMIs and opinions on FAA policy influence were analyzed from each sample of active pilots. While BMI, a confounding variable that could be causing high pilot nephrolithiasis, was used for comparison to the general population and to ensure similarity amongst the samples, questions on hydra-

tion and lavatory habits provided insight on the extent of FAA policy influence. Average water intake and the differences in water consumption in and out of work were calculated for each group. To determine whether pilot dehydration was influenced by the policies, responses to the opinion statements were graphed and compared. These measures allowed for a comprehensive analysis of the influence of the cockpit protocol on pilot nephrolithiasis.

Results

The collection of 280 cargo and passenger pilot surveys resolved several crucial questions about the impact of FAA regulations on pilot nephrolithiasis. To properly interpret the findings of this study and establish a focus on the FAA regulations’ influence on nephrolithiasis in pilots, it is first necessary to establish the differences identified between the two sample groups. As mentioned previously, both cargo pilots and passenger pilots were sampled, and the final randomized groups which were analyzed consisted of 100 individuals each. The following table displays basic background information for each group:

	PASSENGER PILOTS	CARGO PILOTS
Mean Age	40.1	45.1
Mean # of Years Spent as Pilot	13.9	21.0
Mean Flight Hours Accumulated	7,533	10,088
Gender Distribution	6% female 94% male	1% female 99% male

Table 1
Comparison of Cargo and Passenger Pilot Samples

Cargo pilots had a higher mean age and number of piloting years. Accordingly, the cargo flight hour mean was 2,555 greater than that of passenger pilots.

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Although both groups were disproportionately male, a considerable difference was that the passenger pilot sample consisted of five more females.

The primary objective of this project was to identify the true proportion of pilots who have had kidney stones, so this frequency was established for each sample group. Of the 200 individuals in the samples, a total of nine cargo and eighteen passenger pilots reported having had kidney stones, a proportion that appears to be increasing for passenger pilots when graphed:

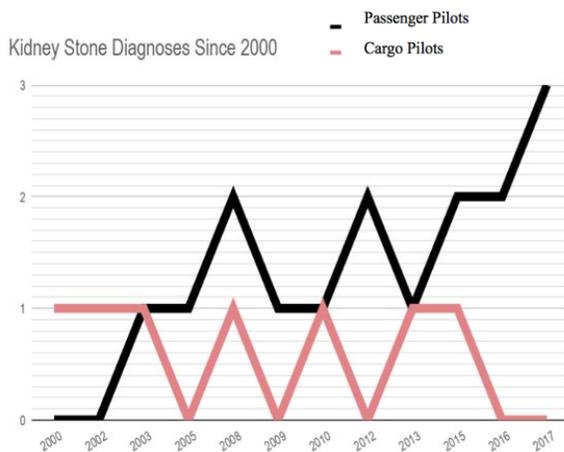


Figure 1. Kidney Stone Count by Year (cases before 2000 not depicted on graph)

A 2-proportion z-test for $P_{\text{passengerpilot}} - P_{\text{cargopilot}}$, where P represented the true proportion of pilots with kidney stones, was performed to determine whether pas-

senger pilots had a higher nephrolithic rate, which would imply that the adherence to FAA policies was correlated with exacerbated kidney stone development. Based on the test (see Appendix B), conducted at the five percent significance level, passenger pilots experienced a greater rate of kidney stones, with a P-value of .031 being lower than the alpha of .05.

While the incidence of nephrolithiasis appeared rather different amongst the two groups, it was also necessary to evaluate pilot opinions, as pilots are ultimately responsible for determining how FAA policies impact their in-flight habits. Fluid intake and bathroom usage were addressed to determine both current and prospective risk for nephrolithiasis. Based on survey responses, 85% of passenger pilots reported that FAA rules deterred them from using the bathroom, making them more susceptible to nephrolithiasis:

In conjunction with avoiding the lavatory more often, passenger pilots were more likely to report that they intentionally decreased their fluid intake while flying a plane: these subjects reported drinking an average of 3.7 cups on piloting days, compared to 6.3 cups outside of work. In contrast, cargo pilots slightly increased fluid intake while flying, drinking 5.1 cups on workdays and 5.0 cups on non-workdays. These factors, combined with the large gap in kidney stone prevalence between the samples, suggest that FAA policies have made passenger pilots more susceptible to nephrolithiasis.

As mentioned previously, one of the additional goals of this survey was to identify a BMI distribution for

PASSENGER PILOTS: "The pilot cockpit protocols enforced by the FAA keep airline pilots from using the bathroom as often as they would like."

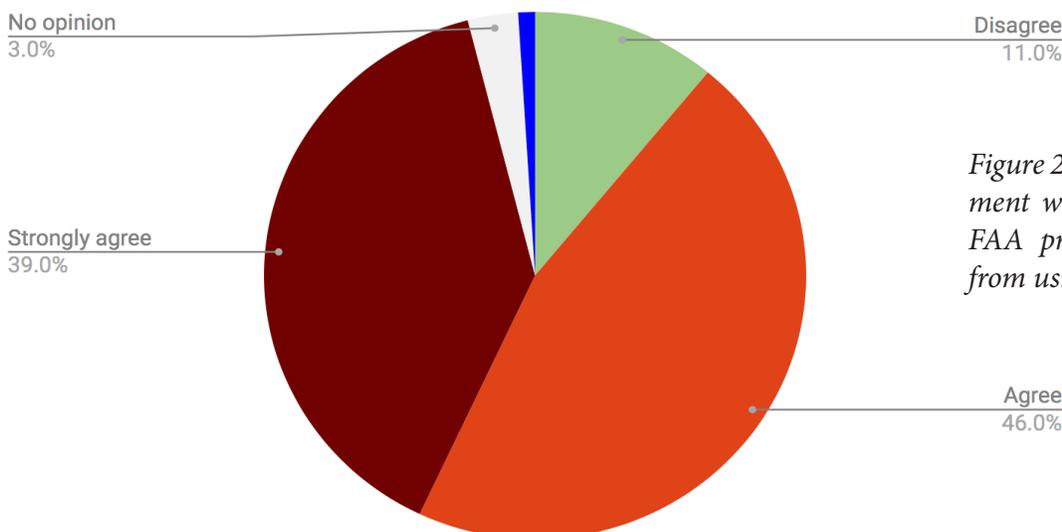


Figure 2. Passenger pilot agreement with the statement that FAA protocol prevents pilots from using the lavatory.

pilots, since high BMI was identified as a risk factor unrelated to FAA policies that could be exacerbating nephrolithiasis. Here, the samples were only somewhat similar, as 66% of passenger pilots and 78% of cargo pilots were overweight or obese.

Discussion

Analysis of Results

Based on extensive investigation, it was found that the post-9/11 cockpit protocol enforced by the FAA has exacerbated in-flight dehydration in passenger pilots, and is, therefore, a likely promoter of pilot nephrolithiasis. The results of this study addressed the rate of kidney stone development as well as the influence of modifiable risk factors for pilots. According to the aforementioned hypothesis test conducted, there was statistically significant evidence to suggest that passenger pilots have a higher rate of nephrolithiasis than cargo pilots. Considering the idea that the FAA cockpit protocol is the only systematic difference between the two populations, this supports the primary hypothesis that FAA rules cause higher stone rates. While overall nephrolithic prevalence was greater amongst passenger pilots, the samples were not large enough to truly support hypothesized time trends for stone development, though this would have been ideal. Alongside the finding a rate of kidney stones, the understanding of BMI and dehydration in pilots was also refined.

Contrary to the findings of Bryman and Mills (2007), both pilot samples displayed rather normal BMI distributions, disagreeing with the hypothesis that BMI would be higher amongst pilots than non-pilots. Surprisingly, the cargo pilot group, which reported a slightly higher rate of obesity than passenger pilots, experienced a lower incidence of nephrolithiasis, though this difference may have been due to small sample size. While this shows that high BMI was generally common, neither group was unusual in comparison to non-pilots. According to a report by the Center for Disease Control (2017), 70.7% of American adults are overweight or obese, implying that commercial pilots do not have substantially greater risk for kidney stones as a result of high BMI than does the general population (“Obesity and Over-

weight,” 2017). Therefore, other modifiable factors have been aggravating pilot nephrolithiasis, of which the most notable appears to be dehydration.

The results of this survey suggest that the majority of passenger pilots avoided water as a direct result of the FAA cockpit regulations. In conjunction with the findings of Werfelman (2014), both samples consumed less than the recommended eight cups of water daily, implying that dehydration is an issue for all pilots regardless of FAA rules. More significant to this research, this survey showed that passenger pilots deliberately reduced water consumption while working, a habit identified by Goldfarb (2016) as the primary mechanism for nephrolithiasis in transportation workers due to uric acid accumulation (Goldfarb, 2016). Furthermore, 85% of passenger pilots agreed that FAA cockpit rules deter pilots from going to the lavatory, verifying that FAA cockpit protocol is a hindrance for passenger pilots who wish to use the bathroom. These pilots have accordingly reduced water intake, making them more susceptible to nephrolithiasis.

Limitations

Several limitations to this research should be considered, with the most prominent being the potential for voluntary response bias: pilots who have had nephrolithiasis may have been more interested in the research and participated more frequently than others, implying that the samples may not have been fully representative. Furthermore, most participants were forum users: pilots who were not active on forums during the two weeks of survey collection lacked representation. Due to potential bias and the survey’s reliance on participant honesty, further studies should be done to verify results.

In addition to bias, there exists the issue of underlying risk factors which were not controlled for: diet and other health issues may have been different between the two samples due to sampling variability. Such factors were not examined due to limited time and resources, meaning the analysis of risk factors was incomplete. Finally, due to small sample size, the results may contain inaccuracies, and could not be used to identify time-trends in nephrolithiasis. Nonetheless, this project gives direction to the issue of the FAA regulations’ influence on pilot nephrolithiasis.

Conclusion and Future Directions

The findings of this survey provide several interesting implications for the aviation industry. Most importantly, it is apparent that passenger pilots are likely more susceptible to nephrolithiasis as a result of FAA cockpit regulations, which have amplified the prevalence of a modifiable risk factor, dehydration, by causing pilots to reduce fluid intake to use the lavatory less frequently. Additionally, this study contradicts former hypotheses that American pilots are more overweight and obese than non-pilot Americans (Bryman & Mills, 2007).

From this study, there remain several questions regarding the future of American airline pilots, and additional research should be conducted to address the extent of FAA influence on nephrolithiasis. The FAA's CAMI research branch, given its resources and access to pilots, should most likely address this issue. A time-trends analysis should be conducted to estimate current and future nephrolithiasis rates, and this survey or a similar study should be repeated on a larger scale. Furthermore, if future research supports the findings of this survey, a solution to the issue of nephrolithiasis must be established, via an acceptable amendment to FAA protocol granting pilots unhindered access to the lavatory. If a high rate of nephrolithiasis persists, thousands of pilots will not only be physically harmed but also face losing certification. Additionally, undetected pilot kidney stones may result in the endangerment of hundreds of passengers. Further investigation into this issue is critical: without it, the health and safety of countless individuals may suffer, and the future of the aviation industry may be compromised as it ascends into this new stone age.

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FLIGHT TO THE STONE AGE: KIDNEY STONES IN AMERICAN AIRLINE PILOTS

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Appendix A

Airline Pilot Survey 2018

*All responses to this survey will be anonymously recorded and stored safely by the researcher. Your participation in this research study is voluntary. You may choose not to participate. If you decide to participate in this research survey, you may withdraw your survey at any time. If you decide not to participate in this study or if you withdraw from participating at any time, you will not be penalized. You are not obligated to answer every question in this survey. The data acquired from this survey will be used in a research paper and presentation regarding kidney stone prevalence in pilots and FAA cockpit rules, in which no survey participant names will be disclosed. Thank you for your time, and with any questions or request to cancel survey, please submit in the questions section of this survey or contact the author or research advisor at: *****@***** and *****@*****.*

Please provide age and gender:

AGE: _____

GENDER: _____

Please verify your current occupation:

Airline Pilot (passenger)

Airline Pilot (cargo)

Retired Airline Pilot

Other (please specify) _____

Under what Airline are/were you most recently employed? _____

Under which country are you currently employed?

Please select all that apply.

United States of America

Other (please specify) _____

How many years have you been a pilot? If retired, please include date of retirement: _____

Have you ever had a kidney stone?

Yes

No

Prefer not to answer

If your answer to the previous question was yes, in what year were you diagnosed? _____

Have you ever been treated for a kidney stone?

Yes

FLIGHT TO THE STONE AGE: KIDNEY STONES IN AMERICAN AIRLINE PILOTS

No
Prefer not to answer

Please select your current flight route type:

- Domestic
- International
- Both domestic and international

Approximately how many hours of flight have you accumulated? _____

How many cups of water (8 fluid ounces), on average, do you drink on a day where you are in flight? _____

How many cups of water (8 fluid ounces), on average, do you drink daily outside of work? _____

What is your approximate height ? _____
ft _____ in

What is your approximate weight? _____ lbs

Do you use the bathroom less frequently during your flight as a result of FAA cockpit rules?

- Yes
- No

To what extent do you agree or disagree with the statement: *"I avoid drinking beverages while flying a plane to avoid needing to go to the lavatory"*

- Strongly disagree
- Disagree
- Agree
- Strongly agree
- No opinion

To what extent do you agree or disagree with the statement: *"The pilot cockpit protocol enforced by the FAA keeps airline pilots from using the bathroom as often as they would like."*

- Strongly disagree
- Disagree
- Agree
- Strongly agree
- No opinion

Does your family have a history of kidney stones?

- Yes
- No
- Not sure

Which region of the United States do you currently live in?

- West
- Midwest
- South

- Northeast
- Alaska
- Hawaii
- Other (please specify) _____

If you have any questions for the researcher, please include them in the following response box, along with your e-mail:

All data is stored in a password protected electronic format. To help protect your confidentiality, the surveys will not contain information that will personally identify you. The results of this study will be used for scholarly purposes only. By clicking "Submit my responses," I understand that my survey answers may be used in a research paper and presentation. I verify that my answers are as I wish them to appear.

If you do not wish to participate in the research study, please decline participation by clicking on the "Cancel" button.

Appendix B

Inference procedure: 2-Prop z-Test for
 $P(\text{Passengerpilot}) - P(\text{Cargopilot})$

Let $P(\text{Passengerpilot})$ = the true proportion of
 American passenger pilots who have had kidney
 stones

Let $P(\text{Cargopilot})$ = the true proportion of Ameri-
 can cargo pilots who have had kidney stones

Null hypothesis: $P(\text{Passengerpilot}) = P(\text{Cargopilot})$

Alternate hypothesis:

$P(\text{Passengerpilot}) > P(\text{Cargopilot})$

Conditions:

Pilot surveys included in both samples are ran-
 domly selected

Each pilot being diagnosed with kidney stones is
 likely independent of other pilots both within and
 between the samples

The 100 cargo pilots and 100 passenger pilots in
 the samples include less than 10% of the total pilot
 population for their respective group

I expect both successes and failures to result in at
 least

$.14(100) = 14$ and $.14(100) = 14$ successes, and

$.86(100) = 86$ failures and $.86(100) = 86$ failures.

Both successes and failures of both groups are
 at least 10, so sample size is large enough for us to
 proceed with the procedure.

Mechanics: $z = 1.86$

$p^{\wedge}(\text{cargopilot}) = .09$ and $p^{\wedge}(\text{passengerpilot}) = .18$.

$p^{\wedge}(\text{pooled}) = .135$. P-value: .0321279.

Conclusion: I reject the null hypothesis that the
 true proportion of American passenger pilots who
 have had kidney stones is equal to the true proportion
 of American cargo pilots who have had kidney stones,
 with an alpha of $.05 > p$ -value of $.03$. There is statistical-
 ly significant evidence at the 5% level to support the
 hypothesis that the true proportion of American pas-
 senger with kidney stones is greater than the true pro-
 portion of American cargo pilots with kidney stones.

Evolution of an Invasive Species

Solidago Canadensis in Europe and the Americas: A Comparison Using Ribosomal ITS and 5S-NTS Sequences

Rachael Dickenson

The genus *Solidago* is recognized as an invasive species around the world. This study aims to identify emerging genetic divergence in the species *Solidago canadensis*, a well-established species in both Europe and North America. Since the introduction of *S. canadensis* to Europe is far too recent for any changes to be manifested in morphology of the species, ribosomal ITS and 5S-NTS DNA were sequenced from samples collected on both continents and compared to identify genetic variations. Geneious 8.1.8 software was used to align sequences, identify genetic variations, and build phylogenetic trees. Comparison of phylogenetic trees lacked sufficient reliability regarding location of origin and development of populations, therefore no definite conclusions were reached regarding time and manner of introduction or rate of variation. However, an understanding was reached that more research on both ITS and 5S-NTS sequences is necessary before drawing full conclusions regarding origin and development of *S. canadensis* populations.

Keywords: *Solidago canadensis*, invasive species, rDNA sequencing, phylogenetic analysis, Internal Transcribed Spacer (ITS), 5S-Non-Transcribed Spacer (NTS)

Introduction

The genus *Solidago*, commonly called Goldenrod, contains over 100 species and is considered highly invasive in nearly all areas of the world outside of its native region in Midwestern North America (excepting one species native to Europe) (Sheppard, 2006). Observation of the frequency of *Solidago* and similarly invasive herbaceous plants gives rise to questions regarding how quickly and in what manner such genera change to dominate a previously unintroduced area.

Most simply, invasive species are defined as “any nonnative species that significantly modifies or disrupts the ecosystems it colonizes” (Rafferty, 2015). Human globalization in recent decades has greatly spurred the introduction and encroachment of invasive species on native habitats around the world.

Notable consequences of this intrusion include destruction of native keystone species and subsequent ecological imbalances, disruption of natural landscapes, decreased biodiversity, and adverse effects on human food sources (Stace & Crawley, 2015). Current combative measures to eliminate invasive species include physical removal and use of biological controls, but unfortunately reestablishment of balanced ecosystems is often difficult to achieve. Several species of the genus *Solidago*, including *S. canadensis*, are labeled by the Royal Horticulture Society in the British Isles as invasive species commonly sold for ornamentation but can quickly become unmanageable when introduced to a native ecosystem (Stace & Crawley, 2015). Therefore, a question can be posed as to not only how these *Solidago* species affect a given area as an invasive species, but how (if at all) their genetics change to facilitate invasion of an area once introduced.

Emergence and Relevance of Intraspecies Variation

Genetic information of living organisms is known to spontaneously mutate through errors in DNA replication during meiosis and mitosis, resulting in beneficial, detrimental, or neutral impact on organism survival. In other words, organisms with beneficial mutations for their given situation are more likely to survive and those with detrimental mutations are less likely to survive, while those with neutral mutations will continue to exist as before (Hillis, Sadava, Heller, & Price, 2012). For invasive species such as those of the genus *Solidago*, introduction and invasion of new areas means exposure to different environmental factors, thereby affecting whether a given mutation is considered beneficial, detrimental, or neutral upon its spontaneous occurrence. Dong Yu, and He (2015) concluded that “climate and recipient communities explained 71.39% species impact of *S. canadensis*,” indicating that movement into regions with different characteristics likely results in different expression of *Solidago* genes and eventual evolutionary divergence by development of mutations beneficial, detrimental, or neutral to a given *Solidago* species in a new area.

Genetic changes in plants require millions of generations to accumulate and become manifested in morphology (Schaefer, 2015). Therefore ribosomal Internal Transcribed Spacer (ITS) and 5S-Non Transcribed Spacer (NTS) sequences were selected for use in this study. ITS and 5S-NTS are ribosomal non-coding DNA, proven to be one of the most variable sequences and commonly used in phylogenetic comparisons (Alvadhani *et. al.*, 2012 ; Álvarez and Wendel, 2003). Use of these sequences in phylogenetic comparison, while unable to identify full mutations emerging in a given species, allows identification of intraspecies variation and divergence of new populations. Phylogenetic comparison involves comparison of characteristics, in this case genetic characteristics, for the purpose of determining an organism’s evolutionary history. Diagrams known as phylogenetic trees display these differences by creating “branches” to show which organisms first developed genetic differences from the most recent common ancestor (see Results for further explanation).

In the context of this study, it is important to understand how such noncoding DNA as the ITS and 5S-NTS sequences are related to coding sequences: In each tandem repeat of ribosomal DNA (rDNA), ITS1 and ITS2 flank the 5.8S subunit of coding DNA while 5S-NTS exists in the Intergenic Spacer (IGS) region between each complete repeat of rDNA (see Fig. 1), filling the unused spacer regions with additional repeats of code.

Solidago canadensis was selected as a well-established species of the genus *Solidago* in North America and Europe. Alternative species considered under the given time and seasonal restrictions of this study were *Solidago gigantea* (also native to North America and invasive to Europe) and *Impatiens glandulifera* (native to the Himalayan region and invasive to Europe), included in the first iteration of sequencing but thereafter discarded when it was determined that *S. canadensis* displayed the most variability. Stability of *S. canadensis* populations in both North America and Europe increases the likelihood of mutations having been developed in Europe since its introduction as a garden plant in the mid-1700s (Sheppard, 2006) or that mutations have emerged in North America since the time of its passage to Europe. Furthermore, identification of differences between varying populations in Europe may suggest whether a single large introduction or several smaller introductions of *S. canadensis* resulted in its eventual establishment as an invasive species. Discovery of which populations were able to subdue native species may also provide insight into which areas of Europe and North America need

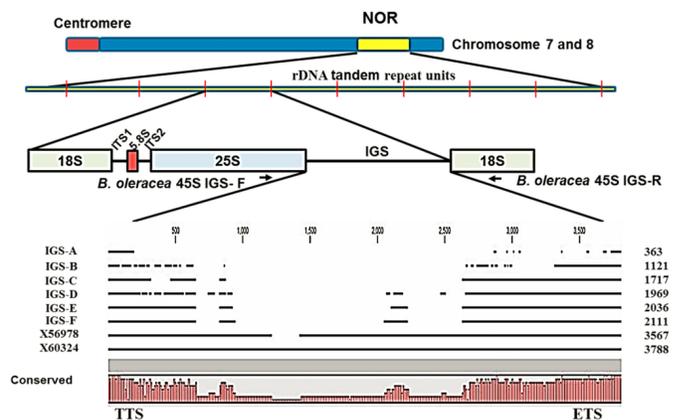


Fig. 1 Arrangement of sequences within rDNA repeats

improved management of *S. canadensis* to prevent further introduction outside the native range of the species.

Literature Review

Previous research has been done regarding changes in *Solidago* DNA sequences and its development following introduction to an environment, particularly by Schaefer (2015) in his examination of morphological and genomic sequence differences between Azorean *Solidago azorica* and other *Solidago* specimens from North America to determine ancestry and optimal nomenclature for *S. azorica*. His use of phylogenetic trees derived from ITS sequence differences to identify *S. azorica* origin prior to its emergence as a distinct species demonstrates the practicality of such methods of comparison for use in this study. Schaefer's use of morphology as a basis of comparison between *S. azorica* and its potential parent species is used effectively to support his conclusion, but is impractical in the context of this study due to my focus on a given species, members of which are morphologically indistinguishable.

A similar method of molecular analysis is used by Laureto and Barkman (2011) to identify origins of the *S. houghtonii* hybrid, including ITS sequences and phylogenetic trees to determine ancestry of the given species. Morphology is not used as major support for conclusions of their study, but multiple other regions of genetic information such as cpDNA intergenic spacers (within IGS region depicted in Figure 1) were used to further support results. Time and monetary restrictions made sequencing of more than two regions impractical in the context of my study; however, use of GenBank as done by both Schaefer and Laureto and Barkman was considered feasible. GenBank is a publicly available database of genetic sequences by researchers around the world, allowing additional data points to be added without the researcher needing to obtain a separate sample from the given location and go through the process of DNA extraction and sequencing. One of the few concerns regarding inclusion of sequences from GenBank is that samples cannot be verified as having been correctly identified and sequenced, meaning that an unreliable sequence could possibly be included as a viable data point

and lead to false conclusions or results. Regardless, Laureto and Barkman (2011) describe it as necessary for comparisons to determine parentage, necessitating "at least one species from each section and subsection of the genus... to represent the phylogenetic breadth of the genus" in their own successful study involving *Solidago* species ancestry and phylogenetic development.

Despite extensive use of ITS sequences by Schaefer, Laureto and Barkman, and their predecessors, some concern is expressed regarding the reliability of ITS sequences for phylogenetic trees due to their biparental nature and continual homogenization with unstable pseudogenes (Álvarez & Wendel, 2003). Otherwise stated, ITS sequences may contain unequal amounts of genetic material from maternal and paternal lineages, and constant recombination of repeats due to the repetitive nature of these sequences may result in combination of recent ITS sequences with disused and decaying ones. This combination has been shown to result in lower guanine-cytosine (GC) content, meaning less stable DNA structure and thus less reliable sequencing results (Álvarez & Wendel, 2003). The main focus of the study by Álvarez and Wendel (2003) is a comparison of ITS region sequencing against that of other viable sequences to determine which is most reliable in phylogenies. The ITS region was determined to be the least reliable of those examined due to it being the most homoplasious of the study, meaning that it is the most likely to have gene similarities not resulting from shared ancestry. One possible explanation for these similarities is convergent evolution, an occasion in which organisms of different ancestry develop similar characteristics through evolution under similar living conditions. These similarities result in skewed phylogenetic conclusions regarding species origin and development. Nevertheless, research by Ritland, Ritland, & Straus (1993) aims to distinguish between closely related species of the *Mimulus* family using ITS-based phylogenies and attempts to account for possible misleading results of ITS sequences by including three "clone" samples (plants of the same species, collected from the same location) of each species examined. Multiple clones of each test sample may have limited undue influence of ITS based results, but limited Ritland et al. (1993) to eight samples for comparison. This number of samples was sufficient in his case

EVOLUTION OF AN INVASIVE SPECIES: SOLIDAGO CANADENSIS

for distinguishing between eight given species, but insufficient for identifying differences between populations of varying geographic locations (especially since samples of the same location could be from different populations) as was the goal of my study.

Importance and Purpose of Study

Very few studies have been published to compare intraspecies variation between populations and attempt to pinpoint methods of introduction into a given environment. Weber and Schmid (1998) examined morphological variations of *Solidago* species introduced to Europe with a study featuring statistical analysis of shoot growth, leaves, and inflorescence measurements from 24 rhizome system samples gathered from different regions and climates between 44 and 61 degrees latitude on the continent. Their results stated that “only a small and insignificant proportion of variation among field populations could be explained by correlation between the characters and latitude” (Weber and Schmid, 1998), suggesting that not all differences between population could be explained by geographic latitude differences. Differing parent populations prior to introduction into Europe provides a possible explanation of these variations. Gradual morphological change correlating to latitude change of sample collection was observed in controlled garden cultivation over the subsequent two years of the study, but the lack of correlation in field populations was never analyzed as to whether such variations were genetic differences (developed since introduction to Europe or present due to differing parent populations) or simply characteristics of an identical genome expressed differently under varying environmental conditions across Europe (as seems assumed by the authors).

The purpose of this experiment is to draw conclusions about introduction and variability of *S. canadensis* between Europe and North America using ITS and 5S-NTS sequencing and phylogenetic comparison in a manner similar to that of Schaefer (2015) with gel electrophoresis techniques based off those of Ritland et al. (1993). The hypothesis is that if ITS and 5S-NTS rDNA are sequenced, aligned, and examined from both European and North American samples of *S. canadensis*, then variations will be observed in both spacer sequences and use of comparison techniques

such as phylogenetic trees will make it possible to determine origins and development of the species since its introduction to Europe.

Materials and Methods

Acquisition of Sample Materials

Samples of *S. canadensis* to be used in this experiment were requested via email from various institutions across the United States and Europe (see acknowledgements), as well as several samples from the herbarium at the Naturkundemuseum Stuttgart. All tissue samples were either silica-gel dried or herbarium specimen leaves, with the exception of a single silica-dried root sample.

Three separate iterations of DNA extraction, Polymerase Chain Reaction (PCR), and sequencing were carried out over the course of this study. The first iteration contained eight tissue samples, four of which were *S. canadensis* (the others being *Solidago gigantea* and *Impatiens glandulifera*), and sequenced ITS, ITS1, ITS2, and 5S-NTS. The second iteration contained nine samples and the third contained eight samples of

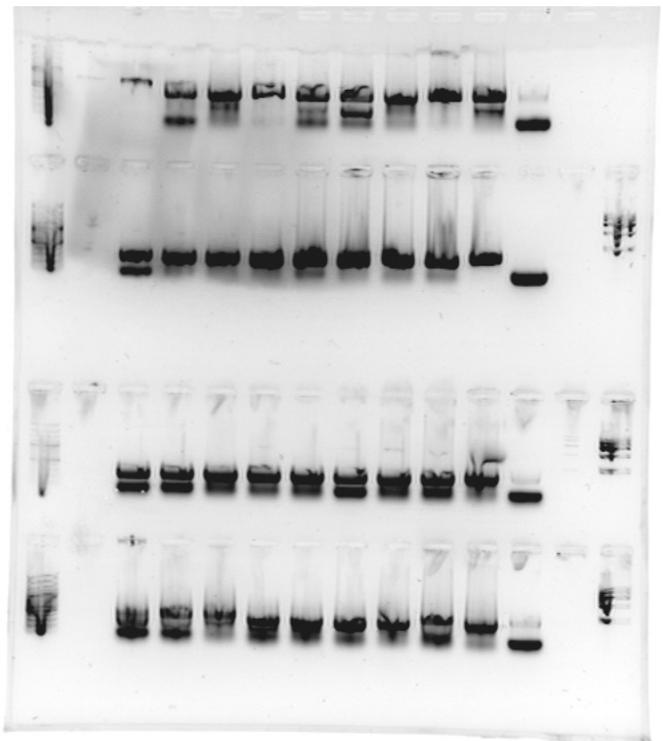


Fig 2: Gel electrophoresis image of first iteration samples

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S. canadensis. Second and third iterations sequenced only complete ITS and 5S-NTS due to monetary constraints and expediency of results, since sequencing of ITS forward and reverse was both cheaper and less time consuming than sequencing ITS1 and ITS2 separately and combining the two.

Sequences of *S. canadensis* and other *Solidago* species from GenBank for the ITS region were obtained and utilized in an additional phylogenetic tree (see Results) despite concerns expressed in the introduction of this study.

General Procedure

Initial cell lysis and DNA extraction from sample tissue was conducted following Macherey-Nagel protocols for extraction of genomic DNA from plant tissue using Nucleospin® Plant II kit (Macherey-Nagel, 2014). Prior homogenization of samples was completed using metal beads and a high throughput homogenizer. Subsequent DNA was prepared for PCR with the addition of 0.2µl Taq Polymerase (isolated from *Thermus aquaticus* bacterium), 2.5µl 10X buffer (green, with 20mM MgCl₂), 2mM deoxynucleotide triphosphate solution (dNTPs), 1.0µl forward primer, 1.0µl reverse primer, and 16.8µl nuclease-free water for each 1.0µl sample of DNA template. Forward and reverse primers were arranged in four combinations as follows: ITSA/ITSB for entire ITS amplification, ITSA/ITSC for ITS1 amplification, ITSB/ITSD for ITS2 amplification, and 5S-NTSF/5S-NTSR for entire 5S-NTS amplification.

PCR thermocycling program for ITS strands began with 5 minutes at 95°C (for denaturation), followed by 10 cycles of the following: 30 seconds at 95°C, 45 seconds at 60°C (decreasing 0.5°C each cycle), and 1 minute at 72°C. Next was 30 cycles as follows: 30 seconds at 95°C, 45 seconds at 55°C, and 1 minute at 72°C. The program was completed with 5 minutes at 72°C.

PCR thermocycling for 5S-NTS began with 5 minutes at 95°C (for denaturation), followed by 10 cycles of the following: 30 seconds at 95°C, 45 seconds at 64°C (decreasing 0.5°C each cycle), and 1 minute at 72°C. Next was 30 cycles as follows: 30 seconds at 95°C, 45 seconds at 59°C, and 1 minute at 72°C. The program was completed with 5 minutes at 72°C.

Once PCR had been completed, agarose gel elec-

trophoresis was performed and examined in inverted color as shown in Fig 2, with the inclusion of a positive and negative control to reveal possible contaminations and determine which samples had enough replications of the desired noncoding region to be sent for full sequencing. Sufficient samples of the first and third iterations were sent as part of a 96-well plate (along with samples of unrelated projects by the Naturkundemuseum), and thus did not require manual purification by the researcher. In the second iteration, preparation for sequencing of selected samples was performed in accordance with procedures listed in PCR clean-up manual by Macherey-Nagel (2014). Following clean-up (when necessary), samples were labeled and sent to LGC Genomics for their “Flexi Run” service, yielding complete forward and reverse ITS and 5S-NTS sequences.

After receiving raw ITS, ITS1, ITS2, and 5S-NTS data for samples of the first, second, and third iterations, a bioinformatics software called Geneious 8.1.8 (created by Biomatters Ltd.) was used to align forward and reverse strands into full DNA sequences. In the case of the first iteration, ITS1 and ITS2 sequences were combined to yield full ITS sequence for comparison with those of the second and third iterations. Forward strands of 5S-NTS in all three iterations were thoroughly garbled by sequencing errors, forcing the researcher to use only reverse sequences in 5S-NTS comparisons. Furthermore, 5S-NTS reverse direction of second iteration samples of required resequencing due to unreadability of sequences. The Geneious 8.1.8 program was then used to create phylogenetic trees of the ITS and 5S-NTS regions (see results), as well as a phylogenetic tree based on ITS region sequences along with *Solidago* ITS sample sequences obtained from GenBank.

Results

Unclear portions containing errors in sequencing, generally located at the start and end of the sequences, were removed along with sequences of primer DNA so only true differences would appear when DNA was compared between samples. These differences could then be viewed in alignments by the Geneious 8.1.8 program, as indicated in Figure 3 (below) by differences between rows of sequencing.

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Clear differences in sequences between samples are indicated by different color box and letter (indicating name of nitrogenous nucleotide base), such as that in position 5 of Figure 3, where thymine is clearly indicated while all other samples at that position displayed cytosine. Less certain differences are displayed as gray boxes where the sequencing was unclear or detected two base pairs at that position. One explanation for this uncertainty is the possible existence of two variations of code within the several tandem repeats of a given specimen's sequence, both of which would then be amplified by the PCR and detected by sequencing software.

Furthermore, the Geneious 8.1.8 software was used to produce phylogenetic trees of both ITS and 5S-NTS sequences (see Fig. 4 and 5). Numbers listed at the divisions in the tree are known as "bootstrap values" and indicate how many out of the 100 possible phylogenetic trees the program creates that particular division was present in. Thus, higher bootstrap values indicate higher certainty that a given division or dividing variation occurred at the indicated point. The number at the bottom of each image (not attached to

any particular division) is the changes per base pair for each phylogenetic tree. Samples which appear to diverge on the farther left side of each image (earlier in the tree) are those with the most differences from the consensus alignment of the sequences, therefore calculated to be the most genetically different in comparison to the others in the tree.

Sample titles as listed in Figures 4 and 5 are formatted as follows: uppercase letter R followed by iteration number, underscore followed by sample number within the previously specified iteration, underscore followed by name of region sequenced (ITS for combined ITS forward and reverse primers or 5S-NTS reverse primer), then underscore followed by abbreviation for country of origin.

Notable areas of the ITS tree depicted in Figure 4 include the three lowermost samples, four samples to the left and below the bootstrap value 56, and the sample R3_7_ITS_US located in the upper right.

Notable areas of the 5S-NTS tree depicted in Figure 5 include the two lowermost samples, as well as the location of all samples in the tree relative to their locations in the ITS tree (Fig. 4).

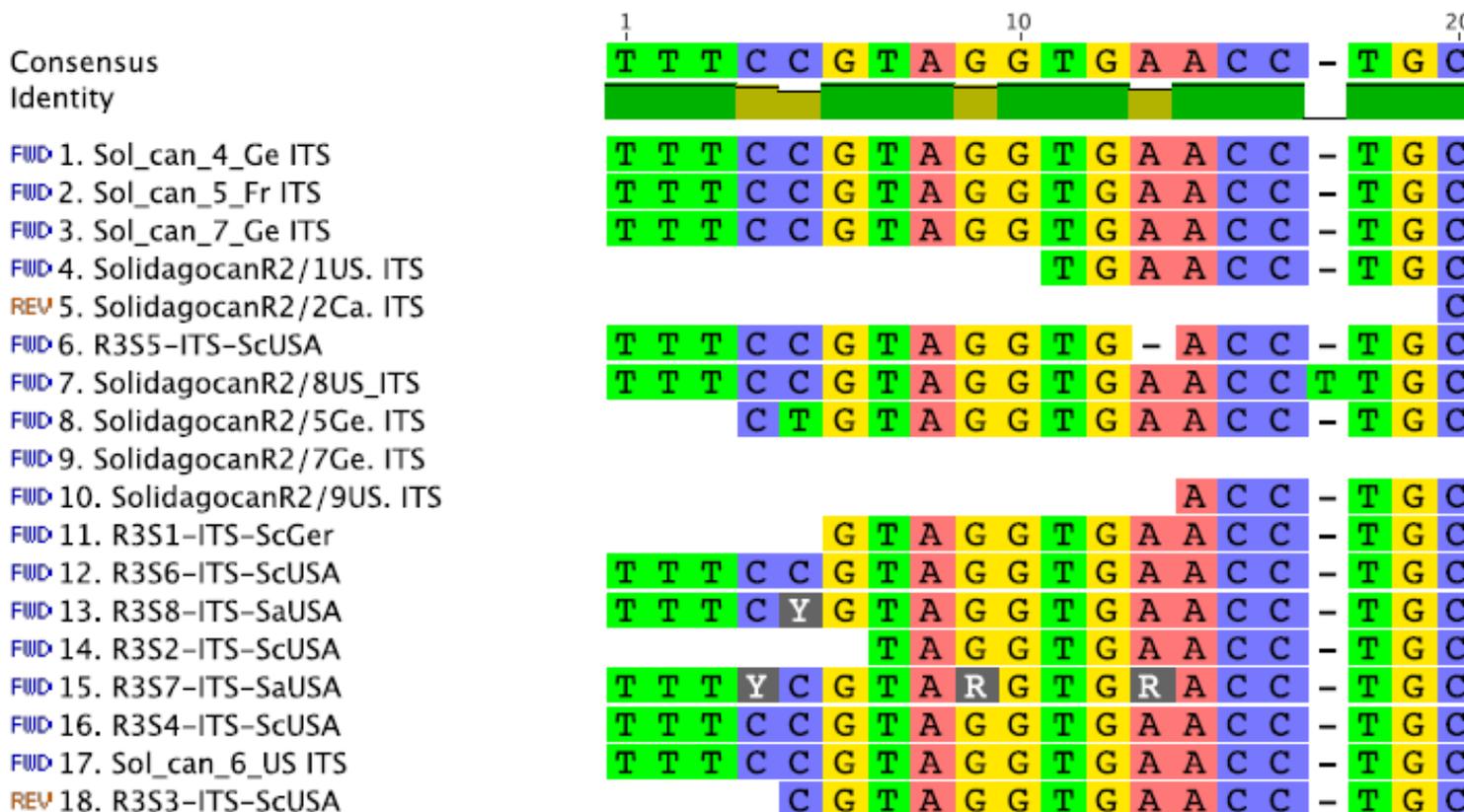


Fig 3: Initial 30 base pairs in ITS alignment of all samples

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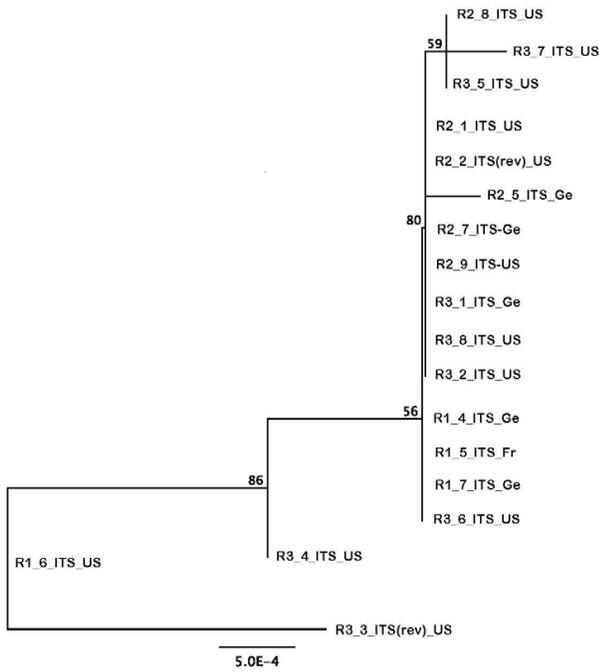


Fig 4: Phylogenetic tree for ITS sequences

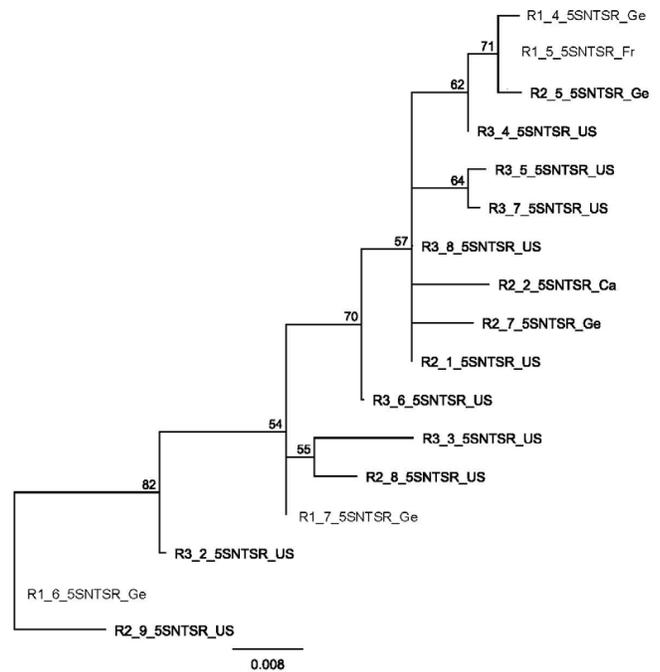
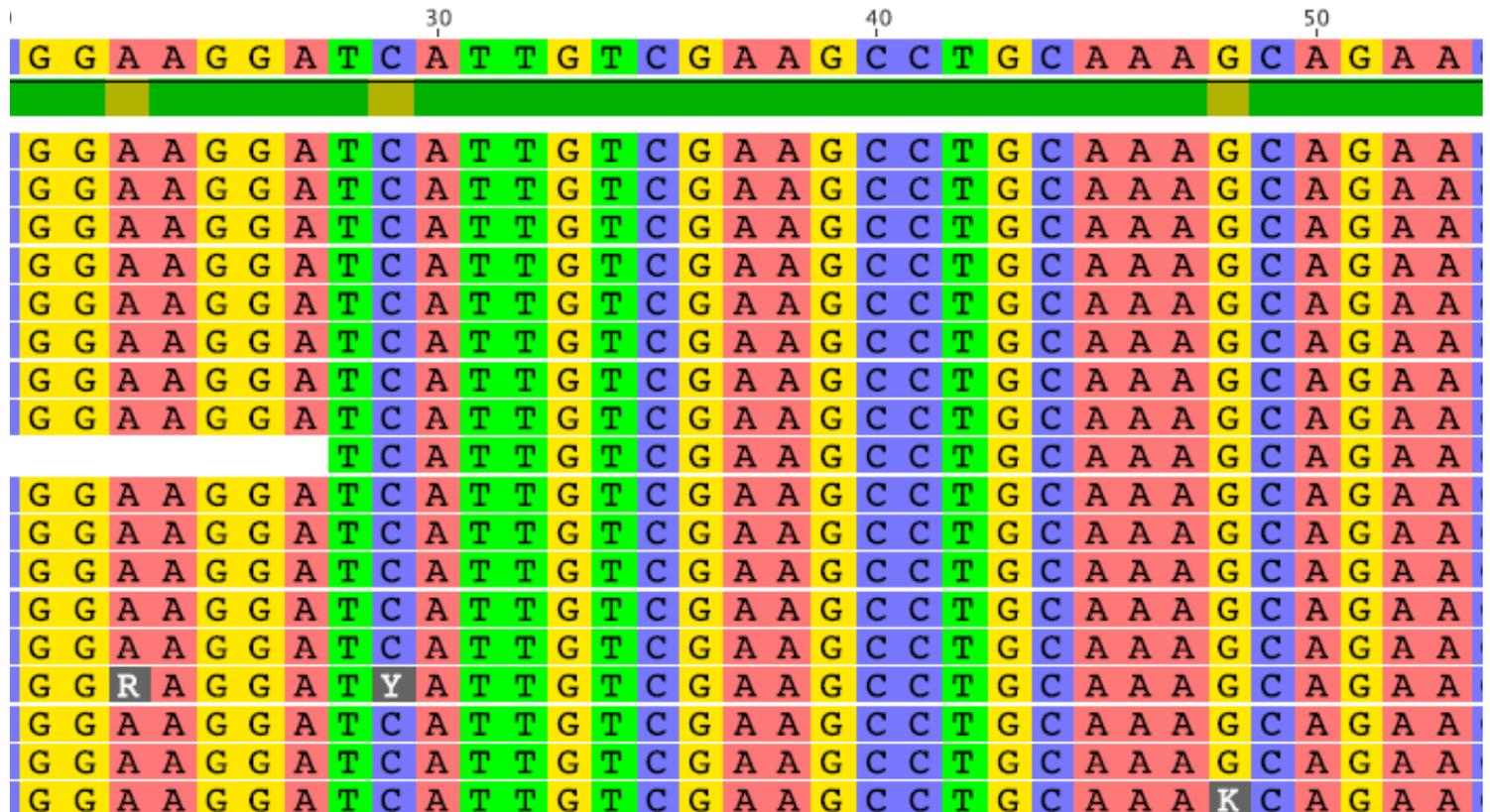


Fig 5: Phylogenetic tree for 5S-NTS sequences

As considered necessary for comparisons to determine ancestry by Laureto and Barkman (2011), ITS sequences from GenBank were used in compilation

with the results seen in Figure 4 (5S-NTS sequences being too short and unreliable) to create the phylogenetic tree in Figure 6 (below).



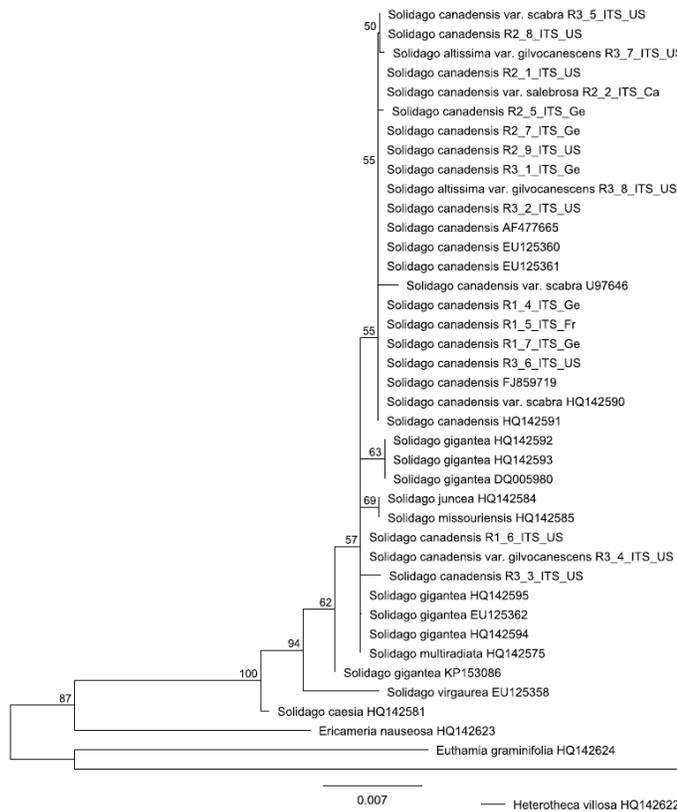


Fig 6: Phylogenetic tree for ITS with GenBank samples

Inclusion of three non-*Solidago* plants allowed for what is referred to as “rooting”: a visual representation of how different populations are when compared to much less closely related species such as the bottom three specimen included in Figure 6. Formation of an outgroup by use of 1-3 samples of a differing genus occurred also in studies by Schaefer (2015), Laureto and Barkman (2011), and Ritland et al. (1993). The amount of difference between given samples of *S. canadensis* and outgroup species also provides clear indication of directionality of evolutionary change (Kinene, Wainaina, Maina, & Boykin, 2016) thus clarifying which variations likely occurred earlier than others, due to their similarity to a species which diverged from the last common ancestor of the tree much earlier. Inclusion of this phylogenetic tree confirms that all *S. canadensis* samples used in the study are closely related as populations within a given species should be, thus ruling out possibilities of incorrect identification by the researcher in sample acquisition.

Analysis and Discussion

Analysis of ITS phylogenetic tree (Fig. 4) reveals that ITS samples R3_3, R3_4, and R3_6, collected from U.S. states Colorado, Wyoming, and New York, respectively, are significant due to their high amounts of variation from the ITS consensus sequence (see Fig. 3). The lowest two samples of the ITS tree (R1_6 and R3_3, of Vermont and Colorado respectively) are grouped together because they have an identical variation of cytosine rather than guanine in position 546 of ITS. Sample R3_4 ITS_US of Wyoming is located between the two lowermost samples and the rest of the samples because it is unsure at the same position (Fig. 7). The lowermost sample, R3_3 ITS_US from Colorado, is farthest removed because it has another definite difference in a different position not shared by any other samples in the alignment. Laureto and Barkman (2011) utilized a similar method involving analysis of sequence alignment and comparison of specific base pairs to supplement explanations of phylogenetic differences displayed in the constructed trees.

Based off these differences, there is a possibility that sample R1_6 ITS_US from the state Vermont may have been introduced to its area of collection from Colorado, where sample R3_3 ITS_US was collected and within the native range of *S. canadensis*, before developing an additional variation. Furthermore, sample R3_4 ITS_US (collected in Wyoming) indicates uncertainty possibly due to its collection location in the native region of the species, where populations and gene pools are larger, causing greater variation and possibly resulting in the presence of two different DNA sequence repeats within the same sample.

A grouping of four samples (titles beginning with R1_4, R1_5, R1_7, and R3_6) are grouped in the same clade of the ITS tree, indicating an identical amount of difference from the ITS consensus sequence and thus the possibility of a shared origin. The sample R3_6 ITS_US was collected in the US state of New York, meaning that it would be the parent population of the group. The other three samples of the group were collected in France and Germany, close enough together for the possibility that their populations could have developed following a single introduction from the

EVOLUTION OF AN INVASIVE SPECIES: SOLIDAGO CANADENSIS

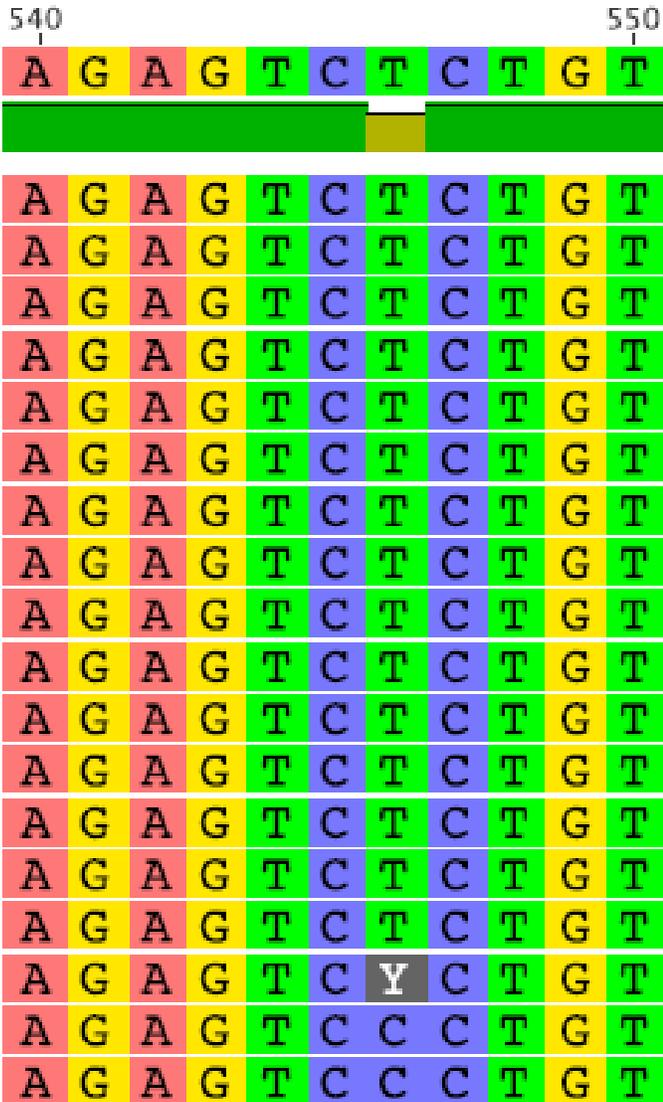


Fig 7: Position 546 of ITS alignment

New York population of the species into Europe. Similar groupings were identified by Schaefer (2015) and supported by the multiple regions he sequenced, thus allowing him to make practical conclusions regarding parent populations of *S. azorica*, the species whose ancestry he was trying to determine.

The sample R3_7_ITS_US was collected from the US state of Colorado, within the native range of the species. Its significant difference from other samples of the ITS tree (see Fig 4) could be due to a lack of sequence clarity. Many base pairs in this sample were noted as uncertain, the sheer amount (as can be observed in Fig 3) of which suggests sequencing errors unrelated to the sample or research methodology, rather than the presence of two base pairs at one position in a single sample's set of DNA repeats (as would

typically be responsible for an unclear base interpretation). Such possible sequencing errors are also noted by Laureto and Barkman (2011) in agreement with earlier statements by Álvarez and Wendel (2003) regarding PCR errors and false variable sequences due to the recombinant nature of tandem repeat regions such as ITS.

Due to the unclear forward primers of the 5S-NTS sequences and the shorter nature of that noncoding region, ITS results obtained by the researcher can be considered more reliable and more likely to be accurate than those of the 5S-NTS region. Additionally, many areas of the reverse 5S-NTS sequences were unclear (similar to the forward primer but less severe) and thus were cut out by the researcher in editing prior to alignment, further shortening the number of positions available for comparison. Conflicting phylogenies between the two regions were noted immediately in the two trees (Fig 4 and 5), including most obviously the difference in the two lowermost samples from the ITS sequence results. Sample R2_9_5SNTS_US, collected from the US state of North Carolina, was depicted the sequence most variable from the consensus, suggesting that it may have also originated from the same R3_6 sample population from Colorado. However, this same suggestion regarding the R2_9 sample is not reflected in the more reliable ITS tree, leading the researcher to discard this evidence. Laureto and Backman (2011) avoided this conflict in their study involving sequencing of multiple regions by determining percent variation for regions such as chloroplast DNA rather than focusing on individual placement of samples within a phylogenetic tree. This was made possible by their study's goal of differentiating between species, which can be indicated by overall variance rather than specific base pair polymorphisms. Unfortunately, this study's goal of determining emergence of populations (that is, when and in what manner populations came to differ within a given species), as indicated by emergence of polymorphisms, means that such avoidance was not possible.

Conclusion

ITS and 5S-NTS rDNA were successfully sequenced, aligned, and examined from both European and North American samples of *S. canadensis* with use

of comparison techniques such as phylogenetic trees. Previous studies in the field were used as models for methodology and analysis, but key differences exist when compared to conclusions by Shaefer (2015) and Laureto and Barkman (2011) in that the purpose of their studies was simple ancestry and nomenclature, while my study aimed to identify emerging variations in a species and their geographic locations of origin. The hypothesis for this study was partially proven in that variations were observed in both ITS and 5S-NTS sequences. However, due to sequence unreliability and insufficient evidence, the researcher was unable to determine with certainty the origins and development of *S. canadensis* populations since its introduction to Europe.

Future Directions

Given more time and financial resources, full re-sequencing of the 5S-NTS region would benefit this research with the inclusion of clear and reliable data for meaningful comparison to ITS results. Also, inclusion of other *S. canadensis* genetic information, such as the ETS region of nrDNA recommended for use in conjunction with ITS by Laureto and Barkman (2011) or single-copy nuclear genes suggested by Álvarez and Wendel (2003) as a more stable alternative to ITS sequencing for creation of phylogenetic trees, could prove useful in confirming conclusions made from ITS and 5S-NTS results. Additional samples from the same and more varied locations across Europe and the Americas would allow for more comparisons between a larger number of potential populations and introductions.

On a larger scale, it has been made clear through my research that more exploration should be done to determine the origins and development of invasive species *S. canadensis* as was done by Schaefer (2015) regarding the origins of *S. azorica*. As pointed out by Stace and Crawford in their documentation of species invasive to the British Isles (2016), “this topic [development of *S. canadensis* and *S. gigantea* since introduction to Europe] has been almost totally neglected by researchers in Europe... DNA technology might change that”, meaning that many advances in this field of study are yet to come with the refinement of existing sequencing and comparison technology. Perhaps

such sequencing errors as were an obstacle in this study can be prevented in the future through development of more advanced DNA. Although no definite conclusions regarding *S. canadensis* variation were determined through my study, the lack of present research in this area, demonstrated by studies such as that by Weber and Schmid (1998), continues to stand as a reason for additional exploration into how invasive species are changed by the communities which they come to dominate.

Acknowledgements

I would like to thank the Staatliches Museum fuer Naturkunde Stuttgart for allowing me to conduct my research in their laboratory facilities and use of their equipment, as well as providing training in laboratory procedures and a number of tissue samples for analysis. My thanks are especially due to Dr. Mike Thiv, Ms. Anne-Kristin Schilling, and Ms. Cornelia Krause of the Naturkundemuseum for their help in planning and supervising my laboratory work. I would like to thank Ms. Katie Stuble of the Holden Arboretum, St. Andrews University, Dr. Egan of the Elmira College Herbarium, Professor Fishbein of Oklahoma State University, Mr. Battaglia of Eckert Herbarium, and Dr. Rohwer of the Universität Hamburg for providing sample tissue. Thanks also to Mrs. Stephanie Payne and Mr. Philip Bailey for their constructive feedback and encouragement, and Department of Defense Education Activity and Stuttgart Community Spouses Club for their financial support of my research. Finally, I would like to thank Mr. Daniel Coapstick, my teacher, for his instruction and experience in organization and composition of a research project.

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Smoke Break: Electronic vs Tobacco Cigarettes

Isaac Gallogly

The use of electronic cigarettes is beginning to surpass the use of tobacco cigarettes, especially among youth and young adults. Because there is ample evidence that tobacco cigarettes are intrinsically more dangerous, the potential impacts of the growth of electronic cigarettes is often ignored. In order to make inferences about the aforementioned impacts, a comparison was done between the chemical components of e-cigarette aerosol, using the Pax Labs JUUL™, and that of tobacco cigarette smoke. The analysis was done through the use of a gas chromatograph mass spectrometer. It was found that there are a number of potentially dangerous compounds that are present in both types of aerosols. Therefore, the health effects related to these compounds can affect users of either type of cigarette. Based on the findings of this study, electronic cigarettes need to be considered as much of a public health concern as tobacco cigarettes, and action must be taken accordingly. The social and political campaign against tobacco cigarettes that has been witnessed in the United States should be used as a model for a similar campaign against electronic cigarettes.

Keywords: electronic cigarette, tobacco cigarette, aerosol, health, JUUL

Introduction

Concurrent with modern advancements in technology, electronic cigarettes are replacing conventional tobacco cigarettes. Setting them apart, e-cigarettes do not use tobacco and are comprised of a battery of varying voltage, a container in which a flavoured solution is stored, a heating element such as a coil, and a mouthpiece through which a user inhales an aerosol.¹ For the purposes of this study, only electronic cigarettes which use a nicotine-containing solution will be examined, and variances in battery voltage will be deemed negligible. Many questions about the safety of this emerging technology, especially when compared to conventional tobacco cigarettes, have been raised.

Like tobacco cigarettes, e-cigarettes expose users to a number of chemicals that have the potential to cause adverse health effects; however, these consequences are not fully understood.^{2,3} A number of studies have attempted to explain the potential impacts of electronic cigarette usage through the use of gas chro-

matography, a method of gas analysis, but many have failed to evaluate modern electronic and tobacco cigarettes in the same experiment. In addition, a relatively new electronic cigarette known as the JUUL™, which uses nicotine salts found in leaf tobacco as opposed to free-base nicotine, has not been studied extensively, yet has become one of the most popular products on the market.⁴ The purpose of this study is to evaluate to what extent the chemical composition of e-cigarette aerosol, using the JUUL™, compares to that of tobacco cigarette smoke and to evaluate what implications particular similarities may have for the health of users and the future of the electronic cigarette industry.

Electronic Cigarette Industry

Growth

Recent studies reveal that the electronic cigarette industry is growing exponentially, evidenced by the sales of electronic cigarettes. In 2015, the e-cigarette/vapor industry amassed \$3.3 billion in sales, showing

incredible market value.⁵ In the same year, an observational study done on a number of specialty vape shops showed that 58.2 million nicotine-containing electronic cigarette units were sold.⁶

The usage of electronic cigarettes has increased considerably among youth and young adults. Usage amid those aged 18-24 surpassed that of adults aged 25 or older in 2014, and the gap continues to grow.⁷ Additionally, electronic cigarettes have surpassed conventional cigarettes in popularity among this age group.⁷ This trend resembles the trend identified in the 1964 Surgeon General *Report on Smoking and Health* wherein conventional tobacco cigarette usage was most popular among young adults.⁸ Such a resemblance may suggest that the scope of the electronic cigarette industry will, at its height, be similar to that of the tobacco industry in the mid 1900s. As these numbers continue to grow, and electronic cigarettes continue to be used in place of tobacco cigarettes, it is increasingly vital that a comprehensive comparison between the two be done in order to assess any potential impacts of the large-scale transition.

Reasons for Growth

Accumulating research indicates that the increase in popularity of electronic cigarettes, especially among youth, can be attributed to three factors. The first of which is accessibility and appeal. Producers of electronic cigarettes intentionally design flavourings, like; fruit, cotton candy, and mint, to appeal to the group that has the most interest in their products, young adults aged 18-24.^{2,9,10} Additionally, the accessibility of e-cigarettes resembles that of tobacco cigarettes as both are available for purchase at various convenience stores, gas stations, specialty shops, and online.² Currently, the FDA does not have the authority to regulate electronic cigarette advertising, and has yet to implement a labeling system for e-cigarette packaging. On the contrary, the FDA, a U.S. regulatory agency, does both for tobacco cigarettes. Consequently, consumers living in the U.S. are not exposed to a printed record of the potential risks associated with electronic cigarettes, like they are with tobacco cigarettes, during the purchase.¹⁰ This lack of regulation effectively increases the appeal and accessibility of electronic cigarettes.

Second, the efficiency of nicotine delivery has con-

tributed to the growth of the electronic cigarette industry. A study done to determine the concentration of nicotine in the blood of users of electronic cigarettes and that of users of tobacco cigarettes concluded that the two amounts are very similar.¹¹ These findings suggest that the two modes are equally as effective at delivering nicotine. Therefore, the health effects associated with nicotine in e-cigarettes are generally the same as for typical tobacco cigarettes.² This is of particular concern because nicotine has been found to affect brain development of adolescents which are the most common users of electronic cigarettes.^{7,8} On top of that, nicotine is a highly addictive drug and is the catalyst in the transition from experimentation with electronic or conventional cigarettes to sustained smoking.⁸

Third, the public perception of e-cigarettes is conducive to the prolonging of their use. A number of studies have shown that the most commonly cited reasons for first using electronic cigarettes among youth and young adults are flavouring/taste, curiosity, and most importantly, low perceived harm as compared to tobacco products. The use of e-cigs as a means to quit smoking tobacco cigarettes was not found as a commonly cited reason among this age group.^{2,12,13,14,15} According to these findings, electronic cigarettes as a whole are perceived as less harmful than conventional cigarettes, and this is leading people to begin using them for recreational purposes rather than to stop smoking tobacco cigarettes.²

As the electronic cigarette industry continues to grow, the tobacco cigarette industry continues to decline. Regulation, on both the product itself and advertising, has played a large role in this decline.¹⁶ Similar regulation does not currently exist to this degree for electronic cigarettes but may be an effective means at curbing the further growth of the industry.

Existing Knowledge

A recent study conducted by affiliates of the University of Rochester Medical Center substantiates that exposure to e-cigarette aerosol causes measurable oxidative and inflammatory effects in human lung cells.¹⁷ The scope of these consequences is unknown because the topic is so modern. A prospective study on the current generation of e-cigarette users is needed to truly understand the effect of the inhalation of elec-

tronic cigarette aerosol on the human body.

However, there are a number of potentially harmful compounds that are known to be found in said aerosol. Based on several exploratory studies, these can be grouped into three categories of carbon-based compounds.^{3,10,18} The first of which is carbonyls. One study observed the presence of carbonyls in 47 of 51 electronic cigarette flavourings tested.¹⁰ The second category is volatile organic compounds. A separate study found that multiple VOCs were found present in electronic cigarette aerosol.¹⁸ The third category is carcinogens. A study done by researchers at the Institute of Occupational Medicine and Environmental Health showed that the levels of formaldehyde, a carcinogen, observed in select electronic cigarette aerosols was in the range of levels reported in tobacco smoke. These researchers concluded that in some cases, electronic cigarettes “might expose their users to the same or even higher levels of carcinogenic formaldehyde than tobacco smoke.”³ This collection of studies also revealed that a number of metals are found in electronic cigarette aerosol, including nickel, cadmium, and lead.¹⁸ However, for the purposes of the current study, metals will not be analyzed or considered.

Though there are indeed harmful compounds found in electronic cigarette aerosol, there are significantly more found in tobacco cigarette smoke.^{3,17,18} Because of this, it is important to note that the use of electronic cigarettes is a popular strategy for the cessation of tobacco cigarette smoking. The effectiveness of this strategy varies.¹⁹

Hypothesis

Because of the presence of the previously mentioned compounds in electronic cigarette aerosol, the popularity of these devices is a public health concern.² Exacerbating the problem, electronic cigarettes are perceived to be significantly less harmful than tobacco cigarettes.² In addition, the use of electronic cigarettes is high among youth, paralleling trends in tobacco cigarette usage at the height of their popularity.²⁰ These factors illustrate the need for more information about electronic cigarettes in order to avoid the impacts of another cigarette smoking epidemic. The current investigation has been designed to com-

pare the composition of electronic cigarette aerosol to the composition of tobacco cigarette smoke. It is hypothesized that the results will support the assertion that e-cigarettes are harmful to the user’s health. Such a finding would promote initiatives leading to a public health campaign to curb their use and the growth of the industry, similar to the campaign against the tobacco industry.

Methodology

Initially, a qualitative method involving the survey of high school students in regards to their use of electronic and tobacco cigarettes was considered. However, because the medical field is concentrated with surveys of this nature, a quantitative scientific design was chosen instead and modelled after a number of studies which detail the use of gas chromatography to observe the presence of various compounds within an aerosol.^{18,21} Gas chromatography was chosen because it is widely considered to be one of the most effective methods of analyzing gaseous samples.³³ The intent of this design was to gather data that could clarify the link between electronic cigarette usage and potential health risks in order to show the possibility of impacts on public and individual health similar to those of tobacco cigarettes.

Setup

For the purposes of this study, an electronic cigarette and tobacco cigarette were acquired. The JUUL™ e-cigarette was chosen because of its patented use of nicotine salts as opposed to free-base nicotine and the lack of available research involving this new technology.⁴ The JUUL™ utilizes disposable cartridges to store the electronic cigarette flavouring liquid called pods. Each pod contains .7 mL of liquid and is 5% nicotine by weight. Four flavours of JUUL™ pods were chosen: mint, fruit, mango, and creme brulee. A Pall Mall Menthol cigarette was chosen as the tobacco sample because of its affordability and convenience. All steps of the procedure were done in a local environmental laboratory specializing in the analysis of air and dissolved gases.

Procedure

The first step of the experiment involved designing a device that would mimic human inhalation in order to extract gas from the two types of cigarettes. For this, a number of devices were tested, all taking advantage of pressure differences to force the gas out of the cigarette and into a syringe. The final design of the apparatus was modelled after the device described in “Universal electronic-cigarette test: physiochemical characterization of reference e-liquid.”²¹ It was comprised of a soft plastic tube that was stretched around the mouthpiece of the electronic cigarette or the tip of the tobacco cigarette and then connected to a valve. A 45 mL disposable plastic syringe was connected to the valve to measure the amount of gas being collected. The valve was included to ensure that no gas would escape the device during the extraction procedure. Once the syringe was full, the gas was then transferred to a 22 mL evacuated glass vial using a disposable needle. The syringe was discharged entirely. The collection process was repeated twice for each of four flavours of JUUL™ pods as well as for the Pall Mall Menthol cigarette. The vials were labeled accordingly and recorded on the chain of custody form.

One vial of each type of sample was run through a gas chromatograph specifically built for the lab in use and calibrated to analyze a specific set of volatile organic compounds. This particular calibration only included compounds expected to be found in contaminated ambient air, so it did not test for many of the compounds that are generally found in e-cigarette flavourings. Regardless, the test was run to ensure that a more thorough and expensive test using a mass spectrometer was truly necessary in order to gather useful data.

Because the results of the first test were inconclusive, the remaining five vials (one for each JUUL™ flavour and one for the Pall Mall Menthol cigarette) were sent through a PerkinElmer GC/MS/ATD Work Station, equipped with a Clarus SQ8T Mass Spectrometer as well as a Clarus 680 Gas Chromatograph, configured to run EPA Method 325B which tests for a much larger array of organic compounds than the first gas chromatograph that was used. This test was successful for the mango, mint, and fruit JUUL™ pods as well as for the Pall Mall sample; however, the test

was unsuccessful for the creme brulee JUUL™ pod. The mass spectrometer assigned a retention time to every measured compound. Each retention time was then matched to the name of its specific compound using a reference library unique to the machine. The mass spectrometer also produced a graph for each sample which matches each retention time to the relative abundance of that particular compound. Relative abundance peaks can only be compared quantitatively within the same sample.

Results

Using gas chromatograms retrieved from the mass spectrometer (see Figure 1 in the appendix), the top fifteen most concentrated compounds found within each sample were compiled. Only fifteen were chosen because at lower concentrations, health effects are of a far smaller magnitude and not pertinent to this study. Each compound was then cross-referenced with all four individual samples in order to observe possible overlap. The results of this analysis are reflected in Table 1. Unsurprisingly, a number of the same compounds were found within each of the electronic cigarette samples. Comparatively, nine of the compounds observed in the tobacco cigarette sample were also observed in at least one of the electronic cigarette samples. These particular compounds are highlighted in red in Table 1.

Health Hazards

The Globally Harmonized System (GHS) for Hazard Communication is a United Nations initiative which works to create a uniform method for the classification and labeling of chemicals. The United States is an active participant.²² With respect to this system, and with reference to the National Center for Biotechnology Information's PubChem database, each compound was paired with its corresponding GHS hazard codes and its potential inhalation symptoms. This data can be found in Table 2. This table also includes the PubChem CID of each compound which can be used to quickly find the compound in the database.

SMOKE BREAK: ELECTRONIC VS TOBACCO CIGARETTES

Table 1. Compounds Found in E-cig and/or Tobacco Cigarette Aerosol

Compounds highlighted in red were found in the tobacco cigarette sample as well as at least one of the electronic cigarette samples.

Compound	Average Retention Time (s)	Mint	Fruit	Mango	Tobacco
1-Aziridineethanamine	1.82		X	X	X
1,3-Pentadiene	8.02				X
2-Methylfuran	11.93				X
2-Methylquinolin-6-amine	37.11	X	X		
2-Nitroethanol	7.57	X	X	X	
2,2-Dimethoxyethanol	12.38		X		
5-Amino-6-nitroso-1H-pyrimidine-2,4-dione	8.81		X		
5-Methylhexan-2-amine	32.39			X	
Acetaldehyde	5.59	X	X	X	X
Acetone	8.57	X	X	X	X
Acetonitrile	9.23				X
Benzene	14.74				X
Benzoic Acid	29.78	X	X	X	X
Benzothiazole	30.86	X			
Butane	5.26				X
Carbon dioxide	3.89	X	X	X	X
Chloromethane	4.91				X
cis-1,2-Dimethylcyclopropane	8.22				X
cis-2,3-Epoxybutane	12.77		X		
Ethyl Cholate	26.63	X		X	
Ethyl Formate	8.08			X	
Formamide	8.91	X			
Hydrazoic Acid	8.82	X			
Isobutane	4.82				X
Isobutylene	5.19			X	X
Isopropanol	8.25	X	X	X	
L-Cysteinesulfinic acid	4.74		X		
Levomenthol	29.45	X			
Methyl ethyl ketone	12.67				X
Nicotine	33.13	X	X	X	X
Nicotyrine	37.09			X	X
Propane	4.31				X
Propionaldehyde	8.41	X	X	X	X
Propylene Glycol	20.82	X	X	X	
Toluene	19.43				X
Triacetin	32.35	X			

SMOKE BREAK: ELECTRONIC VS TOBACCO CIGARETTES

To evaluate the potential health effects of a specific compound, each GHS hazard code corresponds to a hazard statement; the statements offer a brief description of a possible health effect resulting from exposure to the compound. Most of the compounds are associated with multiple hazard statements which range in severity. For a list of the GHS hazard codes identified in Table 2 and their corresponding hazard statements, see Table 3 which can be found in the appendix.

The total number of GHS hazard codes/statements associated with each compound is communicated in Figure 1. Found in all three of the JUUL™ samples as well as the tobacco cigarette sample, acetaldehyde is one of the most dangerous compounds identified. It can be associated with fifteen GHS hazard statements, including but not limited to the following: “toxic if inhaled,” “may cause drowsiness or dizziness,” and “may cause cancer.” Also found in all four samples was ac-

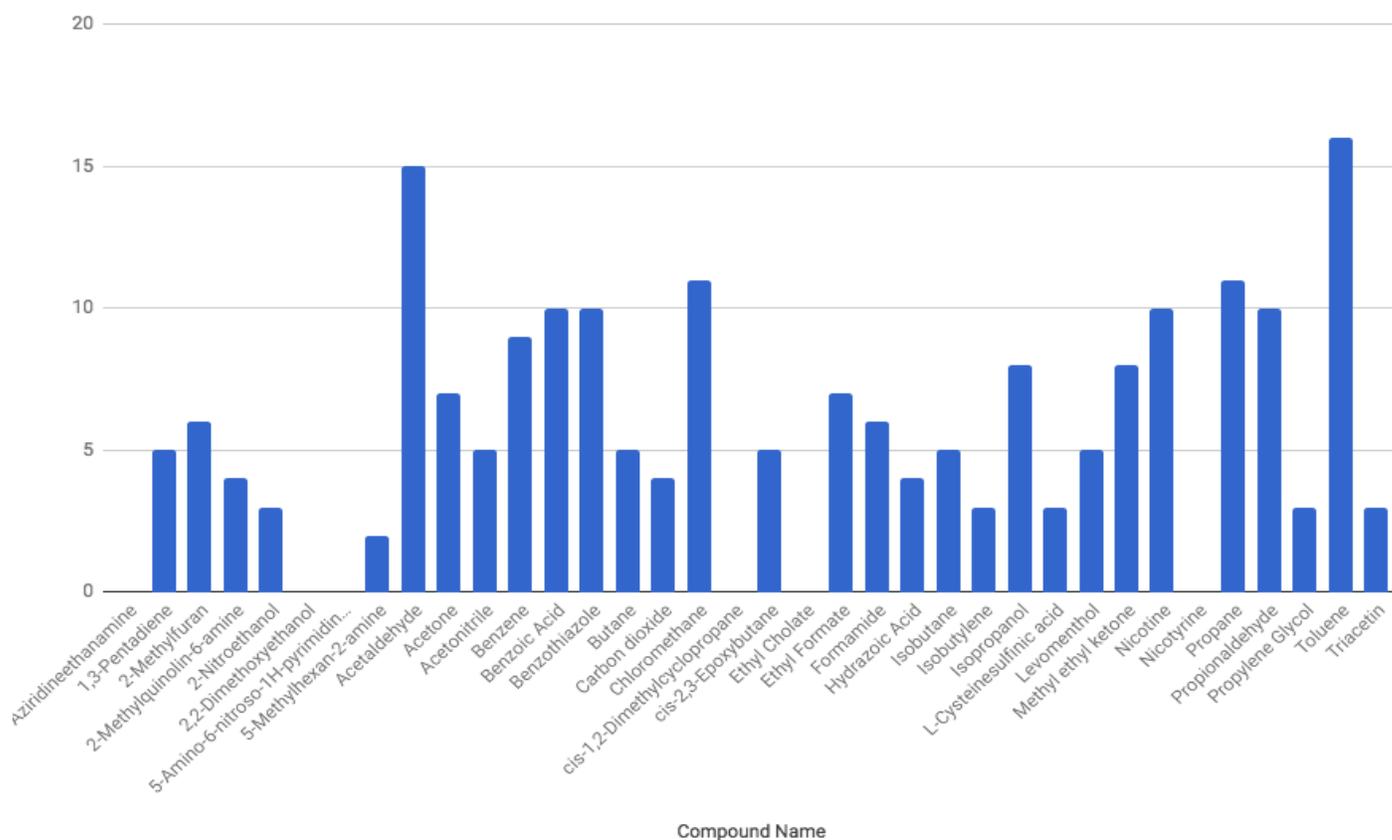
etone, often used in nail polish remover because of its solvent properties.²⁴ Acetone is associated with seven GHS hazard statements including “may cause respiratory irritation,” “suspected of damaging fertility or the unborn child,” and “caused damage to organs through prolonged or repeated exposure.” Found only in the tobacco cigarette sample, toluene can be associated with the most GHS hazard statements (sixteen).

Discussion

Prior to the completion of this study, very little scientific research surrounding the composition of vapor generated by the JUUL™ electronic cigarette existed even though it has become one of the most popular products in the e-cigarette market. Its patented use of nicotine salts found in tobacco leaves, as opposed to freebase nicotine, makes it difficult to draw conclu-

Figure 1. Frequency of GHS Hazard Codes by Compound

Frequency of hazard codes calculated using information from the National Center for Biotechnology Information PubChem compound database.²³



SMOKE BREAK: ELECTRONIC VS TOBACCO CIGARETTES

sions about the JUUL™ based on past studies.⁴ However, the JUUL™ is just one of many products that comprise the e-cigarette industry—an industry which is currently experiencing a growth that resembles that of the tobacco cigarette industry in the twentieth century.^{5,6} The data collected and presented in this study allow for comparisons to be made between the health hazards of the two types of cigarettes. With this in mind, the following was hypothesized before any collection of data was done: e-cigarettes contain types and quantities of molecules with the potential be harmful to the user’s health.

Tobacco Cigarettes

In terms of health risks, this study confirms that the inhalation of tobacco cigarette smoke is more dangerous than the inhalation of electronic cigarette aerosol. The top four most hazardous compounds (as measured by frequency of hazard codes) were found in the tobacco cigarette sample. These compounds are toluene, acetaldehyde, chloromethane, and propane. Comparatively, the only one of these four found in any of the electronic cigarette samples is acetaldehyde.

In general, the most severe hazard statements can be

Table 2. Potential Health Hazards and Inhalation Symptoms of Identified Compounds

Information retrieved from the National Center for Biotechnology Information PubChem compound database.²³

Compound Name	PubChem CID	Inhalation Symptoms	GHS Hazard #s
1-Aziridineethanamine	97697	N/A	N/A
1,3-Pentadiene	62204	N/A	H225, H304, H315, H319, H335
2-Methylfuran	10797	N/A	H225, H301, H319, H330, H331, H411
2-Methylquinolin-6-amine	103148	N/A	H302, H315, H319, H335
2-Nitroethanol	12252	N/A	H315, H319, H335
2,2-Dimethoxyethanol	542381	N/A	N/A
5-Amino-6-nitroso-1H-pyrimidine-2,4-dione	536741	N/A	N/A
5-Methylhexan-2-amine	34204	N/A	H226, H314
Acetaldehyde	177	Cough	H224, H302, H311, H317, H319, H332, H336, H341, H350, H360, H370, H372, H402, H335, H351
Acetone	180	Sore throat. Cough. Confusion. Headache. Dizziness. Drowsiness. Unconsciousness.	H225, H319, H336, H320, H335, H361, H372
Acetonitrile	6342	Sore throat. Weakness. Chest tightness. Shortness of breath. Dizziness. Nausea. Vomiting. Convulsions. Unconsciousness. Symptoms may be delayed.	H225, H302, H312, H319, H332
Benzene	241	Dizziness. Drowsiness. Headache. Nausea. Shortness of breath. Convulsions. Unconsciousness.	H225, H304, H315, H319, H340, H350, H372, H401, H411
Benzoic Acid	243	Cough. Sore throat.	H302, H315, H318, H319, H372, H318, H361, H373, H402, H335
Benzothiazole	7222	N/A	H301, H302, H311, H319, H332, H310, H316, H320, H402, H412
Butane	7843	Drowsiness. Unconsciousness.	H220, H280, H336, H340, H350,
Carbon dioxide	280	Dizziness. Headache. Elevated blood pressure. Increased heart rate. Suffocation. Unconsciousness.	H332, H335, H280, H336
Chloromethane	6327	Staggering gait. Dizziness. Headache. Nausea. Vomiting. Convulsions. Unconsciousness.	H220, H332, H351, H361, H373, H280, H302, H336, H360, H370, H372
cis-1,2-Dimethylcyclopropane	252321	N/A	N/A
cis-2,3-Epoxybutane	92162	N/A	H225, H315, H319, H335, H341
Ethyl Cholate	6452096	N/A	N/A
Ethyl Formate	8025	Cough. Shortness of breath. Headache. Drowsiness.	H225, H302, H319, H332, H335, H336, H370
Formamide	713	Drowsiness. Headache. Nausea. Diarrhoea.	H351, H360, H373, H360Df, H320, H360D
Hydrazoic Acid	24530	N/A	H200, H319, H335, H370
Isobutane	6360	Shortness of breath. Suffocation.	H220, H280, H332, H336, H371
Isobutylene	8255	Dizziness. Drowsiness. Lethargy. Nausea. Unconsciousness. Vomiting.	H220, H413, H280,
Isopropanol	3776	Cough. Dizziness. Drowsiness. Headache. Sore throat.	H225, H319, H336, H335, H361, H370, H372, H373
L-Cysteinesulfonic acid	1549098	N/A	H315, H319, H335
Levomenthol	16666	Irritation of the mucous membranes and upper respiratory tract. Cough. Headache. Dizziness. Nausea. Vomiting. Drowsiness. Numbness. Laboured breathing.	H315, H319, H320, H336, H402
Methyl ethyl ketone	6569	Burning sensation. Nausea. Vomiting. Convulsions. Abdominal pain. Diarrhoea. Headache. Sweating. Weakness. Dizziness. Confusion.	H225, H315, H319, H332, H335, H336, H371, H372
Nicotine	89594	N/A	H301, H310, H400, H411, H300, H335, H361, H370, H319, H330,
Nicotyrine	10249	N/A	N/A
Propane	6334	Drowsiness. Unconsciousness.	H220, H332, H340, H350, H360, H373, H302, H315, H335, H336, H280
Propionaldehyde	527	Cough. Sore throat.	H225, H302, H315, H319, H332, H336, H371, H373, H402, H335
Propylene Glycol	1030	Dry throat. Cough.	H302, H319, H410
Toluene	1140	Cough. Sore throat. Dizziness. Drowsiness. Headache. Nausea. Unconsciousness.	H225, H304, H315, H336, H361, H373, H320, H332, H335, H360, H362, H370, H372, H401, H412, H361d,
Triacetin	5541	N/A	H226, H315, H317

associated with compounds found in the tobacco cigarette sample. The most serious hazard statements include “fatal if inhaled (H330),” “fatal if swallowed (H300),” “may damage fertility or the unborn child (H360),” and “causes damage to organs (H370).” All four of which can be associated with at least one compound found in the tobacco cigarette sample. Additionally, the tobacco cigarette sample contained more compounds suspected of being human carcinogens than any of the three electronic cigarette samples. The compounds found in the tobacco cigarette sample associated with GHS hazard statements relating to carcinogenicity include the following: propane, benzene, butane, chloromethane, and acetaldehyde. Three of the five—benzene, chloromethane, and acetaldehyde—were identified in past studies as potential human carcinogens.^{18,25} Relatedly, a number of studies confirm that combustible tobacco cigarettes are inherently more dangerous to the user’s health than electronic cigarettes.^{3,17,18}

Electronic Cigarettes

Though the tobacco cigarette sample contained the majority of the most dangerous compounds, each of the electronic cigarette samples also contained a number of compounds associated with alarming GHS statements. All three of the samples contained acetaldehyde, acetone, benzoic acid, propionaldehyde, and isopropanol which were among the compounds associated with the most severe GHS hazard statements. These compounds have been identified in electronic cigarette vapour in past studies; the following information summarizes these findings:

- Acetaldehyde was detected unanimously.^{26,27}
- Acetone was detected in all studies in which it was tested.^{26,27}
- One study which tested JUUL™ vapour for the presence of benzene found a large concentration of benzoic acid in the sample, concurrent with the findings of this experiment.²⁸ However, benzoic acid has not been identified in samples of other types of electronic cigarettes.
- Propionaldehyde was only found in samples from electronic cigarettes when the battery voltage was increased considerably.
- There is no evidence that isopropanol has been found in any e-cigarette vapour samples before this study.²⁶

In some cases, the highly toxic compounds formaldehyde, acrolein, and o-methyl benzaldehyde were detected in e-cigarette vapour; however, they were not detected in this screening of the JUUL™.^{26,27}

The compounds found in the electronic cigarette samples varied slightly. There were 13 compounds found in only one of the e-cigarette samples. This variation likely exists in order to give each pod its unique flavour. For example, levomenthol, which was found only in the mint sample is derived from mint oils and is commonly used as a flavouring.²⁹

Comparison

A specific goal of this study was to evaluate an electronic cigarette and a tobacco cigarette in the same experimental environment and through the same analytical lens. Past research has shown similarities between the composition of electronic cigarette vapour and combustible cigarette smoke, but has been wary to relate the health effects of the two. However, the GHS communication system provides a way to compare the potential, rather than the observed, hazards of each compound found in the aerosols. These hazards can be compared because in both types of cigarettes, the compounds are being heated and burned to a similar temperature in order to produce an aerosol that is then inhaled into the lungs.

Specific to this study, there were nine compounds that were detected in the tobacco cigarette sample and at least one of the three electronic cigarettes. This includes acetaldehyde, one of the most dangerous compounds detected. Across the nine compounds (highlighted in red in Table 1), there are a large number of severe GHS statements that can be attributed to the vapour/smoke generated by both types of cigarettes. In addition, past studies, compiled in the PubChem compound database, have identified a number of potential inhalation symptoms that are associated with the compounds that are consistent between the tobacco cigarette and electronic cigarette samples including sore throat, cough, drowsiness, dizziness, headache, and nausea to name a few.²³ This compositional congruity provides convincing evidence that the chemicals found in electronic cigarettes are significantly similar to those found in combustible tobacco cigarettes; consequently, inferences can be made about the

health effects of e-cigarette use based on the effects of tobacco cigarette use that have already been identified.

With this in mind, it is important to evaluate the full scope of these health effects as they may impact users and public health in general. In the twentieth century, combustible tobacco cigarettes grew in popularity until the growth plateaued in response to new information about the health effects associated with their use. Shortly after, ad campaigns and new FDA regulated labelling assisted in further halting the cigarette smoking epidemic, but these new cessation methods did not take into consideration electronic cigarettes.²⁰ Now, the United States is experiencing a similar phenomenon; however, instead of combustible tobacco cigarettes, the use of electronic cigarettes is growing. Based on the findings of this study, this growth could prove to be as substantial and impactful as the growth of the tobacco industry many years ago.

Furthermore, the health effects of electronic cigarettes have the potential to be nearly as harmful to the human body as tobacco cigarettes; therefore, action must be taken to curb the growth of the electronic cigarette industry and the use of its products in order to avoid impacts similar to those caused by tobacco cigarette use. This could likely be accomplished by targeted ad campaigns and FDA regulated labelling which have already proven to be successful in reducing the number of tobacco cigarette smokers. For example, the first federally funded anti-smoking media campaign, coined "Tips From Former Smokers," reportedly motivated 1.64 million Americans to try to quit smoking in the year 2012 alone.³⁰ A similar offensive must be taken against electronic cigarettes to reduce their use, especially amongst youth and young adults.

Another tactic that could potentially assist in achieving this is the implementation of regulated labelling on, and the disclosure of chemicals found within, electronic cigarettes and related products. Though the FDA has made it clear that warning labels will be made mandatory in the near future, the content of these labels is still unknown.³¹ Ideally, e-cigarette warning labels will be modelled after those that can be found on packs of tobacco cigarettes. Also worth noting, consumers are generally unaware of the chemicals found within electronic cigarette juice and the health hazards associated with them. A system resembling the Globally Harmonized System of hazard

communication, which makes the chemicals found within specific products public knowledge, could potentially assist in notifying consumers of the dangers of using these products and motivate producers to be more careful about the chemicals that are used. Ultimately, targeted ad campaigns, regulated labelling, and public ingredient lists should be implemented with the intention of creating a more informed and wary consumer base in order to decrease the use of electronic cigarettes and stifle the growth of the e-cigarette industry.

Future Direction

Limitations

The GC Mass-Spec used in this experiment was only capable of identifying carbon-based compounds, as its intended purpose is to measure environmental contaminants. Therefore, the presence of metals in the vapour samples was not determined. There are a number of metals that can potentially be dangerous when inhaled, so a test for the presence of these metals would contribute to a more thorough understanding of the effects of electronic cigarette use. In addition, only one sample of each flavour of JUUL™ Pod and one sample of the Pall Mall cigarette were run through the GC Mass-Spec because of the cost associated with operating the machine. A study which conducts multiple trials may add credibility to the findings, but the lack of trials should have no effect on the results presented in this report because the Mass-Spec is extremely accurate. However, it is unable to quantitatively compare the concentration, or abundance, of a compound between multiple samples; it can only do so within the same sample. Thus, in order to provide uniformity, only the top fifteen most abundant compounds found in each sample were documented.

Most importantly, the only electronic cigarette that was tested in this study was the JUUL™ and the only tobacco cigarette was a Pall Mall Menthol. The JUUL™ was the subject of this study because of its popularity and originality, but it does not necessarily represent the entire population of e-cigarettes. In order to fully understand the impact that this technology may have on public health, more e-cigarettes would need to be tested. In terms of the tobacco cigarette,

it is likely that there are many different compounds found in other brands of cigarettes, but the variation has no effect on the conclusions made in this study.

Conclusion

Overall, the findings of this study confirm that electronic cigarettes should be a concern for public health. The presence of hazardous compounds in vapour generated by the JUUL™, which are simultaneously present in smoke generated by the Pall Mall menthol cigarette, shows that some of the deleterious health effects that have been previously associated with tobacco cigarette smoking can potentially also be associated with e-cigarette use. Based on the similarities identified, the electronic cigarette industry should be highly regulated in an attempt to dissuade consumers from using them, especially youth and young adults. Using the campaign against tobacco cigarettes as a model, targeted advertising, publicly available ingredient lists, and accurate warning labels may prove to be effective in achieving this goal. Ultimately, though, change will only occur if significant attention is brought to the health effects of electronic cigarettes. Therefore, continuing research is needed to keep up with technological innovation and an ever-changing market as well as to inform the public of the dangers of electronic cigarette use.

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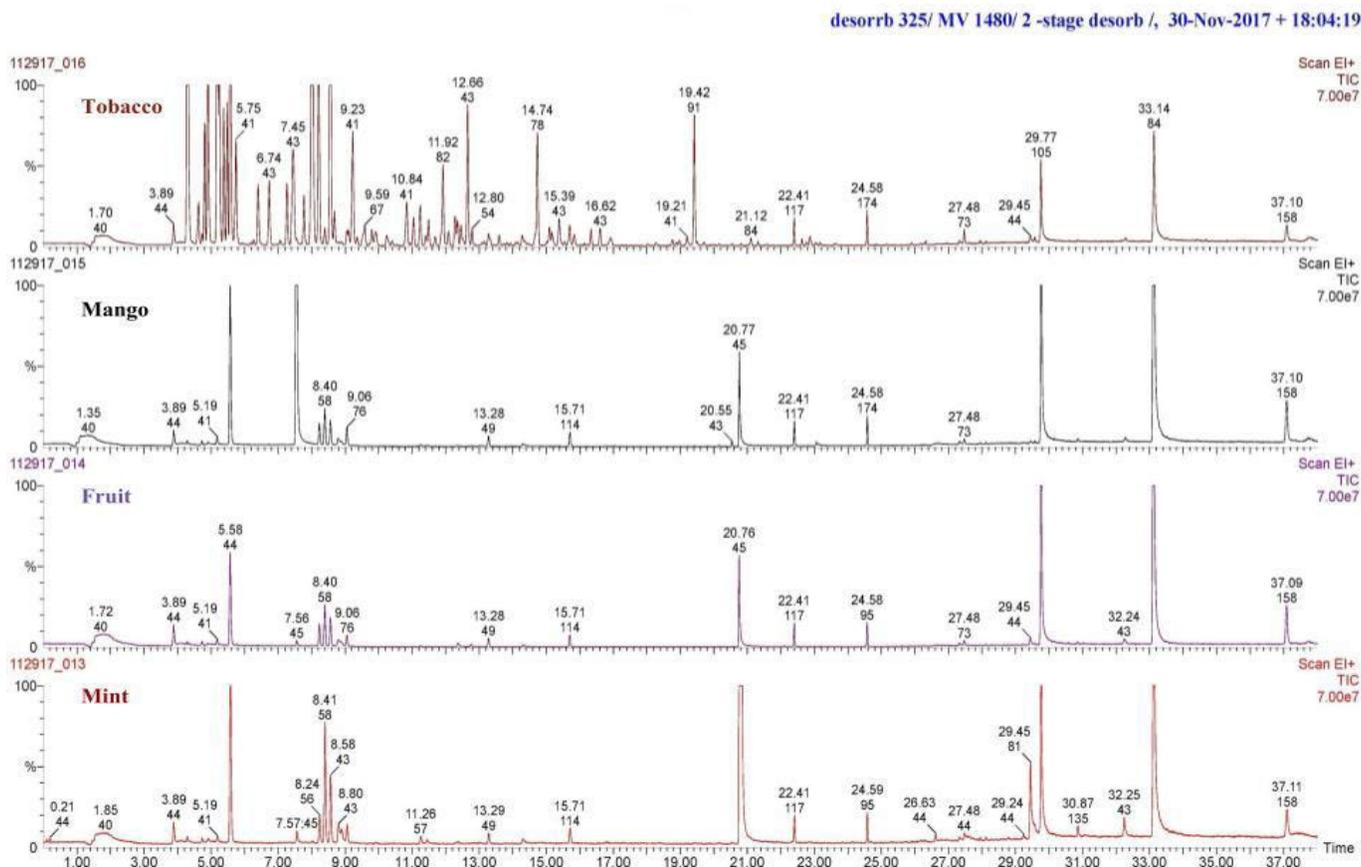
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Appendix

Figure 2. Compiled Sample Chromatograms



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Table 3. Hazard Codes and Corresponding Statements

Information retrieved from GHS seventh revised edition, published by the United Nations Economic Commission for Europe³²

GHS #	Hazard Phrase
H200	Unstable explosive
H220	Extremely flammable gas
H224	Extremely flammable liquid and vapor
H225	Highly flammable liquid and vapor
H226	Flammable liquid and vapor
H280	Contains gas under pressure; may explode if heated
H300	Fatal if swallowed
H301	Toxic if swallowed
H302	Harmful if swallowed
H304	May be fatal if swallowed and enters airways
H310	Fatal in contact with skin
H311	Toxic in contact with skin
H312	Harmful in contact with skin
H314	Causes severe skin burns and eye damage.
H315	Causes skin irritation
H316	Causes mild skin irritation
H317	May cause an allergic skin reaction.
H318	Causes serious eye damage.
H319	Causes serious eye irritation.
H320	Causes eye irritation
H330	Fatal if inhaled
H331	Toxic if inhaled
H332	Harmful if inhaled
H335	May cause respiratory irritation
H336	May cause drowsiness or dizziness
H340	May cause genetic defects
H341	Suspected of causing genetic defects
H350	May cause cancer
H351	Suspected of causing cancer
H360	May damage fertility or the unborn child
H360D	May damage the unborn child
H360Df	May damage the unborn child. Suspected of damaging fertility.
H361	Suspected of damaging fertility or the unborn child
H361d	Suspected of damaging the unborn child
H362	May cause harm to breast-fed children
H370	Causes damage to organs
H371	May cause damage to organs
H372	Caused damage to organs through prolonged or repeated exposure
H373	May cause damage to organs through prolonged or repeated exposure
H400	Very toxic to aquatic life
H401	Toxic to aquatic life
H402	Harmful to aquatic life.
H410	Very toxic to aquatic life with long lasting effects
H411	Toxic to aquatic life with long lasting effects
H412	Harmful to aquatic life with long lasting effects
H413	May cause long lasting harmful effects to aquatic life

Effectiveness of Cognitive Behavioural Therapy for Major Depression in Stage II Pancreatic Cancer Patients from the *Clínica General del Norte* in Barranquilla, Colombia

Hector Cure

Depression is a disorder with specific symptoms that include prolonged periods of sadness. Oncologic (cancer) patients usually present depressive symptoms due to the effects of cancer and the aggressive treatments that can take a toll on their health. Additionally, pancreatic cancer can induce a significant amount of psychological distress in its patients. For this reason, this qualitative content analysis study aims to determine if cognitive behavioural therapy is the most effective treatment for major depression, characterized by at least two weeks of low-mood, in stage II pancreatic cancer patients. Cognitive behavioural therapy focuses on making patients understand that their thoughts can influence their emotions, which then ends up influencing behaviour and affecting relations. The responses to 16 semi-structured post-treatment interviews are assessed in order to determine the effectiveness of the therapy. Cognitive behavioural therapy was able to address main issues in oncologic patients, such as fostering new optimistic perspectives (81.25%), providing empathetic listening (75.00%), and helping patients feel calmed (68.75%). These results suggest that cognitive behavioural therapy is effective for treating major depression in stage II pancreatic cancer patients.

Keywords: Cognitive behavioural therapy, major depression, stage II pancreatic cancer, Colombia

Introduction

Cancer is a chronic disease that affects countless people worldwide. This disease consists of the abnormal and uncontrolled division of cells in the body, invading different tissues and organs. Different types of cancer can aggressively affect patients and decrease their lifespan exponentially by forming masses of tissues called tumours in functional organs, disrupting the organs' optimal functioning (National Cancer Institute). Therefore, doctors have to provide patients with treatments to reduce the pain this disease may inflict. However, cancer can deteriorate the mental health of patients by inducing psychological distress,

leading to a variety of disorders, including major depression (National Cancer Institute, 2017).

Depression is a medical illness that negatively affects thoughts, emotions, and behaviour as it is characterized by prolonged periods of sadness, loss of interest in activities once enjoyed, and feelings of guilt or worthlessness (American Psychiatric Association, 2017). For every ten patients diagnosed with cancer, two of them become depressed (National Cancer Institute, 2017). This occurs when patients learn they have cancer, feel unmanageable pain, are physically weakened by the disease, view themselves as a burden to others, or take cancer medicines that have been correlated to depression, such as amphotericin B, procarbazine, interferon alfa, l-asparaginase, corti-

costeroids, and interleukin-2 to treat this malady (National Cancer Institute, 2017). Consequently, it is of utmost importance that doctors treat depression for oncologic patients to maintain a healthy immune system, prevent suicide attempts, and reduce depressive symptomatology.

In Colombia, studies there are a few studies that depict effective treatments for depression in cancer patients. For instance, Rodriguez, Amboage, Blazquez, Torres, and Gaviria (2015) describes the effectiveness mindfulness, relaxation, and visualization techniques for treating major depression in cancer patients (Rodriguez et al., 2015). In addition, one of the most prominent studies conducted in Colombia by psychiatrists Mauricio Murillo and Ariel Alarcón, provide the steps doctors should take for effectively treating depression in oncologic patients by advising them to use different psychotherapy interventions (Murillo & Alarcón, 2006). However, none of these studies in Colombia has addressed the effectiveness of a specific type of therapy in a single type of cancer. After all, there are about 45 influential schools of thought in psychology, and each one has their way of managing mental disorders like depression through the techniques and interventions advised by the researchers in the studies mentioned previously. Additionally, both of these studies do not take into account the various type of cancer but instead base on the assumption that the treatments for major depression are as equally effective for all types of cancer. This study aims to specify the knowledge in the field by focusing on the effectiveness of a single type of therapy, cognitive behavioural therapy, and one of the most aggressive types of cancer, pancreatic cancer, as 33-55% of its patients face depression (Massie, 2004).

The general purpose of this research study is to analyze the effectiveness of cognitive behavioural therapy for treating stage II pancreatic cancer patients in the *Clínica General del Norte* in Barranquilla, Colombia. The *Clínica General del Norte* is the site for this study as it is one of the largest hospitals in Colombia treating gastroenterological diseases and pancreatic cancer. A content analysis and interview method are used to evaluate the content of the responses to 16 semi-structured post-treatment interviews on stage II pancreatic patients to determine the effectiveness of the therapy. For this study, a sample of pancreatic cancer patients in stage II is collected due to the advice of

the staff at the hospital because there are not as many patients in further stages in conditions to speak entirely for an interview.

Literature Review

When it comes to identifying a type of cancer, staging is used to describe the cancer size and how far it has grown, providing essential information to develop a plan for treatment. For pancreatic cancer, the stages range from stages I (1) through IV (4) and the higher the stage, the more advanced the cancer is (American Cancer Society, 2017). In stage I the tumour is confined to the pancreas and is smaller than two centimeters; in stage II cancer might have spread to three or less nearby lymph nodes (small swellings throughout the body's lymphatic system that aid in immune responses); in stage III cancer can reach a size of over four centimeters and may have spread to four or more lymph nodes; and in stage IV cancer can be any size and has spread to distant sites, such as the peritoneum (lining of the abdominal cavity), liver, lungs, or bones (American Cancer Society, 2017). As cancer in the pancreas advances, several problems begin to present and these include, but are not limited to, jaundice (yellowing of the skin and eyes), pain in the abdomen and back, nausea and vomiting, gallbladder and liver enlargement, blood clots, and diabetes (American Cancer Society, 2016).

At the Johns Hopkins Oncology Center, Clark, Loscalzo, Trask, Zabora, and Philip (2010) investigated the psychological distress experienced by patients diagnosed and treated with pancreatic cancer, the fourth major cause of cancer deaths in the United States. These researchers determined that pancreatic cancer patients usually demonstrate elevated levels of distress when compared to other types of cancer due to the physical symptoms that become present (Clark et al., 2010). In fact, while depression is seen in 20% of cancer patients in general (National Cancer Institute, 2017), this percentage elevates to 33-55% when it comes to pancreatic cancer patients (Massie, 2004). Although there is no current data regarding depression rates for pancreatic cancer in Colombia, this percentage remains in 17% and 28.8% when it comes to other Spanish speaking countries like Spain and Ec-

uador (Rodríguez et al., 2015; González et al., 2015).

Pancreatic cancer patients who suffer from depression deal with certain difficulties in their daily lives. Specifically, psychologists from Edinburgh pinpointed five main troubles cancer patients with major depression face: concern for other people's well-being (65%), difficulties in interpersonal relations (61%), loss of interest in activities (56%), low mood (55%), and fears of cancer recurrence (54%) (Kleiboer, 2011). Consequently, current psychotherapies should address these issues that cancer patients experience.

Today, there is a variety of treatments for depression in oncologic patients in Colombia. Colombian psychiatrists Mauricio Murillo and Ariel Alarcón in their article, "Psychosomatic Medicine Treatments in Cancer" provide a current guideline for Colombian psychiatrists to effectively treat depression in oncologic patients. They summarize the process of psychological treatment for oncologic patients, which includes: a psychoeducative group, an initial psychological diagnosis, common psychological follow-ups, group psychotherapy (focusing mostly on psychodynamic therapy), individual psychotherapy (focusing more on expressive and supportive psychotherapy), family intervention, and "unspecified" interventions (Murillo & Alarcón, 2006). Rodríguez et al. (2015) also explicitly state the usefulness of using mindfulness, relaxation, and visualization interventions for treating major depression in cancer patients after reviewing the premise of each one of them and how in theory it can help reduce depressive symptomatology (Rodríguez et al., 2015). Although many types of interventions are recommended and used to treat depression in oncologic patients in Colombia (Murillo & Alarcón, 2006; Rodríguez et al., 2015) no studies indicate which treatments are the most effective for treating depression in patients with aggressive cancers, such as pancreatic, or focus on a specific type of therapy.

At present, the most common types of psychotherapies include cognitive behavioural therapy, supportive-expressive group therapy, problem solving therapy, cognitive-existential group therapy, supportive therapy, and mindfulness-based stress reduction. However, cognitive behavioural psychotherapy is the main psychological intervention performed on patients with pancreatic cancer at the *Clínica General del Norte* in Barranquilla due to the hospital's positive

experience with this treatment over the past years. Barrera and Spiegel (2014) define this treatment as a therapeutic modality that focuses on making patients understand that their thoughts can influence their emotions, which then ends up influencing behaviour and affecting relations (Barrera & Spiegel, 2014). This type of therapy includes psychoeducation, which is one of the key aspects in psychotherapies in Colombia as described by Mauricio Murillo and Ariel Alarcón (2006) in "Psychosomatic Medicine Treatments in Cancer." Although there is a variety of cognitive therapies, all of them follow the same assertion: a person's negative reasoning most likely will lead to illogical thinking and, therefore, dysfunctional behaviour (American Psychological Association).

Recent studies support the effectiveness of cognitive behavioural therapy for treating depression in cancer patients. At Comprehensive Cancer Center at the Ohio State University, Brothers, Yang, Strunk, and Andersen (2011), found that 61% of 36 cancer patients had significant clinical changes after receiving cognitive behavioural therapy for treating major depression after being diagnosed with cancer, deeming the treatment effective (Brothers et al., 2011). Additionally, in another study at the Imam Reza hospital in Birjand, Khodai, Dastgerdi, Haghghi, Sadatjoo, and Keramati (2011) concluded that cognitive behavioural therapy is essential for decreasing depression in patients with cancer. They claimed the treatment worked because it fostered positive thoughts as in a sample of 24 patients receiving the therapy there was a statistically significant decrease in depression, while no change in the control group (Khodai et al., 2011).

Arguments regarding the effectiveness of psychotherapies versus antidepressants have emerged over time. The American Psychiatric Association Practice Guidelines (APAPG) claim that cognitive behavioural therapy and interpersonal psychotherapy are the most adequate treatments for major depression as their effectiveness compares to that of antidepressants (Horne & Watson, 2011). Additionally, in some cases psychotherapies are preferred over antidepressants as in the Brown University Child & Adolescent Psychopharmacology Update it is stated that the FDA urged manufacturers to include warning statements and recommendations in antidepressant containers, which are correlated to an increase in certain depressive symptoms and suicidality ("FDA Cautions Use of Antidepressants; APA Responds," 2004).

Determining the usefulness of a treatment for depression in patients suffering from a specific type of cancer is of utmost importance to improve the patient's mental and physical health. In fact, at the University of Malaya, Chan, Ahmad, Yusof, Ho, and Krupat (2014) found that depressed cancer patients are 4.31 times more susceptible to death than those who don't suffer from depression (Chan et al., 2014). Hence, this study attempts to determine if cognitive behavioural therapy is effective for treating major depression in patients suffering from stage II pancreatic cancer, filling the gap in the field by addressing a specific type of cancer and therapy.

Methods

Overview

For this study, a content analysis and interview method are used in which inferences are made by interpreting and coding material, in this case, interviews. The cohort consisted of adult patients suffering from stage II pancreatic cancer and depression at the *Clínica General del Norte* in Barranquilla, Colombia, who received five sessions of cognitive behavioural therapy. The interviews evaluate the efficacy of this treatment by providing insight into its advantages and disadvantages. This methodology is adapted from Orengo-Aguayo and Segre (2010), in their study, "Depression treatment delivered at the point-of-care: a qualitative assessment of the views of low-income US mothers." Orengo-Aguayo and Segre (2010) evaluate the views of low-income US mothers facing depression on a psychological intervention called "Listening Visits" through content analysis and interview method with participants' responses to a post-treatment semi-structured interview assessing their views of the intervention (Orengo-Aguayo & Segre, 2010). Therefore, these researchers were able to determine the strengths and weaknesses of the psychological intervention provided to the patients, and the possibility of making it available for a larger population. For this reason, the content analysis and interview methods are applicable for this research study as they would elucidate the views of stage II pancreatic cancer

patients facing depression regarding the effectiveness of the cognitive behavioural therapy delivered at the *Clínica General del Norte*.

The data used in the present study was gathered from Colombian patients who underwent cognitive behavioural therapy. At the *Clínica General del Norte*, 16 patients were enrolled to receive the psychological therapy in 2018. The Institutional Review Board (IRB) approved the procedure for this study, and all patients signed an informed consent form. In general, patients were screened by a psychologist at the hospital for depression using the Hospital Anxiety and Depression Scale (HADS), a fourteen-item scale that yields data related to levels of anxiety and depression (Snaith, 2003). Those pancreatic cancer patients scoring ≥ 11 were flagged as having reported major depressive symptoms (Snaith, 2003), and their doctors, with the permission of the participants, kept the patients' medical information in a designated folder at the hospital to take them into account for the study. The HADS scores were reviewed once again by the psychologist and interviews were conducted to verify that patients who did not meet the criteria were excluded from the study (criteria for exclusion includes current alcohol or substance abuse, psychotic symptoms, history of depression, or any other type of chronic disease). Since all patients met the criteria for participating in the study, the patients were enrolled by asking them to sign an informed consent form that described the purpose of the study. The patients received five cognitive behavioural therapy sessions within a three-week timeframe. Following advised practices from Mayo Clinic, due to the fact that it is a top-rated institution in the United States, the number of cognitive behavioural therapy sessions at the *Clínica General del Norte* usually varies between five to twenty, depending on whether the treatment is short, medium, or long-term; the level of depression, gravity of symptoms, speed of progress, and amount of stress; and the extent of support from relatives (Mayo Clinic, 2017). The choice of five sessions was feasible for the Colombian-based trials at the *Clínica General del Norte* as the study was to be conducted in three weeks. For cognitive behavioural therapy at the hospital, there is no stipulated protocol for the number of sessions, but all participants completed five sessions initially. Patients at the hospital usually receive the therapy for as long as they are

hospitalized and after the study was completed with the initial five sessions, the patients who remained hospitalized continued to receive the treatment. For research assessments, patients completed two interviews. The hospital's psychologist conducted the first one to diagnose major depression in the patients. The second and final interview was conducted at the end of the three-week time frame for the assessment of the treatment.

Participants

The patients in this study underwent cognitive behavioural therapy sessions. In the sample of 16 participants, the average age was 63 years-old; most people are diagnosed with pancreatic cancer are within this range (45 years-old and above) (American Cancer Society, 2016). All of these patients suffered from major depression and stage II pancreatic cancer. Additionally, they all were undergoing chemotherapy. All of the patients were Colombian.

Procedure

Of the 16 participants, all of them agreed to be interviewed to share their views regarding cognitive behavioural therapy, and the qualitative assessment was completed after these interviews. For the interviews, the participants' responses were recorded in the form of notes. All the interviews were recorded in Spanish, Colombia's official language, and then transcribed into English. Afterward, the notes were passed into an electronic document and organized in tables for further extraction and analysis. The interviews were not audio-recorded because this population of patients in critical situations might be reluctant to participate if they were audio-recorded. Instead, it was decided to safeguard the environment of trust in the study by making an effort to record the patients' responses verbatim in written form. In the interviews, the patients were encouraged to share their honest opinion about cognitive behavioural therapy and were told how valuable their feedback was for the improvement of the service.

Measures

In the interviews, patients were asked three open-ended questions that can be used to assess the participants' views about the treatment for major depression around three main domains: participant impression (Q1: *When your healthcare provider first told you that he or she wanted to do this treatment with you, what did you think about this idea?*), treatment success (Q2a: *What do you think about the treatment in terms of its helpfulness?* and Q2b: *Can you describe for me a specific example of how the treatment was helpful to you?*), and suggested improvements (Q3: *What would you change about the treatment?*) (Orengo-Aguayo and Segre, 2010).

Data Analysis

Using qualitative content analysis, the themes that surfaced from the patient's responses to the questions were recorded and defined. This analytic approach is optimal to identify the themes that appear in the data and classify them systematically by using codes, thus emphasizing the patients' perspectives on a given topic. This method included a two-step basic approach, congruous with a "goal-free" evaluation (Scriven, 1991) where the patients' views about the helpfulness of cognitive behavioural therapy were assessed. In the first step, a coding manual was developed to code the interviews. All responses to each question were read to generate themes that arose from the data. The themes were then transformed into a group of clear codes for each question. Moreover, each code was defined by including important characteristics of what to include if seen in the responses. The objective was to create an understandable manual where the codes for the questions express the themes that were seen in the responses. In the second step, the responses were coded. The object of analysis was the response provided by a patient to a question coded. The responses to the three questions were coded independently. Finally, the analysis of the data analysis was expressed through a quantitative abridgment of the patients' coded responses that reflect how many of the participants favoured a code (Table 1). Additionally, paraphrased responses are presented for illustrative purposes and, each one contains the patient's number in the study.

Results

The data is organized in Table 1 in such way that the themes in the responses to the three questions are shown. For each of the items, the percentage of participants endorsing each thematic code is presented along with the number of times the theme was recurrent in the responses of the 16 patients.

Initial Views

The patients' initial impressions of cognitive behavioural therapy are expressed in the four main themes that came up from the data (Table 1). The majority of the patients (75.00%) endorsed cognitive behavioural therapy.¹ Some of the patients (12.50%) expressed some uncertainty about cognitive behavioural therapy but were open or curious to receive the interven-

Table 1.
Themes: Patients' Responses in Interviews (N = 16)

Initial Views About Cognitive Behavioural Therapy	Number (percentage)
Endorsed	12 (75.00)
Uncertain Positive	2 (12.50)
Uncertain Negative	1 (6.25)
Negative	1 (6.25)
Examples of How Cognitive Behavioural Therapy Was Helpful	Number (percentage)
Fostered a new perspective (positive thoughts)	13 (81.25)
Supplied empathy and a space to talk	12 (75.00)
Were calming or relaxing	11 (68.75)
Turned to faith	9 (56.25)
Gave advice and were helpful in problem-solving	9 (56.25)
Improved communication in interpersonal relationships	7 (43.75)
Helped patient feel less isolated	6 (37.50)
Medical information was provided	3 (18.75)
Participant did not find cognitive behavioural therapy helpful	1 (6.25)
Suggested Changes to Cognitive Behavioural Therapy	Number (percentage)
No change	9 (56.25)
Longer sessions/more sessions	6 (37.50)
Medication	1 (6.25)

* Note: Patients could address several themes, therefore, percentages can exceed 100%

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tion.² One patient (6.25%) was skeptical of receiving cognitive behavioural therapy because she didn't feel comfortable talking about something so personal but decided to go forward with it.³ Additionally, one patient (6.25%) had a negative view regarding the treatment as he denied the usefulness of psychological therapies but did not care about having to receive the treatment.⁴

Examples of How Cognitive Behavioural Therapy Was Helpful

In the sample, one patient (6.25%) claimed that cognitive behavioural therapy wasn't useful. However, the majority (81.25%) of participants in the study indicated that cognitive behavioural therapy helped them view their situation with a new positive perspective (Table 1).⁵

The second most frequently disclosed helpful aspect of cognitive behavioural therapy, noted by 75.00% of participants, was that the intervention provided empathy (the empathy displayed by the psychologist) and a space to talk. Additionally, most patients felt calmed after the sessions (68.75%), reducing their feelings of anxiety. Turning to the Catholic faith (56.25%) and reduced

feelings of loneliness (37.50%) were mentioned as well.⁶ Some patients thought that the therapy was useful in terms of providing advice and solving problems they might face (56.25%); it improved their relations with their relatives (43.75%), especially communication; and that they received medical information in case they had questions about their health situation (18.75%).⁷

Suggested Changes

When the patients were asked if they would change anything about the treatment they received, about half (56.25%) said that they would not change anything.⁸ Of the patients who did suggest any changes, 37.50% stated that they would have liked more or longer sessions.⁹ One patient (6.25%), who did not believe that cognitive behavioural therapy helped him, suggested he be prescribed antidepressant medication to help him neurobiologically.¹⁰

Discussion

Stage II pancreatic cancer patients received cognitive behavioural therapy as a possible intervention to

1 Yes, I accepted this treatment because I knew I needed to face my problems in order to move on, and even more when I have four children waiting for me to come home. (Patient 2)

2 I was apprehensive of receiving the treatment at first because I wasn't really sure what it was, but decided to go for it because I wasn't feeling well internally. (Patient 6)

3 I was doubting if I should receive the treatment at first because I didn't feel comfortable talking about this difficult situation with other people. In the end, the doctor was able to convince me to go forward with it. (Patient 10)

4 Initially, I was confused about receiving a psychological treatment because I've never gone to a psychologist in my life. I didn't care, but I knew I wasn't crazy and that God would give me strength to move forward. (Patient 12)

5 I felt that I was treated well. It was helpful in every aspect, especially because I was now seeing life in a beautiful way. I realized that I want to enjoy the rest of my time with family. (Patient 11)

6 The sessions helped keep a positive attitude. I always believed that God would take me out of this dark hole. As time passed, I began feeling less anxiety and more open to talk, especially because of the empathy I received from the psychologist. I finally understood I wasn't alone in this situation. (Patient 6)

7 I felt that I wasn't alone and that I was being heard and understood by the psychologist. Initially, I was extremely anxious and worried, but I received clear and pertinent information about my disease. The doctor advised me to turn to family for support as well. (Patient 8)

8 I wouldn't change anything. As it is, it is excellent. Everyone was so professional and it was oriented to what I needed. It helped me get out of a crisis. (Patient 2)

9 I would like to have more sessions because they really helped me a lot, and I believe there is more work to do in order to feel even better. (Patient 8)

10 If you wanted to treat my depression, why not just give me medication. Everything is in the brain and works through chemicals. I didn't need the therapy. (Patient 12)

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treat major depression. This study assessed their views toward this type of therapy, delivered by a psychologist from the hospital where they were admitted. To condense the results, three main findings were observed. First, when the doctor first discussed therapy with the patients, the majority (75.00%) had supportive views of the therapy. Second, the patients indicated that the most helpful component of cognitive behavioural therapy was that it provided them with a new perspective on their situation, allowing them to think positively. Third, a little more than half of the patients would not change anything about the delivery of the treatment, and 37.50% would have liked longer or more sessions.

After reviewing patient responses, the high percentage of positive views most patients had when introduced to cognitive behavioural therapy could be attributed to the fact that the patients were able to internalize that they were not themselves anymore and that their diagnosis completely changed their mentality. For instance, both patients 3 and 5 voiced their concerns of had similar responses as both addressed their concern of not feeling mentally well, mentioning that they knew they “needed the support from a psychologist” because they knew they were “in a bad mental state” (Patient 3) and were not “feeling like [themselves] anymore” (Patient 5). Moreover, some patients mentioned their endorsement towards the treatment because they had hopes of going back to their families and enjoying the time they had left with them. Patients 1 mentioned that he had “the encouragement to move forward for [his] family” (Patient 1) and patient 2 claimed that she realized she had to face her problems and “even more when [she has] four children waiting for [her] to come home” (Patient 2). The most common views of hope and cancer seen in the media express that patients with cancer only have the hope of a cure (Duggleby et al., 2010). However, the sample expressed hope for improving their quality of life, which encouraged them to receive psychotherapy. These findings are in agreement with European psychologists who found that oncologic patients’ meaning of hope focused on comfort, maintaining relationships, and peace (Duggleby et al., 2010). Furthermore, there was only one patient in the data that presented negative views regarding the treatment. This outlier could be attributed to the patient’s lack of understanding of the treatment and high religious value as he stated that he had never gone to a psycholo-

gist before and believed that God would help him move forward, especially after mentioned that he “wasn’t crazy and that God would give [him] the strength to move forward” (Patient 12). This patient didn’t believe the treatment helped him and wanted antidepressant medication instead by stating that “everything is in the brain and works through chemicals” (Patient 12).

After receiving cognitive behavioural therapy, 81.25% of the patients in the study noted that the most helpful aspect of the therapy was that they were encouraged to adopt a new perspective of their situation, especially one that is generally positive. This majority showcases that the therapy implemented served its purpose as it focuses on the importance of the patient understanding how obstructive thought patterns as a consequence of an adverse situation can affect their behaviour (Barrera & Spiegel, 2014). Therefore, since the treatment helped the patients generate new positive thoughts about their situation, it means that their cognition was transformed to think and, therefore, act in a healthy way to cope with their disease, following the premise of cognitive behavioural therapy. For example, one of the patients mentioned that the therapy was “helpful in every aspect, especially because [she] was now seeing life in a beautiful way” and “realized that [she] wanted to enjoy the rest of [her] time with family” (Patient 11). Additionally, 75% of the patients claimed that the intervention supplied empathy and a space to talk. Most of these patients deal with high levels of stress, especially because pancreatic cancer has the highest rate of psychological distress when compared to other cancer groups (Clark et al., 2010). Hence, they might need to vent their concerns during the sessions. In effect, the *Handbook of Psychotherapy in Cancer Care: The International Psycho-oncology Society’s Training Guide* indicates that in the first step of cognitive behavioural therapy, patients should have the opportunity to talk for “simple ventilation of emotions,” as it allows to build trust between the patient and psychologist and to clearly establish that the patient feels the need to change their coping mechanisms (Horne & Watson, 2011, p. 45). As most patients felt they had an ample opportunity to talk and share their story with the psychologist, building trust for further effective sessions. Patients also mentioned feeling extreme pain, which made them feel stressed and anxious most of the times. Both chronic and acute pain can generate elevated amounts of stress in onco-

logic patients, and these events, as well as knowing the diagnosis, can lead to extreme levels of disquietude or demoralization; but cognitive behavioural therapy can be useful in managing pain (which is associated to uncertainty, death, and disease progression) by helping patients to not view the pain as a sign that the disease is advancing, helping reduce worrisome feelings (Horne & Watson, 2011, p. 44). The results show that the sessions of this psychological therapy helped the patients feel more calmed and relaxed, making their levels of anxiety decrease. Patient 4 was one of the 68.75% of the patients who mentioned that the treatment helped reduce feelings of anxiety or worry: "In the beginning, I felt full of angst, but [the psychologist's] words calmed me down... Also, there were a lot of resources to understand more my condition, which I usually wanted to know about when I felt pain around my stomach as I began to worry" (Patient 4). This depicts how the therapy reduced patient's levels of stress through effective communication with the psychologist and calmed the patient by providing useful information about his or her medical condition.

Recent studies in the Comprehensive Cancer Center at the Ohio State University and the Imam Reza hospital in Birjand have shown that cognitive behavioural therapy has been effective in significantly reducing depressive symptomatology in cancer patients with depression by enabling them to have a more optimistic regarding their situation (Brothers et al., 2011; Khodai et al., 2011). Considering that patients with cancer and depression face certain troubles and concerns in their daily lives, this study might also suggest that cognitive behavioural therapy is effective in diminishing these issues. Some of the main problems faced by depressed cancer patients are "concerns about other people's well-being," "problems in interpersonal relations," "loss of interest," "low mood," and "cancer recurrence" (Kleiboer et al., 2011). However, cancer recurrence does not apply to this study as the patients have not yet recovered from cancer. The results show how that 43.75% of the patients addressed interpersonal relations issues, indicating that they were able to improve their communication with their family members or friends. Further, 56.25% of the patients claimed that the therapy was useful in problem-solving and in giving advice. Patient 15 mentioned that, in the treatment, she received advice on how to deal with knowing that her family is suffering due to her

cancer diagnosis. Thus, concerns about other people's well-being are addressed. Also, feelings of concern for family members can also lead to feelings of isolation or loss of interest. Patient 16, for instance, mentioned that the therapy helped change how she isolated herself from her family: "She [the psychologist] helped me solve problems I had with my family by making me realize that I shouldn't just hide away from them to not make them suffer" (Patient 16). In turn, the data shows that 37.50% of the sample felt that the therapy was vital in reducing their feelings of isolation, which can improve mood or diminish the loss of interest due to new optimistic perspectives and better bonds with family members or friends (Ge et al., 2017).

Furthermore, the data indicate that more than half of the patients (56.25%) strengthened their religious faith after receiving therapy. Most patients, who identified as Catholic, mentioned that they knew that God would help them overcome their current situation. In fact, patient 13 claimed that she prayed with the psychologist: "We also prayed together, and God gave me a lot of hope in this situation" (Patient 13). None of the studies regarding cognitive behavioural therapy mention religious faith as a possible aspect to treat depression or even an outcome after receiving cognitive behavioural therapy. This theme might have emerged due to cultural factors; approximately 80% of the Colombian population is Catholic (U.S. Department of State, 2009). A prevailing trend, which emerged from the data, is the role of religion as a coping mechanism; the cohort expressed belief in a God that has and continues to help alleviate their depression and cancer. Generally, there is a strong positive correlation between hope and spiritual well-being in cancer patients at any stage, and this relationship increased in the fourth (last) stage of cancer (Liaquat et al., 2013). Patients tended to increase their focus on their relationship with God as they experienced pain, giving them hope to cope with cancer and major depression. Henceforth faith can become a key aspect of cognitive behavioural therapy as in several patients it had the potential to change negative thought patterns to ones full of optimism and hope: "I always kept my faith and knew that in one way or another, God would get me out of this situation" (Patient 5). In less religious contexts with medical practitioners or patients who are disinclined to invoke this avenue of therapeutic conversation, it is also possible to solely focus on

optimism regarding their current situation, which is the core of the treatment (Horne & Watson, 2011).

After asking the patients if they would suggest any changes, the majority (56.25%) answered that they would not change anything as their treatment was successful. Furthermore, six patients (37.50%) mentioned that if they were to change anything, they would want longer or more sessions. Cognitive behavioural therapy is known to be a short-term psychological therapy, adjusting to the need of the patient. However, it might be possible that five sessions of cognitive behavioural therapy were not sufficient as in some instances patients might need more sessions to improve their mental health (Mayo Clinic, 2017). Additionally, the hospital would not, in some cases, have the patients hospitalized for more than a month, limiting the psychologists' access to the patient for follow-ups or more sessions. For this reason, it might be ideal to implement programs, such as internet-delivered cognitive behavioural therapy (ICBT). In fact, Swedish psychologists from the University of Gothenburg concluded that ICBT, which can last up to 12 months, was as effective as cognitive behavioural therapy after the first six months (Erikson et al., 2017). ICBT sessions would be more feasible for patients and psychologists as such sessions would consist of seven online modules with activities to be completed and weekly therapist e-mail or telephone support.

Limitations

There are limitations to this study that may have hindered the accuracy of the results. First of all, the sample size of the study was too small. This can be attributed to the rareness and severity of stage II pancreatic cancer. In a study at the Memorial Sloan Kettering Cancer Center in the United States, the researchers were able to collect a sample of 467 patients in 15 years, averaging around 31 patients per year (Argüello, 2006). However, since pancreatic cancer in the United States is two times more common than in Colombia (Argüello, 2006), an appropriate sample for Colombia is approximately 15 patients a year. Also, the severity of pancreatic cancer can take a toll on the patients' health in unpredictable ways, giving

them only a few months left to live. Most patients do not fully recover, and have less than six months to live, while those who do undergo a successful surgical procedure (only 20%) have less than 15 months, which is still significantly low (Argüello, 2006). This might be another significant factor to explain the small sample size. Consequently, having a small sample size raises the question if this study can be generalized to Colombia, therefore, further investigation with a larger and diverse sample size is required to be able to generalize this study to a population beyond this cohort. Moreover, the interviews were not audio recorded. Although the participants' answers were recorded as notes, it was not possible to verify that their exact words were transcribed for verbatim, especially after translating responses from Spanish into English. However, the main idea of what the patients were saying should not have changed much. Another limitation was the fact that hospitals could not usually hold patients for more than a month, making it hard to calculate results based on only five sessions, which probably made the results slightly skewed. For further research, it would be advisable to increase the number of therapies to determine if the results are the same. Additionally, having a control group or an alternate version of the therapy to compare in this study might generate a more accurate analysis of the situation now that the passage of time or an alternative treatment might be as effective as cognitive behavioural therapy. Furthermore, two main biases might have affected the honest response of the patients. First, the patients were told that their feedback was valuable for the improvement of the delivery of the therapy, possibly pressuring them to respond with positive examples of how the therapy worked. The second bias might have arisen from the location of the interviews. Since the interviews were performed in the hospital, it might have influenced patients to endorse the therapy and provide positive feedback regarding the delivery of the treatment as it is possible that they don't feel comfortable answering the contrary while in the hospital that is providing them healthcare.

Conclusion and Future Directions

In this study, the majority of stage II pancreatic cancer patients suffering from depression indicated that they valued cognitive behavioural therapy as an approach to treat depression. Although cognitive behavioural therapy has proven to be successful in treating major depression in cancer patients in recent studies conducted at the Comprehensive Cancer Center at the Ohio State University and the Imam Reza hospital in Birjand (Brothers et al., 2011; Khodai et al., 2011), the significance of these findings are that they present a pioneer study in Colombia; this study suggests that cognitive behavioural therapy as a plausible effective therapy for major depression in pancreatic cancer patients specifically. Studies like the ones conducted by Murillo and Alarcón (2006) and Rodríguez et al. (2015) on therapies for depression in oncologic patients don't address the course of treatment for specific types of cancer (Murillo & Alarcón, 2016; Rodríguez et al., 2015), and the way therapies for depression might differ for patients who suffer from different cancer types. Therefore, further research should examine the views of a large sample of oncologic patients who are treated with cognitive behavioural therapy but suffer from other types of cancer in order to see if there is any variation. This would elucidate the relationship between cancer types and cognitive behavioural therapy by depicting for which type of cancer patients suffering from depression the treatment might work better. Finally, the implementation of cognitive behavioural therapy in other hospitals in Barranquilla as a standard treatment for major depression in pancreatic cancer patients may help to significantly reduce depressive symptomatology and improve quality of life in this type of patients, but further investigation is required.

Acknowledgements

I would like to thank the *Clínica General del Norte* in Barranquilla, Colombia, for giving me their full support to conduct my study there and interview their patients. I would also like to give a special thanks to Dr. Laura Ojeda, the chief psychologist of the hospital, for helping me understand the theory behind cognitive behavioural therapy for facilitating interviews with eligible patients.

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Shifting Perceptions: A Quantitative Experimental Study Regarding the Effect of Positive Kinesics on People Who Stutter

Steven Du

This study examined the effects of kinesics on male adolescents' perception of people who stutter (PWS). Twenty-four male participants from an all-boys high school viewed an audio clip of a person who stutters (PWS), a video of a PWS exhibiting positive kinesics, and a video of a PWS exhibiting negative kinesics. After each clip, the participant completed a semantic differential survey to determine the participant's perception of the speaker. The data was analyzed statistically using XLSTAT. The results indicate that positive kinesics has a significant positive effect on perception, kinesics has a significantly different effect on the perception of male and female speakers, and the perceptions of PWS may have shifted over time. The results did not show that negative kinesics had a significant negative effect on the perception of PWS.

Keywords: stuttering, stutterer, people who stutter, kinesics, perception,

Introduction

For 75 minutes, Philip Garber kept his hand raised, wanting to pose a question about the exploration of the New World to his history class. The professor, however, showed no intention of acknowledging him. In fact, she had already told him not to speak in class, characterizing his speech as “disruptive” and an “infringe[ment] on other students’ time”. Despite his talkative nature, Philip has an acute stutter that makes expressing his ideas difficult. While Philip’s story, as reported by Perez-Pena (2011) in the *New York Times*, is an especially egregious example of discrimination against people who stutter (PWS), it reflects a broader negative perception of PWS.

The current research on the perceptions of PWS

shows that these negative perceptions of PWS have a deleterious effect on their interpersonal relationships, sociality, and employment opportunities (McAllister, Collier, & Shepstone, 2012; Plexico, Manning, & Levitt, 2009; Van Borsel, Brepoels, & De Coene, 2011). Although there are defined consequences that stem from a negative perception of PWS, the factors that affect this perception are less clear. Specifically, there is a gap in knowledge within the understanding of how the kinesics, which are the visual aspects of non-verbal communication, of PWS affect how others perceive them. Previous studies have focused on the perceptions of speech impediments independently of appearance (Rice, Hedley & Alexander, 1993; Mcallister, et al., 2012; Blood & Blood, 2007; Craig & Hancock, 2003; Woods & Williams, 1976). These studies have

concluded that stuttering is perceived negatively with lower ratings ranging from confidence to ability to succeed (Rice et al., 1993; Woods & Williams, 1976). Other studies have determined there is also a negative correlation between stuttering and educational outcomes (McAllister et al., 2012). The results of these studies, which are generally negative, may contribute to the growing negative perception of PWS.

Nonetheless, the current research has also demonstrated that the perceptions of PWS are malleable, especially amongst children and adolescents. For example, studies show that the perception of PWS are sensitive to media portrayals, self-acknowledgement of stuttering, and enrolment in speech therapy (Collins & Blood, 1990; Gabel, 2006; Miller, Mathers-Schmidt, & Fraas, M., 2015). The lack of knowledge regarding the factors that influence the perception of stuttering has led this paper to focus on the relationship between kinesics and perception, thus informing the research question: how do kinesics affect male Canadian high school students' perception of people who stutter?

To investigate the relationship between kinesics and the perceptions of PWS, 24 participants were sampled to participate in an experiment. The participants viewed an audio clip of a PWS, a video of a PWS exhibiting positive kinesics, and a video of a PWS exhibiting negative kinesics. After each clip, the participant filled out a semantic differential survey, which ascertained the participant's perception of the speaker. Based on previous research conducted by Blalock (1982), which showed that positive kinesics improved message reception, the researcher hypothesized that positive kinesics would have a favourable effect on the perception of PWS.

Literature Review

Perceptions of Stuttering

Stuttering is a major physical impairment. Stuttering limits speech and communication, which is a fundamental part of daily social interactions. Stuttering is even considered a disability under the American with Disabilities Act. In spite of its importance, research into stuttering only began in earnest when Marcel Wingate (1964) created a standard definition

of stuttering in the hopes of providing "a basis for a more systematic and efficient approach in the study of stuttering" (p. 489). Since then, many researchers have used Wingate's definition to examine the effect of different listeners (varied by gender, familiarity with stuttering, profession etc.) and different PWS (varied by gender, age, participation in speech therapy, severity of stuttering etc.) on how a PWS is perceived. While there are conflicting results between studies on some subjects, the research consistently shows that listeners react more negatively as the stuttering becomes more severe, and that PWS are rated more negatively than their fluent counterparts are (Boyle, 2017; Collins & Blood, 1990; Gabel, 2006; Von Tiling, 2011; Woods & Williams, 1976). Furthermore, many people hold negative stereotypes of PWS that extend beyond the physical traits of stuttering. When asked to describe stuttering, listeners conflated their own preconceived notions of PWS' personality traits with the physical traits associated with the speech impediment (Hughes, Gabel, Irani, & Schlagheck, 2010). This suggests that listeners' perceptions of PWS are based on not only an adverse response to the act of stuttering but also a negative preconception of PWS.

The negative social stereotypes and stigmas surrounding stuttering were first explored in Woods and Williams (1976). By using a stratified sample of 156 participants from varying backgrounds, Woods and Williams examined the stereotypes associated with male PWS. The researchers found significant differences between the traits ascribed to stuttering and fluent males across all demographics of listeners: "many people expect a stutterer to be different from a non-stutterer in certain undesirable ways" (p. 274). Specifically, the average PWS is expected to exhibit "shyness, anxiety, lack of self-confidence, and social withdrawal" (p. 276). The researchers observed no gender-based differences in the data of listeners' perceptions, which reaffirms previous results from Schroeder (2002). Contrastingly, other studies contend that female listeners judge PWS more positively than male listeners do (Burley & Rinaldi, 1986; Dietrich, Jensen, & Williams, 2001). While there is no consensus on the subject, this paper recognizes the possibility of gender-based perceptions of PWS.

More concerning is the conclusion from Woods and Williams (1976) that educators and speech pathologists were no less likely to hold negative stereo-

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types of PWS. A subsequent study conducted by Rice et al. (1993) found that similar biases and preconceptions existed against children with speech-language impairment. Once again, there was no difference in perception between educators, speech pathologists, employers, and the public. Such a pervasive stereotype of PWS would suggest that PWS actually behave in the undesirable manner in which they are described. However, previous studies into PWS and fluent speakers did not find a significant difference in personality (Beech & Fransella, 1968).

The origins of this seemingly unfounded stereotype of PWS has eluded researchers. Woods and Williams (1976) suggest that listeners wrongly extrapolate the state of anxiety a PWS experiences while speaking to his or her overall personality. More recently, a study conducted by Miller et al. (2015) attempts to find an explanation in media portrayals of PWS. Miller et al. (2015) conclude that stuttering is generally portrayed negatively – often for comedic effect – across all media platforms (e.g. literature, YouTube, television, movies). Moreover, Miller et al. (2015) find that media portrayals are pivotal in shifting listeners' attitudes towards PWS: “[m]ore negative portrayals of stuttering lead to more negative perceptions of PWS while more positive portrayals of stuttering in turn lead to more positive perceptions of PWS” (p. 72). Miller et al.'s (2015) study also suggests that listeners' perceptions are affected by non-auditory factors such as the appearance and behaviour, which has prompted this paper to focus on the relationship between the kinesics of PWS and perception.

Influence of Kinesics on Communication

Although other researchers have examined the influence of body language, anthropologist Ray Birdwhistell formalized the field of study now known as kinesics. Kinesics, derived from the Greek word *kinesis* (motion), is “the systematic study of the visually sensible aspects of nonverbal interpersonal communication” (Birdwhistell, 1970, p. 354). In his seminal work *Introduction to Kinesics*, Birdwhistell (1982) created an annotation system for kinesics based on three classifications: facial expression, body language, and gestures. Furthermore, Birdwhistell (1970) estimates that only about 30% of meaning in social interactions is conveyed by spoken word. By creating a systematic

approach to the study of body motions, Birdwhistell legitimized an otherwise undervalued field of study.

Researchers often study kinesics in a business environment, where effective and efficient communications are paramount. A study conducted by Blalock (1973) investigated the impact of kinesics on message perception between management and employees. Blalock concludes that positive kinesics increases the credibility of a message, while negative kinesics has an adverse effect. These effects are enhanced when positive kinesics is paired with negative verbalization (e.g. poorly phrased, unpleasant delivery) or negative kinesics is paired with positive verbalization. Similarly, Chu, Strong, Ma, and Greene (2005) argue that kinesics are a powerful tool in business negotiations, especially between negotiators of different nationalities. Chu et al. find that negotiators tend to move closer and gesture to emphasize important points, smile to gain cooperation, and make continual eye contact to gain power. The positive effects of good kinesics were also noticed in teaching, another field that emphasizes communication. Orton (2007) explores the use of gestures in the teaching and learning of modern languages, an endeavour that has neglected the use of kinesics in favour of verbal channels of expression. Orton surmised that body motions played an integral role in learning: “language teachers could most beneficially make rich use of gestures in their teaching” (“Conclusion,” para. 1). Not only does kinesics play an integral role in business interactions, its ability to change message perception suggests that it could also have a powerful effect on the perceptions of PWS.

Impact of Stuttering on PWS

The negative perceptions of PWS often create complications that influence their health and social involvement. These ramifications start from a young age and persist through adulthood. Children and adolescents who stutter risk being bullied and excluded at school. A self-reported survey conducted by Blood and Blood (2004) found that 43% of PWS experienced bullying, which was significantly higher than the 11% of fluent speakers who experienced bullying. These negative interactions and attitudes are internalized by PWS, resulting in “lower quality of life, hope, self-esteem, and self-efficacy” (Boyle, 2017, p. 922). The apprehension of potential negative reactions often cause

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PWS to withdraw or limit social interactions (Plexico et al., 2009). The transition into adulthood yields similar consequences. Stuttering continues to have a negative effect on interpersonal relationships, interfering with conversations and dating (Van Borsel et al., 2011). Despite this, a quantitative analysis of adult PWS found no significant differences in educational attainment or employment outcomes (as measured by pay) although PWS were observed to hold positions of lower status (McAllister et al., 2012). The researchers suggest that the propensity for PWS to hold lower-ranking positions “reflect[s] their preference for avoiding occupations perceived to require good spoken communication abilities” (p. 106). This conclusion is supported by a study from Klein and Hood (2004), which reported that half of the stuttering participants sought employment positions that required minimal speaking. The significant ramifications that the negative perception of stuttering has on PWS speak to the importance of further understanding the underlying factors that create such a perception.

The consequences that arise from the negative stereotypes of PWS demonstrate that the perceptions of PWS are formed from factors more complex than the physical trait of stuttering. However, few studies situate stuttering and its perception in a greater context, analyzing stuttering in conjunction with other external factors. None has specifically examined the relationship between stuttering, kinesics, and perception of PWS. This paper will provide a greater understanding of the effect of kinesics on the perceptions of PWS.

Methodology

Experimental Design

In this correlational study, an experiment was conducted to determine the effect of a PWS's kinesics on their perception by male adolescents. While a qualitative method might be able to provide a more detailed description about the perceptions of PWS, there is no consistent process to compare the individual descriptions. As such, this study uses standardized measures to analyze the relationship between these variables quantitatively, where kinesics was evaluated based on the only comprehensive annotation system that was

created by Birdwhistell (1970), focusing on facial expression, body language, and gestures;

stuttering was assessed based on the standard definition of stuttering proposed by Wingate (1964), which has been widely cited by subsequent studies on stuttering (Brundage, Bothe, Lengeling, & Evans, 2006; Conture & Kelly, 1991; Miller et al., 2015); and

listeners' perceptions were determined using a semantic differential scale, which was notably applied to the field of stuttering in Woods and Williams (1976) and has continued to be effective in determining listeners' attitudes of stuttering (Burley & Rinaldi, 1986; Miller et al., 2015; Rice et al., 1993).

In the experiment, participants listened to a PWS without visuals as a control. Then, they were shown two videos: one with a PWS exhibiting positive kinesics, and the other with a PWS exhibiting negative kinesics. As evidenced in Burley and Rinaldi (1986), the sex of the speaker has a significant effect on the listeners' perceptions. Thus, this process will be repeated on videos with a speaker of another sex. The selection and content of these videos will be discussed in a later section. After each video sample, the participants filled out a 10-item semantic differential scale (see Appendix A). The scale consists of numbers (one through seven) assigned to bipolar adjective pairs (negative/positive, dull/intelligent). Positive traits were assigned to the high end of the scale (seven), while negative traits were assigned to the low end of the scale (one). Participants were provided with a definitions sheet to ensure that there was a standardized understanding of the personality adjectives (see Appendix B). Furthermore, participants were given instructions to circle the number that most accurately reflected their perceptions about the personality traits of the speaker. Participants were also given the chance to familiarize themselves with the scale through a sample question so that they knew how to use the scale properly. Upon completion of the survey, the researcher collected the materials for analysis. Statistical analysis was performed on the data to determine if significant differences existed between the trait ratings across video samples.

Video Samples

The videos and audio clips used in this study were chosen from a series of TED Talks about personal

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experiences with stuttering. This allowed for the content and message to be consistent between clips. Furthermore, the videos that were chosen featured speakers with similar appearances to avoid potentially confounding factors like race, body type, or height. The stutter rate was also kept consistent between clips with speakers of the same sex (see Appendix C). Each video clip was shown to a selected panel of two teachers and three grade 12 students that assigned it a kinesics score based on body language, facial expression, and gestures (see Appendix D). The clips were compiled into one video that was shown to each participant individually on the same laptop. After the initial instructions were read (See Appendix E) and any questions were answered, the experimenter left the room to allow the participant to respond individually and uninfluenced.

Participants

Participants for this study were randomly selected from a private boys' high school in a region situated on the outskirts of the Greater Toronto Area. This school was chosen for its convenience of sampling. However, the consistency of demographics within the school allowed for a more representative sample of educated adolescents. Furthermore, due to the potentially confounding effect of the listeners' sex on perception, this study chose to focus on male listeners. (Burley & Rinaldi, 1986; Dietrich et al., 2001). From a random sample of students, the selected individuals were asked to complete a screening survey (see Appendix F). Students who indicated they were performing at grade-level expectations; had no impairments associated with vision, hearing, or language comprehension; and used English as their primary language were selected as participants in the experiment. Most importantly, students were asked whether they stuttered (now or previously), had a family member who stuttered, or had close relations with a friend who stuttered. Students who met any of the above criteria were excluded to obtain a representative perception by reducing the confounding effect of familiarity with stuttering (Woods & Williams, 1976). Consent forms (see Appendix G) were given to the selected students, and those who returned the forms signed by a parent or guardian participated in the experiment. The final sample

consisted of 24 students, with six students from each grade. The desired sample size was calculated using the sample size formula for finite populations:

$$\text{Sample Size} = \frac{\frac{z^2 \times p(1-p)}{e^2}}{1 + \left(\frac{z^2 \times p(1-p)}{e^2 N}\right)}$$

where

$p = 0.5$;

z is the z-score associated with the confidence level;

e is the margin of error; and

N is the population size.

As such, a 24-person sample is sufficient for a desired 95% confidence level and a 20% margin of error, given that the population of the school is 613 students.

The participants maintained full autonomy during the experiment and were allowed to withdraw at any time without penalty. Those who withdrew would have their responses disregarded and omitted from the analysis. The data was recorded without attributing names to responses, and the data was used for the sole purpose of this study.

Results

The data from the experiment were analyzed to determine the effect of kinesics on the perception of PWS. The analysis focused on three main areas of investigation: the holistic effect of kinesics on perception, the effect of kinesics on individual personality traits, and the difference in effect between positive and negative kinesics. Since the data was bifurcated based on the sex of the speaker, a secondary analysis was conducted on the relationship between sex, kinesics, and perception. The data are summarized in the following charts and tables. Statistical analyses were conducted using XLSTAT.

Holistic Effect of Kinesics on Perception

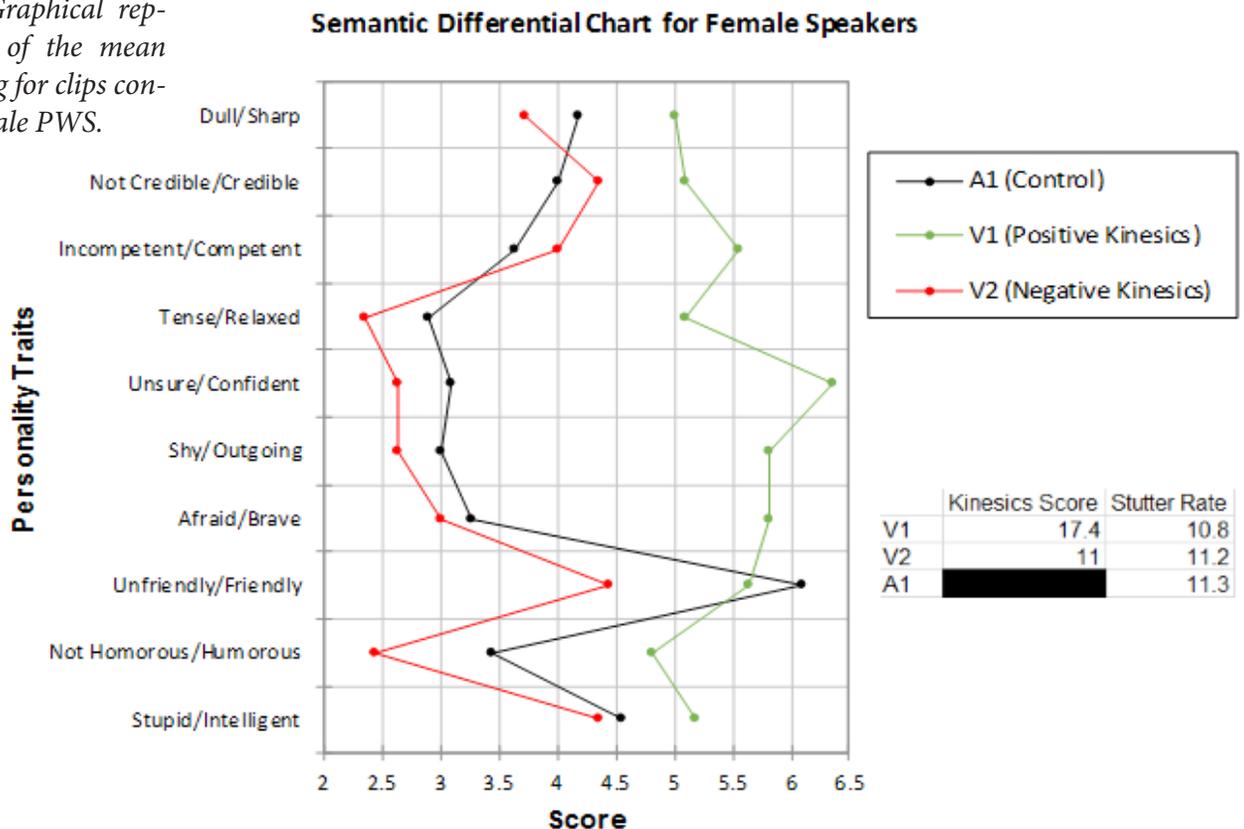
A multivariate analysis of variance (MANOVA) using the Wilk's Lambda test was performed on the datasets to determine the holistic effect of kinesics on perception. Of the six group comparisons (female control-positive, female control-negative, fe-

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Figure 1. Mean Likert ratings across participants for clips containing female PWS.

Adjective Pairs	A1 Female Control		V1 Female Positive		V2 Female Negative	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Dull/Sharp	4.18	0.98	5.00	1.10	3.73	1.01
Not Credible/Credible	4.00	2.05	5.09	1.22	4.36	1.50
Incompetent/Competent	3.64	1.91	5.55	1.04	4.00	1.55
Tense/Relaxed	2.91	1.04	5.09	1.70	2.36	1.03
Unsure/Confident	3.09	1.38	6.36	1.21	2.64	1.29
Shy/Outgoing	3.00	1.18	5.82	0.98	2.64	1.36
Afraid/Brave	3.27	1.62	5.82	0.98	3.00	1.67
Unfriendly/Friendly	6.09	0.94	5.64	0.92	4.45	1.29
Not Humorous/Humorous	3.45	1.57	4.82	1.17	2.45	0.69
Stupid/Intelligent	4.55	1.75	5.18	0.98	4.36	1.36

Figure 2. Graphical representation of the mean Likert rating for clips containing female PWS.

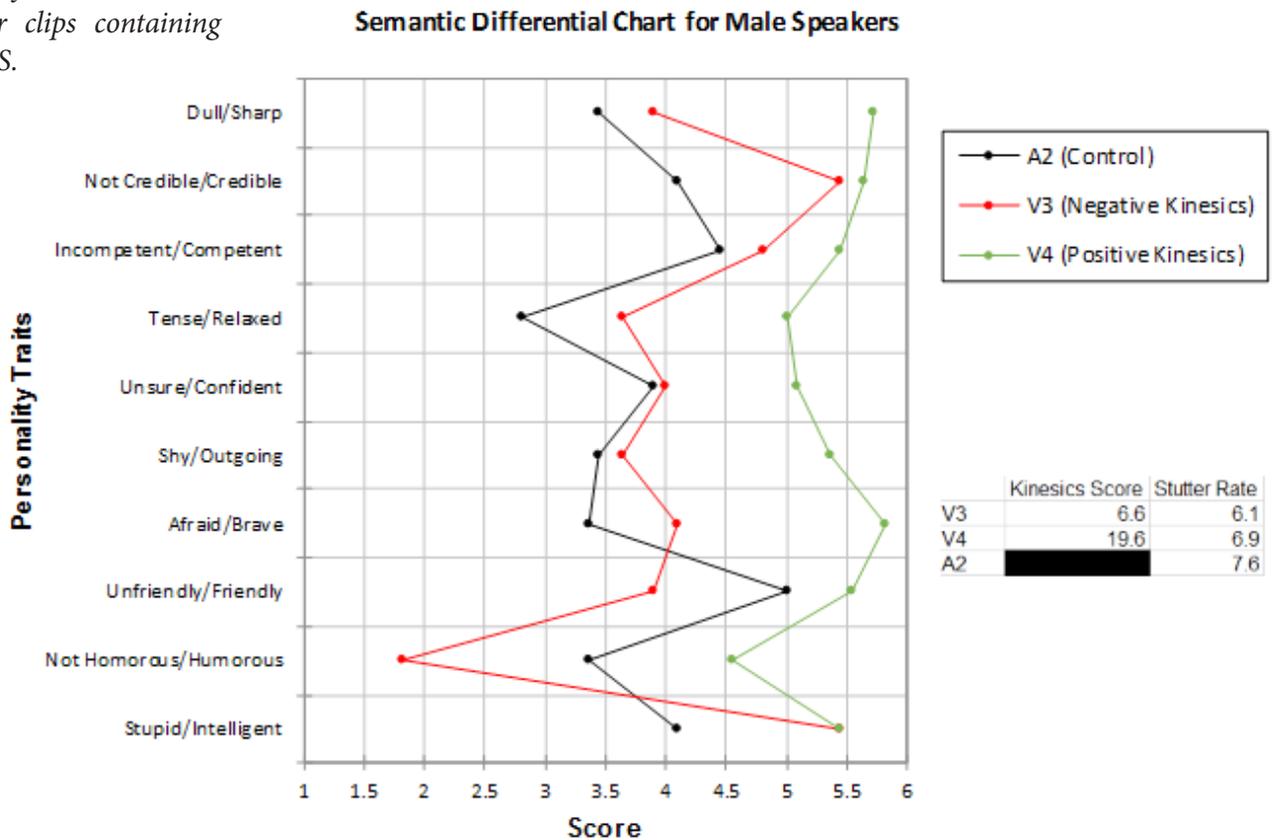


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Figure 3. Mean Likert ratings across participants for clips containing male PWS.

Adjective Pairs	A2 Male Control		A2 Male Negative		A2 Male Positive	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Dull/Sharp	3.45	1.51	3.91	1.81	5.73	0.90
Not Credible/Credible	4.09	1.51	5.45	0.93	5.64	0.81
Incompetent/Competent	4.45	1.69	4.82	1.17	5.45	0.93
Tense/Relaxed	2.82	1.33	3.64	1.36	5.00	1.90
Unsure/Confident	3.91	1.51	4.00	1.18	5.09	1.58
Shy/Outgoing	3.45	1.21	3.64	1.29	5.36	1.29
Afraid/Brave	3.36	1.80	4.09	1.30	5.82	0.98
Unfriendly/Friendly	5.00	1.26	3.91	1.38	5.55	0.82
Not Humorous/Humorous	3.36	1.57	1.82	0.87	4.55	1.29
Stupid/Intelligent	4.09	1.58	5.45	0.82	5.45	1.13

Figure 4. Graphical representation of the mean Likert rating for clips containing male PWS.



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Figure 5. Summary of statistical output for MANOVA using the Wilk's Lambda Test.

Test	d.f.	Lambda	F	p-value	Significant at $\alpha = 0.05$
A1-V1 (Female Control - Female Positive)	10, 24	0.223	3.842	0.018	Yes
A1-V2 (Female Control - Female Negative)	10, 24	0.458	1.304	0.334	No
A1-V2 (Female Positive - Female Negative)	10, 24	0.119	8.114	0.001	Yes
A2-V3 (Male Control - Male Negative)	10, 24	0.243	3.422	0.028	Yes
A2-V4 (Male Control - Male Positive)	10, 24	0.215	4.026	0.016	Yes
V3-V4 (Male Negative - Male Positive)	10, 24	0.269	2.984	0.043	Yes

male positive-negative, male control-positive, male control-negative, male positive-negative), only the female control-negative comparison did not achieve statistical significance. On balance, this indicates that the overall perception of speakers exhibiting positive or negative kinesics is significantly different from the control. A summary of the MANOVA data can be seen in Figure 5.

to be more superficial. Traits that are more associated with cognitive behaviour and mental competence (not credible/credible, incompetent/competent, unfriendly/friendly, not humorous/humorous, stupid/intelligent) did not achieve significance for any of the comparisons. This trend indicates that cognitive trait ratings are less sensitive to a change in kinesics than behavioural trait ratings.

Effect of Kinesics on Individual Traits

For the comparisons to the control that achieved statistical significance, post-hoc testing (with a Bonferroni correction for $\alpha = 0.05$) was performed to determine the effect of kinesics on the individual trait ratings. Despite being holistically different from the male control, none of the individual trait ratings for the male negative sample was significantly different from the control. Contrastingly, three adjective pairs – tense/relaxed, shy/outgoing, afraid/brave – achieved significance for both the female control-positive and the male control-positive comparisons. These traits manifest themselves in surface behaviour and tend

Positive vs Negative Kinesics

A MANOVA was used to compare the negative sample data with the positive sample data for both speakers (see Figure 6). Both comparisons achieved statistical significance, indicating that positive kinesics has a different effect on perception than negative kinesics. As seen in Figures 1 and 3, the mean Likert ratings for the speaker exhibiting positive kinesics are significantly higher than the control. As such, positive kinesics shifts listener perception favourably. Contrastingly, the effect of negative kinesics on perception is negligible. Not only did the MANOVA comparison for the female negative-control fail to achieve signifi-

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Figure 6. Summary of the univariate analyses of variance (ANOVA) conducted on individual trait ratings.

	A1-V1 (Female Control - Female Positive)		A2-V3 (Male Control - Male Negative)		A2-V4 (Male Control - Male Positive)	
	<i>p</i> -value	Significance	<i>p</i> -value	Significance	<i>p</i> -value	Significance
Dull/Sharp	0.080	N	0.530	N	< 0.0001	Y
Not Credible/Credible	0.145	N	0.019	N	0.007	N
Incompetent/Competent	0.009	N	0.564	N	0.102	N
Tense/Relaxed	0.002	Y	0.169	N	0.005	Y
Unsure/Confident	< 0.0001	Y	0.877	N	0.088	N
Shy/Outgoing	< 0.0001	Y	0.737	N	0.002	Y
Afraid/Brave	< 0.0001	Y	0.291	N	0.001	Y
Unfriendly/Friendly	0.267	N	0.067	N	0.244	N
Not Humorous/Humorous	0.032	N	0.010	N	0.068	N
Stupid/Intelligent	0.306	N	0.019	N	0.030	N

Figure 7. Summary of the mean change in trait ratings between the control and positive samples for both speakers.

Adjective Pairs	Mean Difference Between V1 (Female Positive) and A1 (Female Control)	Mean Difference Between V4 (Male Positive) and A2 (Male Control)
Dull/Sharp	0.82	2.27
Not Credible/Credible	1.09	1.55
Incompetent/Competent	1.91	1.00
Tense/Relaxed	2.18	2.18
Unsure/Confident	3.27	1.18
Shy/Outgoing	2.82	1.91
Afraid/Brave	2.55	2.45
Unfriendly/Friendly	-0.45	0.55
Not Humorous/Humorous	1.36	1.18
Stupid/Intelligent	0.64	1.36

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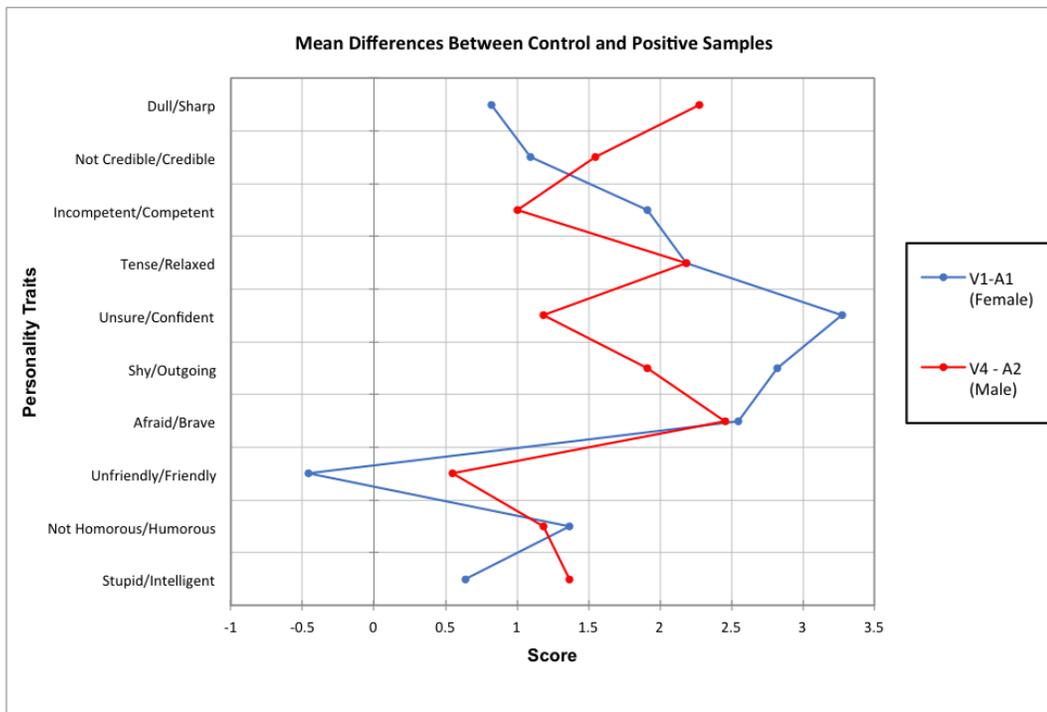


Figure 8. Graphical representation of the mean change in trait ratings between the control and positive samples for both speakers.

cance (Figure 5), but the ANOVA for male negative-control also failed to find significant differences between individual trait ratings (Figure 6).

Sex of the Speaker, Kinesics, and Perception

A separate analysis was conducted to determine if the change in perception for the male speaker was significantly different from that of the female speaker. While the vastly different kinesics scores for the negative samples (6.6 for male, 11 for female) made it inconsistent to compare the changes in perception from control to negative, the similarity in kinesics scores for the positive samples (19.6 for male, 17.4 for female) allowed for a meaningful comparison. The data used for this analysis are summarized below.

A MANOVA, $F(10, 24) = 5.700, p = 0.004$, performed on these data found that there was a significant difference in the change in perception between the male and female speakers. However, post-hoc testing revealed that only the changes in ratings for the unsure/confident adjective pair between the speakers were statistically significant ($p = 0.003$). These trends

suggest that kinesics has a different effect in shifting overall perceptions of male and female PWS. This is especially prominent for ratings of confidence, where the female speaker benefited much more from positive kinesics than did the male speaker.

Discussion

The three main findings from the data are explored below. The results show that positive kinesics has a significant effect on perception, kinesics has a significantly different effect on the perception of male and female speakers, and the perceptions of PWS may have shifted over time.

Effect of Kinesics

The results of this study demonstrate that positive kinesics has a pronounced effect in shifting perceptions favourably, with the largest effect on surface traits associated with behavior (tense/relaxed, shy/outgoing, afraid/brave). On the other hand, cognitive traits (stupid/intelligent, not credible/credible, incompetent/competent) experienced an insignificant posi-

tive change from positive kinesics. While there is no research examining the effect of kinesics on the perceptions of PWS, the findings of this study complement research conducted by Miller et al. (2015) and Blalock (1973). Miller et al. finds that negative media portrayals of PWS had a negative effect on perception and, conversely, that more positive portrayals of PWS had a positive effect on perception. However, Miller et al. found that the positive effect did not reach significance for any individual traits. Blalock's study, which focused on the perception of messages in a business environment, similarly concluded that the use of positive body language had a favourable effect on overall message perception. Furthermore, Blalock reported that the perception of messages from speakers with negative vocalization is not significantly affected by the use of negative kinesics. The results of this study, which show that negative kinesics has a negligible effect on the overall perception of PWS, confirm Blalock's previous findings.

Effect of Gender and Kinesics

This study also concluded that kinesics has a significantly different effect on the perception of male and female speakers. As concluded from the analysis in the previous section, this is especially prominent for ratings of confidence, where the female PWS benefited much more from positive kinesics than did the male PWS. Although not focusing specifically on the effect of kinesics and conducted on subjects of both genders, previous research has suggested that listeners' perception is affected by the sex of the speaker. A study conducted by Burley and Rinaldi (1986) noted that there were significant differences in the rating of male and female PWS, where listeners were likely to rate male speakers more positively. An explanation for the difference in the way kinesics affects the perceptions of male and female speakers observed in this study may be found in another study conducted by Koppensteiner and Grammer (2011). The study examined how the body movements of male and female speakers affected viewers' perceptions of personality. Participants viewed videos of public performances where the speakers' movements were rendered into stick figure movies. The results showed that stick figures representing male speakers were rated more favourably than stick figures representing female speakers. Therefore, the researchers suggest that there

is a difference in the body language of males and females, which influences viewers' perceptions of their personality. The participant responses from this study demonstrate a marked difference in the way kinesics affects the perceptions of male and female PWS, affirming the conclusions from Koppensteiner and Grammer.

Contemporary Perceptions of Stuttering

Lastly, the current study provides evidence that the perceptions of PWS may have shifted over time, reflecting a greater acceptance of stuttering. Although this study found that listeners rated PWS in the control sample to be tense, shy, and afraid, it also found that listeners rated PWS to be friendly, sharp, competent, and intelligent. This contradicts earlier studies, which found that listeners had negative baseline ratings for all personality traits. For example, Woods and Williams (1976) concluded that listeners ascribed undesirable traits, such as stupid and unfriendly, to male PWS. Collins and Blood (1990) drew the same conclusion with female PWS. These differences in findings might be attributed to the fact that the PWS in this study shared personal experiences with stuttering, which could skew the results positively. However, more recent studies conducted by Gabel (2006) and Miller et al. (2015) show that listeners are starting to rate PWS more positively, which is a trend reflected in the results of this study.

Conclusions

Implications, Limitations, and Future Research

Understanding the different factors that affect the perceptions of PWS is key to shifting those perceptions. This study shows that there is a significant positive effect on perception associated with positive kinesics. These findings have larger social and clinical implications that can help PWS improve their interaction with others. PWS can use this information to both empower themselves and change the attitudes of those around them. Furthermore, by integrating positive kinesics building techniques into speech therapy, speech language pathologists have another tool to help PWS build confidence and cope with their speech disorder.

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Several limitations to this current study warrant discussion. First, the small sample size reduces the generalizability of the results. Since the participants were only chosen from an all-boys private boarding school, extrapolation of these conclusions to the greater population would be erroneous. Furthermore, the use of two different speakers may have introduced confounding variables. Although measures were taken to ensure that the speakers featured in the videos had similar physical appearances, not all the visual factors could be controlled. For example, one female speaker was blonde and wore glasses while the other was a brunette woman who did not wear glasses. In addition, one male speaker had slightly darker skin and facial hair. Though these differences may affect the data, there should not be a significant difference in the way the speakers are evaluated.

While this study has established the link between kinesics and the perceptions of PWS, further research could be done to determine the effect of kinesics on listeners' physiological and emotional responses to stuttered speech. A previous study identified that listeners respond negatively to stuttered speech through a variety of physiological responses, such as a lower heart rate, a lower breathing rate, and the tendency to avoid eye contact with the speaker (Guntupalli, Everhart, Kalinowski, Nanjundeswaran, & Saltuklaroglu, 2007). The study also found that listeners responded with negative emotions, such as discomfort, embarrassment, and unhappiness. Given the positive effect of positive kinesics on listeners' perceptions of PWS, there is potential that positive kinesics could also mitigate negative listeners' responses.

Even though there exists a negative perception of people who stutter, this study provides compelling evidence that these perceptions can be favourably changed with positive kinesics. The understanding gained from this study can help inform useful practices in speech language therapy that can help people who stutter to better approach their interactions with others. Indeed, there is still more to discover in the different factors that influence the perceptions of people who stutter. By gaining a greater understanding of these factors, such as kinesics, people who stutter are able to empower themselves and potentially mitigate the complications associated with a negative perception.

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Appendix A – Semantic Differential Survey

Cheetahs are:

Slow	1	2	3	4	5	6	7	Fast
------	---	---	---	---	---	---	---	------

The speaker in the audio clip is:

Dull	1	2	3	4	5	6	7	Sharp
------	---	---	---	---	---	---	---	-------

Not Credible	1	2	3	4	5	6	7	Credible
--------------	---	---	---	---	---	---	---	----------

Incompetent	1	2	3	4	5	6	7	Competent
-------------	---	---	---	---	---	---	---	-----------

Tense	1	2	3	4	5	6	7	Relaxed
-------	---	---	---	---	---	---	---	---------

Unsure	1	2	3	4	5	6	7	Confident
--------	---	---	---	---	---	---	---	-----------

Shy	1	2	3	4	5	6	7	Outgoing
-----	---	---	---	---	---	---	---	----------

Afraid	1	2	3	4	5	6	7	Brave
--------	---	---	---	---	---	---	---	-------

Unfriendly	1	2	3	4	5	6	7	Friendly
------------	---	---	---	---	---	---	---	----------

Not Humorous	1	2	3	4	5	6	7	Humorous
--------------	---	---	---	---	---	---	---	----------

Stupid	1	2	3	4	5	6	7	Intelligent
--------	---	---	---	---	---	---	---	-------------

Appendix B – Definitions Sheet

Definitions

Sharp- Quick thinker; clever

Dull- Slow thinker; foolish; unskilled

Credible- Believable; trustworthy

Not Credible- Not believable; untrustworthy

Competent- Capable; having the ability to do what is needed

Incompetent- Incapable; not having the ability to do what is needed

Relaxed- At ease; free; comfortable

Tense- Tight; rigid; anxious

Confident- Self-assured; believes in oneself

Unsure- Uncertain; hesitant

Outgoing- Friendly; socially confident

Shy- nervous around others; timid

Brave- showing courage; ready to face danger

Afraid- filled with fear; scared

Friendly- pleasant; kind; easy to get along with

Unfriendly- Mean; unpleasant; hard to get along with

Humorous- Amusing; has a good sense of what is funny

Not Humorous- Unamusing; does not have a good sense of what is funny

Intelligent- Smart; quick to understand

Stupid- Not smart; slow to understand

Appendix C – Video and Audio Transcripts

Female A1

People think that I've forgotten their name

03:38

when I hesitate before saying it.

03:42

And it is a very weird thing,

03:45

because proper nouns are the worst.

03:49

If I'm going to use the word "Wednesday" in a sentence,

03:53

and I'm coming up to the word,

03:56

and I can feel that I'm going to stutter or something,

03:59

I can change the word to "tomorrow,"

04:02

or "the day after Tuesday,"

04:04

or something else. You know.

04:06

It's clunky, but you can get away with it,

04:11

because over time I've developed this

04:14

loophole method of using speech

04:18

where right at the last minute you

04:21

change the thing and you trick your brain.

04:26

But with people's names, you can't change them.

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Female V1

the reactions to my
03:14
disability were worst part mom why is
03:20
she taking so long honey Oprah didn't
03:22
have a stutter don't be so nervous just
03:28
talk but I was talking as soon as I saw
03:32
myself as they did the world became
03:34
clear because I got no one valuing what I
03:38
had to
03:39
say I started to devalue my own voice as
03:42
well and as middle school approached I
03:46
did things and acted in a certain way so
03:49
nobody would know I was different and if
03:51
they found out I would act like my stutter
03:53
was no big deal

03:58
little did I know at the time this
04:01
perception of my speech would change my
04:03
life
04:04
stutter talk with continued involuntary
04:08
repetition of sounds especially initial
04:11
consonants that's the definition of
04:15
stuttering but it's so much more than
04:17
just a two syllable word and some
04:21
repetitions stuttering
04:25
is a complex system of vocal and
04:27
neurological work and everyone varies
04:29
from the vocal stutter to the secondary
04:33
motion

Female V2

I get through it that way for my job.
06:22
But as an artist who feels that their work
06:24
is based solely on a platform of honesty
06:30
and being real,
06:34
that feels often like cheating.
06:38
Which is why before I sing, I wanted to tell you
06:41
what singing means to me.
06:45
It's more than making nice sounds,
06:49
and it's more than making nice songs.
06:54
It's more than feeling known, or understood.
07:00
It's more than making you feel the things that I feel.
07:06
It's not about mythology,
07:09
or mythologizing myself to you.
07:14
Somehow, through some miraculous
07:19
synaptic function of the human brain,
07:24
it's impossible to stutter when you sing.

Male A2

and it was finally
00:40
might my turn to introduce myself to
00:47
the rest of the class I stood up and I
00:49
said hi I'm everyone laughed I held in a
00:57
few tears of anger and I sat back
01:00
down I was sixteen years old and I was
01:05
sitting in line at a sonic
01:08
Drive through and as I was in my
01:12
car again I was silently saying to
01:16
myself tater tots tater tots tater tots
01:21
I finally made it up to the small ordering window and
01:27
the young woman behind it she asked me
01:29
hi what would you like and I said hi may
01:31
I please have some t and she said what
01:38
what was that you want what
01:45
I got so frustrated in that moment that I just yelled at her

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Male V3

so where does the stammer
 01:43
 come from all stammers and stammer is
 01:47
 are different but in my case it's a
 01:51
 combination of varying degrees of coming
 01:54
 from a very literary family with a
 01:58
 highly verbose elder brother a genetic
 02:02
 shadow of a stammer in my father to
 02:07
 a lesser degree and the natural innate
 02:11
 self-conscious sensitivity
 02:14
 most stammerers grow out of the
 02:17
 condition by five years old but some
 02:20
 more dedicated stammerers persist **male**
 02:25
stammerers outnumber the females by
 02:27
 about four to one **it's** in fact a basic
 02:31
 miracle that speech production develops
 02:33
 as normally as it usually does

Male V4

I was
 04:35
 taught to speak softer I was taught to
 04:38
 act as if I was singing so the words
 04:40
 would flow better I was taught to take
 04:43
 big breaths so I wouldn't **run** out of
 04:47
 hair at the end of my sentences and for
 04:49
 them **for the** most part it's all worked I
 04:52
 have a good career I have a beautiful
 04:55
 partner and I don't freak out as much if
 04:58
 I have to answer the phone nowadays
 05:00
 however it's **all** been a sham it's
 05:04
 all been a very thin **screen**
 05:09
over what the real problem I had was see

05:12
 now that I'm a little older I've seen a
 05:14
 little more in the world and I've
 05:16
 discovered more about myself I've seen that
 05:23
 everything I was taught **about**
 05:25
 my stutter every single thing it was
 05:28
 100% wrong completely wrong
 05:31
 see **I was** taught to see my stutter
 05:38
as an issue as a problem an obstacle
 05:41
 a challenge as a handicap that I had to
 05:43
 overcome in order to have any kind of a
 05:45
 happy life

Stutter Rates

	Stutter Rate
V1	10.8
V2	11.2
A1	11.3
V3	6.1
V4	6.9
A2	7.6

Appendix D – Kinesics Rating Survey

Bad Body Position/Posture	1	2	3	4	5	6	7	Good Body Position/Posture
Bad Gestures	1	2	3	4	5	6	7	Good Gestures
Bad Facial Expression/Eye Contact	1	2	3	4	5	6	7	Good Facial Expression/Eye Contact

Kinesics Scores

	Kinesics Score
V1	17.4
V2	11
A1	
V3	6.6
V4	19.6
A2	

Appendix E – Participant Instructions

Instructions

Thank you for agreeing to participate in my research project regarding the perceptions of people who stutter (PWS). In this experiment, you will be watching videos of different speakers and rating their personality traits on a semantic differential scale. Some of the personality traits will not be explicitly evident in the video. Please rate those traits based on what you believe they would be based on what you have seen.

On the first survey, you will find an example question that we will fill out together to make sure you know how the scale works. The statement reads “Cheetahs are...” If you believe cheetahs are slow, circle 1. If you believe cheetahs are fast, circle 7. If you believe cheetahs are between fast and slow, circle a number in between. Does that make sense?

Please rate all the traits relative to the previous clips. Please have a look over the definitions sheet to familiarize yourself with the terms.

We will now begin the experiment. You will be watching and listening to a series of 2 audio clips and four videos. After each, please fill out the corresponding survey form (for clip A1, fill out form A1). If you are unsure about any of the personality adjectives, please refer back to the definitions sheet.

Appendix F – Screening Survey

Participant Screening Survey

Are you currently failing an English course?

Yes _____ No _____

Do you suffer from learning disabilities?

Yes _____ No _____

Do you have any hearing or visual impairments?

Yes _____ No _____

Is English your primary language (i.e. English is the language you use the majority of the time)

Yes _____ No _____

Does anyone close to you (family member, close friend) stutter?

Yes _____ No _____

Do you or have you ever stuttered?

Yes _____ No _____

Appendix G – Consent Forms

Dear Parent/Guardian,

I have been selected to participate in the AP Capstone Research Program. The aim of this program is to encourage and support high school students in producing meaningful and valuable research. At the end of the year, this research will be submitted to the College Board, with the goal of being published in an academic journal. This research has been approved by my teacher, _____, and an internal ethics review board.

My research will focus on understanding the different factors that affect the perception of people who stutter (PWS) by adolescents. The participants in my project will be students in grades 9, 10, 11, and 12. Participation in this research will involve the completion of a consent form, screening survey, demographics survey, watching video clips of different speakers, and questionnaires that will determine your son's attitudes towards each speaker. All clips have been screened by adults and other students to ensure that the language use was appropriate. The data collection will take place after class time, and it is expected to take about 30 minutes.

All information collected in my research will be treated confidentially and no participants will be identified by name in any publication resulting from the research.

First and foremost, the results of my research will allow the academic field to gain valuable information about how stuttering is perceived by adolescents. Beyond this, the study may also help participants gain a better understanding of stuttering and increased exposure to the academic research process.

Student participation in this research project is voluntary and no student will be disadvantaged through non-participation. Participants may also withdraw from the study at any time or abstain from answering any question.

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Attached to this information sheet is a form seeking consent from both you and your son for him to participate in this research project. Upon signing, please have your son return the form to me, either by hardcopy or via email.

Please do not hesitate to contact me by email at _____ or by phone at _____ if you have any questions regarding this research. If you have any further questions, you can also contact my research advisor and teacher _____ by email at _____.

Yours sincerely,

Grade 12 AP Research Student

Parent and Student Consent Form

Perceptions of People Who Stutter

I, (Student) consent to my participation in this AP Research Project.

I,(Parent/Guardian) consent to my son's participation in this AP Research Project.

I have read the information sheet provided and understand the purpose and nature of the research.

I give permission for my son to view video clips that do not contain nudity, violence, or explicit language and for any survey data collected from my son to be recorded and analyzed.

I understand that any information or personal details gathered during this research are confidential and that my son's name or any other identifying information will not be used or published in the presentation of the research findings.

I understand that participation in this research is voluntary and that my son can withdraw from the research at any time, knowing that there will be no penalty or discriminatory treatment for doing so.

Signed (Student)
Date.....

Signed (Parent/Guardian).....
Date.....

Race, Wrongful Convictions, and Texas: An Analysis of the Impact of Juror and Defendant Ethnicity on Wrongful Convictions in Texas

William Howard-Waddingham

This study explored how different states are impacted by wrongful convictions, how different races are represented in Texas exonerations, and the connection between juror and defendant ethnicity in Texas wrongful convictions. This study employs a quantitative method. The study finds that Texas, New York, and Illinois are the states most impacted by wrongful convictions, that stark racial disparities exist in Texas exonerations, and that there is no connection between juror and defendant ethnicity in Texas wrongful convictions. These findings imply that House Bill 34 (a critical piece of Texas legislation that will be explored later in this study) will not be entirely successful from its lack of stipulations regarding racism, yet also clarify that racial discrimination does not originate with Texas's jurors, providing a direction for future research.

Keywords: Wrongful Convictions, Racism, Juror Discrimination, Defendant Ethnicity.

Introduction

Despite advancements for racial equality over the past half-century in the United States, racism remains a meaningful issue and continues to impact American society in a number of facets. For instance, implicit racial biases have often been conjectured to impact wrongful convictions in the United States. While wrongful convictions are an important issue in virtually every American state, they are a particularly pressing issue for Texas, as the state ranks first nationally for the greatest number of cases overturned by DNA evidence (The Innocence Project, 2009). According to Gould and Leo (2013), wrongful convictions are mainly attributed to perjured testimony, falsified confessions, or jailhouse informants. While Texas has recently established new legislation (House Bill 34) attempting to curb future wrongful convictions (The Innocence Project, 2017), it remains to be seen whether these new regulations will significantly mitigate wrongful convictions, especially for African

American men. While the new legislation deals with a number of the factors Gould and Leo (2013) mention, notably that of jailhouse informants and forced confessions, it fails to take into account any issues of racism. In not tackling racism, House Bill 34 lies contrary to the findings of Feagin (2013), who establishes that wrongful convictions in the United States have inordinately affected black men.

House Bill 34 has been uniformly praised for its initiative; however, far too little empirical work has been done to corroborate its actions and investigate the impact of race on wrongful convictions. I tackled this issue by questioning House Bill 34's effectiveness through a comparable analysis of wrongful convictions in Texas, while also searching for the causes behind racial discrimination in the Texas judicial system. This analysis was conducted with a quantitative approach. First, the analysis determines the states most impacted by wrongful convictions through a state-by-state analysis comparing exonerations to state population. Further, it compares the propor-

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tion population of each ethnic group in Texas to the percentage of wrongfully convicted people who are of the same ethnicity; this area of the study lent insight into the effectiveness of House Bill 34. Finally, it investigates the possibility of racism in past wrongful convictions by conducting a correlational analysis of all recorded wrongful convictions in Texas, examining the ethnicity of the defendant and the racial composition of the county where each trial was held and attempting to retrieve a connection between the two factors. Although the racial composition of the jury will not equally mirror the racial composition of each county, a good estimate of jury ethnicity can be retrieved utilizing this information (which will be further explained later in this study). While this study does not necessarily hold the answers to crafting a legislation which truly combats wrongful convictions, I hope that my findings provide insight into an important topic, and suggest whether or not House Bill 34 is worthy of the praise it has so far received.

Literature Review

Innocent Until Proven Guilty

The line between innocence and guilt is often blurred in the United States of America; for instance, Ferguson (2016) assesses that too often, the American precept of innocent before proven guilty is not upheld in American judicial systems. Hamer (2007) continues this narrative by establishing the increased prevalence of reverse burdens in Western judicial systems, a concept where the defendant is forced to prove innocence on a balance of probabilities instead of the prosecution establishing guilt beyond a reasonable doubt.

Prevalence of Wrongful Convictions

This worrying trend is reflected in the significant rates of wrongful convictions in the United States. For example, Gross, Jacoby, Matheson, Montgomery, and Patil (2005) find that 340 American inmates were exonerated from 1989-2003. Among this group, 80% had been imprisoned for more than 5 years, and the group had collectively spent 3400 years in prison (an average of 10 years per inmate) for crimes that they did not commit (Gross et al., 2005). Texas has par-

ticularly struggled with this issue, ranking first among American states in convictions overturned on DNA evidence (The Innocence Project, 2009). Additionally, Huff (2002) and Huff (2004) estimate an error rate of 0.5% in the American criminal justice system through a study of prosecutors, judges, and attorneys general. This error rate would result in 7500 wrongful convictions annually in index crime trials (index crimes being those of the highest severity in the United States) (Huff, 2002; Huff, 2004). The numbers would, in fact, be even greater for the entirety of the American justice system, as there would be wrongful convictions in non-index crimes.

Factors Influencing Wrongful Convictions

Through these disturbing statistics, a considerable degree of research has been undertaken to determine the contributing factors within cases of wrongful convictions. Most researchers agree on the basic factors involved in wrongful convictions. According to Acker (2009), Gould, Hail-Jares, and Carrano (2014), Gould and Leo (2010), Huff (2002), and Huff (2004), the most significant contributing factors to wrongful convictions include false confessions, tunnel vision (a phenomenon where police will acutely focus on one suspect while ignoring others), forensic error, ineffective assistance of defense counsel, prosecutorial error, and eyewitness mistakes. Additionally, Gould and Leo (2010) and Neuschatz, Lawson, Swanner, and Meissner (2008) charge that jailhouse informants (prisoners incentivized to lie under oath to convict a peer through the promise of lighter sentencing or increased privileges) often impact trials involving an innocent defendant through providing false testimony. Hewitt and Natapoff (2012) reaffirm this idea, as they find that informants are often used to develop cases and retrieve convictions; the authors also establish that these informants are often beneficiaries for “snitching,” as many have either received money or drugs (for drug-addicted informants) for their efforts. Moreover, lost confessions (the instances in which police are unable to withdraw a confession from a guilty suspect, forcing them to investigate innocents) can often cause police to charge an innocent person with a crime (Cassell, 1998). Since the majority of crimes are solved through police interrogation, lost confessions increase the chances that an innocent person will be

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charged when the guilty suspect is not (Cassell, 1998). Finally, Dervan and Edkins (2013) and Schneider (2013) point to the effect of plea bargain proceedings on an innocent defendant as an important factor in wrongful convictions. For instance, Dervan and Edkins (2013) undertook an empirical research study where over 50% of the innocent participants willingly admitted to guilt when they were offered a benefit (such as lighter sentencing or a lesser charge) for doing so.

House Bill 34

House Bill 34 tackles many of the factors influencing wrongful convictions (The Innocence Project, 2017). For instance, the legislation establishes clear guidelines for decreasing false confessions, mitigating errors in forensic investigation, decreasing the impact of eyewitness error, and regulating the use of jailhouse informants (The Innocence Project, 2017). In many facets, Texas has established the most comprehensive legislation in the United States to decrease wrongful convictions (The Innocence Project, 2017).

Factors Impacting Wrongful Convictions: Correlation or Causation?

However, while the factors previously analyzed have clearly been proven to correlate with wrongful convictions, various researchers conjecture there is no causal relationship between these factors and wrongful convictions. For instance, Gould et al. (2014) and Gould and Leo (2010) admit that their research was limited as they could not determine whether the factors were causal and could not determine whether the factors related to wrongful convictions were also existent in cases involving a legitimately guilty defendant.

Racism as a Causal Factor for Wrongful Convictions

Racism is still a significant issue in the United States of America 50 years after the Civil Rights Movement. For instance, although many fundamental aspects of racial inequity have been mended, inherent and systemic racial biases continue to exist. By utilizing the critical race theory (a critical societal analysis examining the relationship between law, race, and power),

Heilig, Brown, and Brown (2012) analyze that many issues of race still pervade Texas, as although African Americans are afforded many equal rights, they are still marginalized and are only given an “illusion of inclusion” (p. 1). Additionally, Horry, Wright, and Tredoux (2010) suggest that people will often hold inherently racial biases towards different ethnicities. Gross, Possley, and Stephens (2017) find that African Americans in the United States judicial system are impacted in numerous ways from racism, ranging from inherent and unconscious biases and institutional racism to explicit discrimination. These inherent and systemic could act as a cause towards Texas wrongful convictions. This idea is aided by the work of Gross et al. (2017), who empirically prove that wrongful convictions have repeatedly targeted African Americans. For example, African Americans comprise 47% of the 1900 exonerations recorded by the National Registry of Exonerations, despite only accounting for 13% of the American populace (Gross et al., 2017). Further, racial disparities in wrongful conviction rates exist for virtually every major crime (Gross et al., 2017). For murder, innocent African American defendants are 7 times as likely as innocent white defendants to be convicted of murder, African American inmates convicted of murder are 50% more likely to be innocent than fellow convicted murderers, and, while only 15% of murders by African Americans saw a white victim, 31% of wrongfully convicted African Americans were convicted for the murder of a white person (Gross et al., 2017). Sexual assault and drug crimes see similar racial disparities in their conviction and exoneration statistics (Gross et al., 2017). Feagin (2013) reaffirms the work of Gross et al. (2017), as he finds that wrongful convictions have specifically targeted African Americans. African Americans are also significantly more likely to be wrongfully convicted of crimes with a white victim, suggesting that race may act as a causal factor to wrongful convictions (Feagin, 2013).

Remaining Gap in Research

First, a gap in the research exists regarding the states most impacted by wrongful convictions. The Innocence Project (2009) found Texas to have the most wrongful convictions overturned by DNA evidence, but this literature review could not retrieve a study that has categorically examined this issue to retrieve

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the states most affected by wrongful convictions as a whole. As exonerations are prompted by many factors other than DNA evidence, for example through new or different testimony, The Innocence Project (2009)'s work will not have captured the full scope of American exonerations. For this reason, this study conducted a state-by-state analysis to determine the states with the greatest number of exonerations in American history; this finding gives clarity to future research on which states are important ones to analyze wrongful convictions within.

Moreover, while extensive research has been undertaken on the factors related to wrongful convictions and some research has been undertaken on potential causal factors, a significant gap remains in the research, particularly pertaining to racism. For instance, this literature review could only retrieve one empirical, statistically proven, study directly linking wrongful convictions with racism, in the work of Gross et. al (2017). Even with the work of Gross et. al (2017), it was not discovered what prompted the racial disparities in wrongful convictions, as it was only established that a correlation between race and wrongful convictions existed, not why it existed or what caused it. This study attempted to fill this gap by thoroughly investigating one potential factor of wrongful convictions of African Americans. It did so through investigating potential juror discrimination by linking the ethnicity of jurors with the race of the wrongfully convicted defendant. This study also extended the work of Gross et al. (2017) by replicating their work on a smaller scale by examining the racial disparity of wrongful convictions in Texas. While this study does not empirically prove racism in the Texas judicial system, it provides further insight into wrongful convictions and helps to assess the potential success of House Bill 34 in deterring future wrongful convictions.

Method

This study was conducted with a quantitative approach. It first sought to find the states most impacted by wrongful convictions by conducting a state-by-state analysis. This analysis compared the percentage of American wrongful convictions attributed to each state to the percentage of the American population each state accounts for. To study the connection be-

tween race and wrongful conviction, the study first retrieved all Texas exonerations listed by the National Registry of Exonerations, subdivided them based on the race of the defendant, and established the percentage of the sample that was African American. It then contrasted this percentage with the percentage African American population of Texas (utilizing information from the United States Census Bureau) to discover whether African Americans are inordinately affected by wrongful convictions in the state. This process was repeated with whites, Hispanics, and all other ethnicities that have been wrongfully convicted in Texas. These steps mirrored those taken by Gross et. al (2017) but analyzed a narrower sample (Gross et al. (2017) analyzed the United States as a whole while this research just examined Texas).

Furthermore, this study individually analyzed each Texas exoneration by retrieving the race of the defendant and contrasting that with the racial composition of the county where the defendant's trial took place. While many would assume that the racial composition of each jury would match the racial composition of the county, Wheelock (2011) presents a different view. Through an empirical study, Wheelock (2011) discovered that African Americans are severely underrepresented in Georgia juries, as the percentage of African Americans in juries is just under $\frac{1}{3}$ less than the overall percentage of African Americans in Georgia; it was also found likely that similar disparities would exist in other Southern states, including Texas. African American underrepresentation on juries is often owed to the practice of peremptory challenges, which allow for candidate jurors to be barred from jury duty without reason (Naidoff, 2013). Naidoff (2013) reaffirms the work of Wheelock (2011) by assessing that whites are severely overrepresented on criminal trial juries in Texas. One example is of Dallas County, which has dismissed 92% of potential African American jurors by peremptory challenges; only 2.8% of jurors in criminal trials were African American (Naidoff, 2013) despite the county being 23.5% African American (United States Census Bureau, 2016). While research has not been undertaken to allow for a completely accurate assessment of the racial composition of juries in Texas (except for in Dallas County), the estimate of Wheelock (2011) is likely a fair approximation for most Texas exonerations as Wheelock (2011) suggests that African Americans

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are barred from jury duty at similar rates across the Southern region of the United States. Therefore, this study estimated that the percentage African American population is reduced by $\frac{1}{3}$ in each jury in every county besides Dallas County (where I can use exact statistics) while white participation is increased by $\frac{1}{3}$ (while not perfect, Wheelock (2011) suggested that white often fill the spots of the underrepresented African American jurors, a sentiment also expressed by Naidoff (2013)). This study used a correlational method to assess the change in the rates of wrongful convictions of different ethnicities to the racial disparity of the county and the projected racial disparity of each jury. This method was ideal, as it allowed for quantitative assessment of a potential factor related to wrongful convictions in Texas, allowing for one to establish whether the race of the juror and the defendant has impacted rates of wrongful conviction. Through the correlational method, it became clear whether increased rates of white jurors, for example, results in increased rates of wrongful convictions of African American defendants, shedding light on the potential impact of juror discrimination.

This research filled a gap in the knowledge regarding the causations of wrongful convictions in Texas and helped to assess House Bill 34's effectiveness. While this research would not seek to empirically prove that Texas juries are inherently racist, it helped clarify whether race impacts wrongful convictions in Texas and whether House Bill 34 will be an effective deterrent to wrongful convictions in the state.

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Results

Table 1			
<i>Prevalence of Wrongful Convictions Per State</i>			
State	# of Wrongful Convictions	Percentage of USA Exonerations Attributed to State	State Proportion of American Population:
Texas	337	15.7%	8.8%
New York	255	11.9%	6.1%
Illinois	214	10.0%	3.9%
California	195	9.1%	12.2%
Michigan	84	3.9%	3.1%
Florida	70	3.3%	6.5%
Pennsylvania	68	3.2%	3.9%
Ohio	63	2.9%	3.6%
Massachusetts	61	2.8%	2.1%
North Carolina	60	2.8%	3.2%
Louisiana	56	2.6%	1.4%
Wisconsin	56	2.6%	1.8%
Virginia	51	2.4%	2.6%
Washington	48	2.2%	2.3%
Missouri	46	2.1%	1.9%
Oklahoma	34	1.6%	1.2%
Georgia	32	1.5%	3.2%
Indiana	32	1.5%	2.1%
New Jersey	30	1.4%	2.8%
Alabama	27	1.3%	1.5%
Tennessee	26	1.2%	2.1%
Maryland	25	1.2%	1.9%
Arizona	22	1.0%	2.2%
Connecticut	21	1.0%	1.1%
District of Columbia	20	0.9%	0.2%
Mississippi	18	0.8%	0.9%

Utah	16	0.7%	1.0%
Oregon	16	0.7%	1.3%
Iowa	14	0.7%	1.0%
Nevada	14	0.7%	0.9%
Kentucky	13	0.6%	1.4%
Montana	13	0.6%	0.3%
Minnesota	13	0.6%	1.7%
Kansas	10	0.5%	0.9%
Nebraska	10	0.5%	0.6%
West Virginia	10	0.5%	0.6%
Alaska	8	0.4%	0.2%
Arkansas	8	0.4%	0.9%
Colorado	8	0.4%	1.7%
South Carolina	7	0.3%	1.5%
New Mexico	7	0.3%	0.6%
Rhode Island	5	0.2%	0.3%
South Dakota	5	0.2%	0.3%
North Dakota	4	0.2%	0.2%
Wyoming	4	0.2%	0.2%
Hawaii	3	0.1%	0.4%
Maine	3	0.1%	0.4%
Delaware	2	0.1%	0.3%
Idaho	2	0.1%	0.5%
New Hampshire	1	0.05%	0.4%
Vermont	1	0.05%	0.2%
Total	2148	100%	100%

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All data in Table 1 was collected from the National Registry of Exonerations (2018) and the United States Census Bureau (2017). The data in Table 1 was ordered from high to low of the number of wrongful convictions in each state. As seen in Table 1, there have been 2148 recorded exonerations of wrongfully convicted inmates in the United States of America. Texas has accounted for 337 of these exonerations - 15.7% of all American wrongful convictions, and the most of any state. This comes despite Texas accounting for a mere 8.8% of the United States population. These discrepancies also exist in the states with the second and third most wrongful convictions in Amer-

ican history: New York and Illinois. New York has accounted for 11.9% of American wrongful convictions but only 6.1% of the American populace; meanwhile, 10% of all American wrongful convictions have taken place in Illinois despite the state only representing 3.9% of the US population. While small discrepancies exist within other states, all are relatively minor; the percentage each other state accounts for American wrongful convictions lies fairly close to its proportion of the American population, as shown in Table 1.

Table 2			
<i>Wrongful Convictions by Race in Texas</i>			
Race	# of Wrongful Convictions	Percentage of Texas Exonerations Attributed to Each Ethnicity	Ethnicity Proportion of Texas Population
African Americans	163	48.4%	12.6%
White	103	30.5%	79.4%
Hispanic	63	18.7%	2.3%
Asian	7	2.1%	4.8%
Other	1	0.3%	0.9%
Total	337	100%	100%

All data in Table 2 was collected from the National Registry of Exonerations (2018) and the United States Census Bureau (2017). The data in Table 2 was ordered from high to low of the number of wrongful convictions of each ethnicity. Within Texas wrongful convictions, there are sharp discrepancies between ethnic prevalence in the Texas populace and the proportion that differing ethnicities account for Texas wrongful convictions, as evidenced in Table 2. These clear disparities exist for African Americans, whites, and Hispanics. For instance, African Americans only account for 12.6% of the Texas populace, yet account for 48.4% of all Texas exonerations. Whites comprise 79.4% of Texas's population, but only 30.5% of Texas's exonerations. Finally, Hispanics represent only 2.3% of the Texas population but account for 18.7%

of Texas exonerations. These stark disparities do not exist within the other ethnic groups that have experienced Texas wrongful convictions, as Asians comprise 4.8% of Texas's population and 2.1% of wrongful convictions, while other ethnicities (such as Indigenous Americans, Alaskans, or Pacific Islanders) account for 0.3% of Texas wrongful convictions and 0.9% of the populace.

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Table 3

Wrongful Convictions by County: The Effect of Juror and Defendant Ethnicity

County	Projected Proportion of White Jurors	Projected Proportion of African Jurors	Projected Proportion of Minority (non-white) Jurors	Proportion of African American Exonerations	Proportion of Minority (non-white) Exonerations	# of African Americans Wrongfully Convicted	# of Whites Wrongfully Convicted	# of Hispanics Wrongfully Convicted	# of Others Wrongfully Convicted
Harris 2013-2018	76.6%	13.2%	23.4%	51.1%	74.4%	46	23	18	3
Harris 2005-2012	62.9%	12.6%	37.1%	78.1%	87.5%	50	8	6	0
Dallas 1995-2004	75.9%	2.8%	24.1%	73.3%	86.7%	11	2	1	1
Harris 1995-2004	65%	12.2%	35%	44.4%	77.8%	4	2	3	0
Bexar 1995-2004	71.3%	4.8%	28.7%	0%	83.3%	0	1	5	0
Montgomery 2013-2018	87.9%	2.9%	12.1%	0%	25%	0	3	1	0
Travis 1995-2004	71.3%	6.2%	28.7%	25%	50%	1	2	1	0
Galveston 2013-2018	86.6%	8.7%	13.4%	66.7%	66.7%	2	1	0	0
Montgomery 2005-2012	84.9%	2.9%	15.1%	0%	33.3%	0	2	0	1
Dallas 2013-2018	80.4%	2.8%	19.6%	0%	100%	0	0	0	2
Bexar 2005-2012	75.4%	5.0%	24.6%	0%	50%	0	1	1	0
Galveston 2005-2012	81.7%	9.2%	18.3%	50%	100%	1	0	1	0
Lamb 1995-2004	77.5%	2.9%	22.5%	0%	100%	0	0	2	0
Montgomery 1995-2004	89.5%	2.3%	10.5%	0%	0%	0	2	0	0
San Jacinto 2005-2012	85.6%	6.9%	14.4%	0%	0%	0	2	0	0
Tarrant 1995-2004	75.5%	8.5%	24.5%	0%	0%	0	2	0	0
Angelina 2005-2012	82.1%	10.0%	17.9%	0%	0%	0	1	0	0
Bexar 2013-2018	80.9%	5.1%	19.1%	0%	0%	0	1	0	0

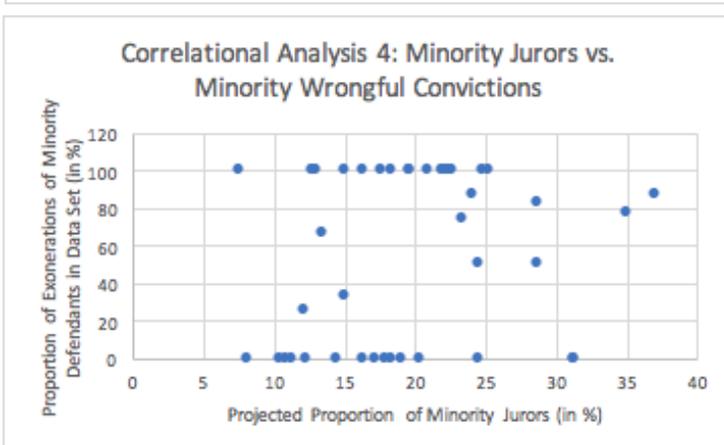
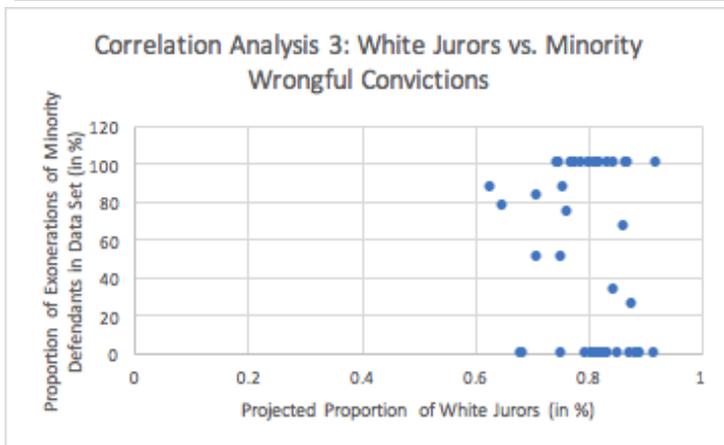
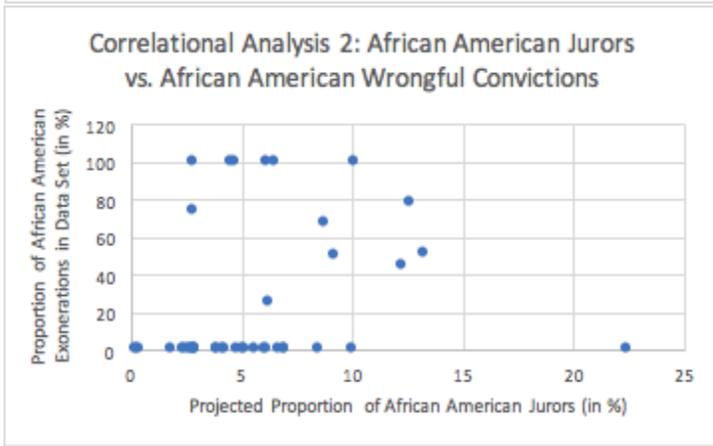
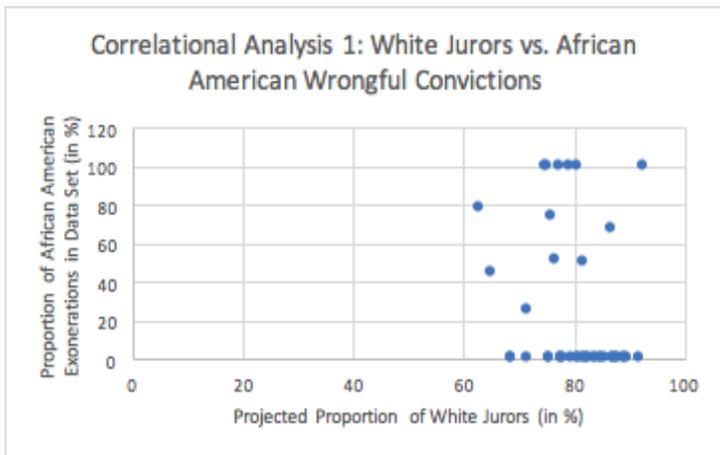
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Brown 2005-2012	87.7%	2.4%	12.3%	0%	0%	0	1	0	0
Cameron 2005-2012	87.2%	0.33%	12.8%	0%	100%	0	0	1	0
Collin 2013-2016	75.1%	6.1%	24.9%	100%	100%	1	0	0	0
Denton 2013-2018	79.6%	6.1%	20.4%	0%	0%	0	1	0	0
Denton 2005-2012	77.8%	5.6%	22.2%	0%	100%	0	0	1	0
Denton 1995-2004	83.7%	3.9%	16.3%	0%	0%	0	1	0	0
Ellis 2005- 2012	81.6%	6.0%	18.4%	0%	0%	0	1	0	0
Hale 1995- 2004	68.7%	3.9%	31.3%	0%	0%	0	1	0	0
Hidalgo 1995-2004	78.0%	0.17%	22%	0%	100%	0	0	1	0
Jefferson 1995-2004	68.5%	22.4%	31.3%	0%	0%	0	1	0	0
Lavaca 2013-2018	80.4%	4.5%	19.6%	100%	100%	1	0	0	0
Leon 1995- 2004	87%	6.9%	13%	0%	100%	0	0	1	0
McLennan 1995-2004	77.3%	10.1%	22.7%	100%	100%	1	0	0	0
Midland 2013-2018	84.9%	4.1%	15.1%	0%	100%	0	0	1	0
Nueces 2013-2018	88.7%	2.6%	11.3%	0%	0%	0	1	0	0
Nueces 2005-2012	82.8%	2.7%	17.2%	0%	0%	0	1	0	0
Nueces 1995-2004	74.8%	2.8%	25.2%	100%	100%	1	0	0	0
Sabine 2013-2018	92.4%	4.7%	7.6%	100%	100%	1	0	0	0
Shelby 1995-2004	79.1%	6.5%	20.9%	100%	100%	1	0	0	0
Upshur 1995-2004	89.1%	6.7%	10.9%	0%	0%	0	1	0	0
Van Zandt 2005-2012	91.8%	1.8%	8.2%	0%	0%	0	1	0	0
Webb 1995- 2004	82.3%	0.27%	17.7%	0%	100%	0	0	1	0
Williamson 2013-2018	83.7%	4.2%	16.3%	0%	100%	0	0	1	0

All data in Table 3, Figure 1, Figure 2, Figure 3, and Figure 4 was collected from the National Registry of Exonerations (2018) and the United States Census Bureau (2016). The data in Table 3 was list-

ed first by the number of wrongful convictions per county, then alphabetically, then reverse-chronologically. Each county was further categorized through restricting the years in which it was examined. It was

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restricted by rounding it towards the nearest analysis undertaken on the racial disparity of each county, using the 2000 census, the 2010 census, and the 2016 American Community Survey (United States Census Bureau, 2016). Table 3 only examined Texas exonerations from 1995 onwards, as the 2000 census is the most recent publicly available census providing county racial disparity information; despite attempts to retrieve information from earlier censuses, I was unable to. As referenced previously, the proportion of African American jurors in Table 3 was projected by multiplying the African American population of each county by 0.667; the lost African American juror population was added to the white population to project the proportion of white jurors. Projected minority juror population was calculated by subtracting the projected white juror population from 100. These steps were taken in accordance to the work of Wheelock (2011) to attempt to accurately project juror ethnicity populations. The only exception to this was for projecting juror population in Dallas county, as the work of Naidoff (2013) allowed for exact knowledge of juror population in Dallas county. There were four correlational analyses conducted after the data was collected, as illustrated above. The correlational analyses were all conducted using the data from Table 3. The first correlational analysis (Figure 1) analyzed the connection between the projected proportion of white jurors (independent clause) and the proportion of wrongful convictions of African Americans per county (dependent clause); this found no correlation. The second correlational analysis (Figure 2) analyzed the connection between the projected proportion of African American jurors (independent clause) and the proportion of wrongful convictions of African Americans per county (dependent clause); this found no correlation. The third correlational analysis (Figure 3) analyzed the connection between the projected proportion of white jurors (independent clause) and the proportion of wrongful convictions of minorities (all non-white ethnicities) per county (dependent clause); this found no correlation. The fourth correlational analysis (Figure 4) analyzed the connection between the projected proportion of minority jurors (independent clause) and the proportion of wrongful convictions of minorities per county (dependent clause); this found no correlation.

Results Alignment

These findings fill a gap in prior research, as they extend and expand on areas of prior research in the field. While the extent of wrongful convictions in the United States are widely known, this is the first known state-by-state breakdown of wrongful convictions. Further, while work has been done on a national scale to analyze the racial disparity of wrongful convictions, in the work of Gross et al. (2017), this is the first known research that has analyzed the racial composition of wrongful convictions in Texas. Through finding stark discrepancies between racial population and racial proportion of wrongful convictions, it confirms that exploring potential race-related motives in Texas wrongful convictions is an important area of study. Finally, through analyzing the connection between racial composition of jury members and the racial disparity of wrongful convictions on a county-by-county basis, this research attempted to provide quantitative answers to an unexplored area of literature surrounding wrongful convictions. It has long been speculated that race may factor into wrongful convictions, such as in the work of Gross et al. (2017), yet no quantitative research had been undertaken to attempt to find the cause of the lopsided wrongful convictions of minorities.

Discussion

Major Results

In the course of this study, three significant results were drawn from the data collected. First, it was established that Texas is disproportionately affected by wrongful convictions in the United States, as the state has registered the greatest amount of exonerations of any American state; the proportion of American exonerations it accounts for also outstrips the proportion of the American populace Texas accounts for. While The Innocence Project (2009) found that Texas accounted for the most wrongful convictions overturned by DNA evidence of any American state, this research clarified that Texas also has the most exonerations of any state, filling a gap in the research.

Further, it was discovered that races are impacted in different ways by wrongful convictions in Texas.

First, this study found that African Americans comprise nearly half of all Texas exonerations, despite only accounting for a small percentage of the Texas population. It also found that whites are severely underrepresented in Texas wrongful convictions, as they make up a far smaller proportion of exonerations than they do of the Texas population. Lastly, it established that Hispanics are also disproportionately affected by wrongful convictions, being wrongfully convicted even at rates higher than that of African Americans in Texas. These findings filled a gap in research regarding Texas wrongful convictions by providing the first known analysis of the ethnic disparity of Texas exonerations.

Finally, this study discovered that juror ethnicity does not correlate to rates of wrongful convictions of non-white defendants. In four correlational analyses undertaken, it was discovered that no correlation existed in the following relationships: white jurors and African American defendants, African American jurors and African American defendants, white jurors and minority defendants, and minority jurors and minority defendants. This filled a gap in the research, as little empirical work has been conducted to analyze the potential effect of racism in wrongful convictions. Through this finding, it seems clear that Texas jurors are not motivated by racism.

Implications

The findings of this study are meaningful as they shed light on a number of pertinent issues. First, it confirms that Texas has the most exonerations of any state, making it a topical state to examine wrongful convictions within. As wrongful convictions are clearly a significant issue in Texas, it is evident that research should continue to be conducted to evaluate the factors influencing wrongful convictions in the state.

Further, the findings of this study clearly evidence a stark racial disparity in Texas wrongful convictions. From this, it appears probable that racial discrimination factors into wrongful convictions in Texas, making it clear that House Bill 34 will not completely tackle the causes of Texas wrongful convictions. This is important as it can act as a guide for future research: while it has now been determined that race impacts wrongful convictions in Texas, further work can be done to clarify where the racial discrimination originates within.

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While House Bill 34 is undoubtedly an excellent piece of legislation that tackles many causes of wrongful convictions and will likely help to mitigate future wrongful convictions, through failing to account for racism it leaves the legislation lacking in its quest to dramatically change Texas's judicial landscape.

Through analyzing the connection between juror ethnicity and defendant ethnicity, this study looked into one potential area of racial discrimination in the Texas judicial system: jurors. To show signs of potential racism, a positive correlation should have existed between white jurors and African American exonerees or between white jurors and minority exonerees. Further, racism could have been suggested through a negative correlation between African American jurors and African American exonerees or between minority jurors and minority exonerees. Ultimately, no correlation was found in any of the analyses. Through this finding, it seems largely confirmed that racial discrimination within the Texas judicial system does not originate within jurors, meaning that one potential cause of racism has likely been eliminated.

Relation to Past Literature

This study extended and clarified the work of a number of key pieces of literature. First, it extended the findings of The Innocence Project (2009) through clarifying how differing states are affected by wrongful convictions. The Innocence Project (2009) discovered that Texas has the greatest number of wrongful convictions overturned by DNA evidence in the United States, but had not determined that Texas accounted for the greatest number of overall exonerations. This study extended the work of The Innocence Project (2009) by proving that Texas was inordinately affected not only by DNA exonerations, but by wrongful convictions in general.

Further, this study narrowed the work of Gross et. al (2017). Gross et. al (2017) discovered in their work that African Americans compose 47% of all American exonerations; this study found the racial disparity of wrongful convictions in Texas, providing a more specific version of the research conducted by Gross et. al (2017). It also extends the work of Gross et. al (2017) through proving that race seemingly impacts wrongful convictions in Texas, as well as in the United States as a whole.

Moreover, this study relates to the work of Horry, Wright, and Tredoux (2010), who found that inherent biases often impact the means by which different ethnicities treat each other. Through the results of this study, it seems clear that this finding does not hold true in the Texas criminal justice system between jurors and defendants.

Finally, this study provides greater depth to the findings of Acker (2009), Cassell (1998), Dervan and Edkins (2013), Gould, Hail-Jares, and Carrano (2014), Gould and Leo (2010), Hewitt and Natapoff (2012), Huff (2002), Huff (2004), Neuschatz, Lawson, Swanner, and Meissner (2008), and Schneider (2013). These authors all conducted extensive research regarding the factors relating to wrongful convictions. This research extends their work by adding a factor that does not impact wrongful convictions, at least in Texas: juror and defendant ethnicity.

Limitations and Alternate Explanations

The results of this study are limited through a lack of information in certain areas of the research. First, although Texas has the most exonerations of any state, it is unknown whether Texas actually has the most wrongful convictions in the United States. One explanation could be that Texas is far more diligent in searching out wrongful convictions than other states, meaning that they would have obviously registered more exonerations. Unfortunately, without the proper information to determine if this is the case, it is impossible to know whether Texas has the most wrongful convictions of any state.

Second, and most importantly, was the lack of conclusive information regarding jury composition. While I was able to estimate jury composition, using the work of Wheelock (2011), it remains unclear whether the approximations were accurate. The situation in Dallas County clearly shows these limits. Dallas County was the only county with conclusive information regarding jury composition, through the research conducted by Naidoff (2013). In that study, it was found that African Americans only accounted for 2.8% of criminal jury members, despite African Americans composing 23.5% of the Dallas County population (Naidoff, 2013). Dallas County's African American proportion population is significantly greater than that of Texas overall (only 12.6% of Tex-

as's population is African American), making Dallas County one of the most African American counties in Texas. Despite this, Dallas County had the eight lowest projected total of African American jurors of all the counties examined in this study. Through these revelations, it becomes clear that similarly large disparities could exist in other Texas counties, and not follow the one-third-reduced rule of Wheelock (2011). Through this, it becomes unclear whether African Americans are barred from jury duty at similar rates to Dallas County in all of Texas. If so, this would suggest state-wide discriminatory practices against African Americans, making it impossible to determine if juror ethnicity played a meaningful role in wrongful convictions. Through this limitation, it is quite possible that the findings could conclude differently; with accurate statistics regarding juror ethnicity, it is well within the realm of possibility that the correlational analyses could find correlations.

Directions for Future Research

Future research on this topic should be defined by the clarifications provided by this study. The research conducted has evidenced that African Americans are disproportionately affected by wrongful convictions, but has also likely ruled out juror discrimination. For this reason, future research should delve further into other potential sources of racism within the Texas judicial system, such as institutional and systemic biases towards African Americans.

Conclusion

Through the course of this study, three significant inquiries were tackled. First, the study provided clarifications on the states most impacted by wrongful convictions through a state-by-state analysis comparing the proportion of American exonerations attributed to each state versus the proportion of the American populace each state accounted for. In this analysis, it was discovered that Texas, New York, and Illinois are the states most affected by wrongful convictions. Next, the validity of House Bill 34 was assessed through analyzing the racial disparity of wrongful convictions in Texas. As African Americans were found to be wrongfully convicted at inordinate rates, it seems likely that

House Bill 34 - which lacks stipulations to tackle factors of racial discrimination - will not be entirely successful. While it accounts for many factors influencing wrongful convictions, its failure to account for racism makes it lacking in its goal to drastically alter the landscape of Texas wrongful convictions. Finally, a potential source of racism within the Texas judiciary, juror discrimination, was thoroughly examined by comparing projected ethnic rates of jurors to rates of African American and minority wrongful convictions. Through the findings of the study, it seems probable that juror discrimination is not the cause of the disproportionate degree of wrongful convictions of African Americans, clarifying the field of literature and providing direction for future research.

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Evaluation of Essential Oils as an Alternative to Conventional Antibiotics

Megan Leinenbach

The purpose of this study was to determine if synergistic interactions between essential oils can increase their antibacterial efficacy by testing essential oils. The following combinations were assessed for effective inhibition of *E. coli* and *S. aureus* growth: tea tree oil as the control solution, tea tree/oregano solution, tea tree/thyme solution, and tea tree/wintergreen solution. A Kirby-Bauer disc diffusion was employed to assess antibiotic efficacy. When comparing the different combinations to the control solution, the results of this study signify the presence of synergistic interactions between select essential oils; the combinations that were proven to increase antibacterial efficacy when compared to the control were the tea tree/oregano and tea/tree thyme solution. The combination with the greatest antibacterial efficacy for inhibiting *E. coli* and *S. aureus* growth was tea tree and oregano oil, which had an average inhibition percentage of 59.53% (*E. coli*) and 55.91% (*S. aureus*). This result was found to be statistically significant ($p < 0.05$) using an ANOVA single factor test.

Keywords: Essential oils, natural medicine, bacterial growth, synergism

Introduction

An increasing number of antibiotic resistant strains of bacteria are posing a health risk to Americans. Since the introduction of penicillin in 1940, antibiotics have been the dominant method used to treat infections; however, antibiotic resistance has been increasing among bacteria populations due to selective pressures (Ott & Morris, 2008). In fact, antibiotic resistance has been detected in bacteria for all of the more than 100 antibiotics in use as of 2008 (Ott & Morris, 2008). The Center for Disease Control (2017) details the impact of antibiotic resistance by stating that at least 2 million people in the United States are infected with antibiotic resistant bacteria each year, and 23,000 people die each year as a direct result of these infections. With antibiotic resistance being the cause of such a large

number of deaths, it is vital that the public is aware of this biological phenomenon and that this be addressed to meet the needs of patients with antibiotic-treatable illnesses.

While multiple bacteria have developed a resistance to antibiotics, this study will focus exclusively on *Escherichia coli* and *Staphylococcus aureus*. *E. coli* is a gram-negative bacterium¹ that is a common inhabitant of the intestinal tract of warm-blooded animals; there are four strains of *E. coli* that can cause diarrheal illness and disease (Jenson, 2003). Further demonstrating this assertion, Christina Gorman (2011) of University of California: Santa Barbara writes that disease-causing strains of *E. coli* are resistant to fourteen different antibiotics and are consistently the subject of research evaluating alternative treatment options. Roa et al. (2015) classify *Staphylococcus aureus* as a gram-positive² bacterium that can be found on the skin and

¹ Gram-negative bacteria have cell walls composed of a thin layer of peptidoglycan, which may affect the way essential oils enter through the cell wall and membrane of the bacteria.

in the nares of humans. Additionally, *Staphylococcus aureus* has become an acute threat to infection control with the increasing prevalence of methicillin-resistant *S. aureus* (MRSA) and its ability to resist multiple drugs (Rao et al., 2015). Although the work of the aforementioned authors focused on two different bacteria, their findings collectively demonstrate the consequences of antibiotic resistance and its implications regarding public health.

Due to the prevalence of antibiotic resistance, various research studies have been conducted to gain a deeper understanding of how this phenomenon occurs. Laxminarayan and Brown (2000), had an environmental perspective when analyzing the mechanisms of antibiotic resistance, asserting that natural selection fosters antibiotic resistance. The authors go further in depth, attributing the development of antibiotic resistant strains to plasmid transfer³, mutation, and the overuse of broad-spectrum antibiotics⁴ in hospitals (Laxminarayan & Brown, 2000). Building on Laxminarayan and Brown's argument, Lauren Richardson, who has a PhD in pharmacology from University of Washington, offers similar information in her PLOS biology article; Richardson identifies the overuse of antibiotics as a primary source of growing antibiotic resistance, indicating that "overuse threatens [antibiotics] efficacy", again, taking an environmental approach to explaining antibiotic resistance. Richardson also ascertains mutation as a mechanism of antibiotic resistance, attributing the mutation of one bacterium to the development of further mutation, leading resistance to become a property of the microbial community (Richardson, 2017). Both Richardson and Laxminarayan's ideas attribute the overuse of antibiotics to the evolution of bacterial communities in response to environmental conditions, meaning that an alternative to conventional antibiotics must be identified in order to limit antibiotic use.

While antibiotic resistance is a concern for the medical community, society itself tends to underestimate the significance of this biological phenomenon; although a multitude of medical discoveries have been made recently, the discovery of new antibiot-

ics is a rare event (Richardson, 2017); thus, the drugs that can be used to combat infections caused by resistant bacteria are limited. In a study done by Adabara (2012), a member of the Department of Microbiology at Federal University of Technology, it was found the 60% of bacterial samples in a Nigerian hospital contained antibiotic resistant bacteria, encompassing many different species. With such a large percentage of collected bacterial isolates being resistant, it is apparent that the resistance of bacterial communities has become a serious issue. The fortuitous results of this study substantiate the critical nature of antibiotic resistance and its global reach. While antibiotic resistance allows all bacterial species to evolve in response to their surroundings, MRSA is the most frequent cause of antibiotic resistant infection in humans Richardson, 2017. Rao et al. elaborate on Richardson's claims about *S. aureus* by stating that it often infects healthy individuals who do not possess risk factors, such as surgery or residence in a long-term facility (2015). The presence of antibiotic-resistant bacteria in both the healthy community and hospitals makes containing the growth of resistance ever more urgent, leading medical researchers to pursue an innovative technique with which to treat antibiotic resistant infections; one of these proposed methods is the application of essential oils.

According to Stephen Bent, a professor at University of California: San Francisco's School of Medicine, the United States has seen a surge in the popularity of herbal products in the last decade, and, as of 2007, were used by twenty percent of the American population for aromatherapy, depression, and nausea among other uses (2008). Various oils and their antibiotic efficacies have been studied extensively; however, in this study, the focus will remain on four essential oils that have been shown to have antibiotic properties: tea tree, thyme, oregano, and wintergreen oil. Tea tree oil, obtained from *Melaleuca alternifolia*, has been found to inhibit the growth of multiple bacteria, including *Escherichia coli* and *Candida* species (Ott & Morris, 2008). Of the multiple essential oils that have been researched, tea tree oil has shown the greatest potential

2 Gram positive bacteria have a cell wall that is composed of a thicker layer of peptidoglycan than gram-negative bacteria.

3 Plasmid transfer: the ability to directly transfer genetic material between bacteria

4 Broad-Spectrum Antibiotics: antibiotics that act against a wide range of disease-causing bacteria, including both gram-negative and gram-positive bacteria.

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as an inhibitory agent for bacterial growth, and oregano oil has been shown to have consistent antibacterial effects against common bacteria (Ott & Morris, 2008). Additionally, both wintergreen and thyme oil are recognized as having antibiotic and antimicrobial properties (European Medicines Agency, 2014). While essential oils are currently popular for their commercial use, they were first used by classical civilizations, most notably in India, for their medicinal properties (Bodeker, Buford, Chamberlain, and Bhat, 2001). Medical professionals have turned to these past treatments in the hopes that they will illuminate the solution to current medical issues, such as antibiotic resistance.

Bodeker et al. (2001) studied the medicinal potential of two popular agroforestry⁵ species; this research revealed that both species were suitable for the treatment of priority diseases in Sub-Saharan Africa, such as AIDS and Malaria, also including treatment of urinary diseases, boils, skin diseases, ulcers, malaria, fever, colic, and inflammation. Research studies evaluating the effectiveness of essential oils to address antibiotic resistance, such as those by Ott, Morris, Bodeker, Buford, Bhat, and Chamberlain, have made it apparent that it is possible to utilize essential oils as a treatment option opposing antibiotics; the use of essential oils could be especially effective in countries where new medical technology has yet to become readily available. While the work of Bodeker et al. does demonstrate the pharmaceutical potential of essential oils, it is relatively generalized, making its application to antibiotic resistance particularly onerous. However, the potential of essential oils as bacterial growth inhibitors was further analyzed by S. Alizadeh-Salteh, K. Arzani, R. Omidgeigi, and N. Safaie in their work discussing how essential oils can be used to inhibit the growth of *Rhizopus stolonifer*, a chief source of destructive postharvest disease of fruit (2010). In addition to the work of Bodeker et al., which generally classified essential oils as having medicinal potential, this new research highlighted the use of essential oils specifically to inhibit bacterial growth rather than treat symptoms of disease. These results further demonstrated the potential of essential oils as bacterial growth inhibitors, demonstrating how essential oils could be advantageous for the inhibition of *R. stolonifer* growth as well as other bacterial species.

The curative potential of essential oils in combating infection by antibiotic-resistant bacteria was addressed by Ott and Morris (2008), in their article *Homeopathic Alternatives to Conventional Antibiotics*. Ott and Morris addressed how a myriad of essential oils and herbs are capable of hindering the growth of microorganisms, including *E. coli* and *S. aureus* (Ott & Morris, 2008). The work of Ott and Morris not only deemed essential oils as effective bacterial growth inhibitors, but it also distinguished between the efficacy of different essential oils. While data obtained prior to this point was limited to a single bacteria or disease, the work of Ott and Morris applied essential oils to multiple bacteria, greatly increasing knowledge regarding natural medicine. The data collected suggests that tea tree oil is an extremely effective inhibitor of *E. coli* growth and identified oregano, thyme, and wintergreen oil as supplementary growth inhibitors (Ott & Morris, 2008).

The present body of research has led to an augmented interest in the homeopathic use of essential oils. In fact, many articles have been published delineating the procedure used to test the efficacy of essential oils as antibiotic inhibitors (Morris, 2010; Schepler, Sethakorn, Styer, 2003). However, the current body of research on this topic has yet to address several gaps in the research of natural medicine, such as the effects of combining essential oils or how the concentration of bacteria affects the efficacy of essential oils. The lack of knowledge about essential oils and natural medicine has led physicians to develop a penchant for conventional antibiotics and become skeptical of the reliability of essential oils as a common treatment (Bent, 2008). While plant pathologists and microbiologists have already conducted considerable research on the efficacy of individual essential oils and their inhibitory effects on multiple strains of bacteria, research concerning the effects of these oils in combination is lacking. By researching the potential synergistic interactions of essential oils, this study can contribute to the growing body of work concerning alternative medicine and address the knowledge gap regarding the effects of combining essential oils. Additionally, the use of gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria in the research will allow for a deeper understanding of how the classifi-

⁵ Agriculture incorporating the cultivation and conservation of trees

cation of bacteria may affect the efficacy of essential oils in combination. This fostered the question: *How do the synergistic interactions of select essential oils – tea tree, thyme, oregano, and wintergreen oil – affect their antibacterial efficacy as an Escherichia coli and Staphylococcus aureus growth inhibitor?* Analyzing the results of prior studies and the efficacy of individual essential oils led to the hypothesis that a combination of tea tree and oregano oil would have the greatest antibiotic efficacy.

Methodology

The methodology chosen for this study was a modified Kirby-Bauer disc diffusion, which was chosen due to its success in producing results for prior studies conducted by the aforementioned researchers. This method was chosen because a Kirby-Bauer disc diffusion poses minimal risk to healthy individuals and is a standard procedure used in clinical laboratories to test the susceptibility of patient's bacterial isolates to antibiotics (Scheppeler et al., 2003); Since this methodology is often used to test antibiotic susceptibility and the goal of this study was to test susceptibility to essential oils, a Kirby-Bauer disc diffusion was an appropriate choice for this study. Additionally, this method utilizes filter paper discs, which can absorb any solution, making the creation of precise essential oil combinations and their exposure to bacteria a relatively simple process. Finally, the quantitative nature of a Kirby-Bauer disc diffusion allows for easy comparison between control and test groups. Overall, this was an optimal methodology for this study because it provided a safe way to obtain accurate data comparing the inhibitory potential of essential oils in combination.

Materials

Four essential oils that are commonly used for their medicinal properties and commercial purposes [tea tree (*leaf*) (*Melaleuca alternifolia*), oregano (*flower*) (*Origanum vulgare*), thyme linalool (*flowering tops*) (*Thymus vulgaris*), and wintergreen (*leaf*) (*Gaultheria procumbens*)] were purchased from Plant Therapy at planttherapy.com; one 10 mL container of each essen-

tial oil was ordered. The brand “Plant Therapy” was chosen because of their company imposed regulations to ensure the consistency and quality of their oils; Plant Therapy tests their oils multiple times, sending them to a third-party laboratory to undergo Gas Chromatography and Mass Spectrometry tests. In addition, two microorganisms were used to determine the inhibitory properties of these essential oils; the bacterial strains that were used included *Escherichia coli* (ATCC 11775) and *Staphylococcus aureus* (ATCC 29213).

Disc Diffusion

Plate Preparation

The susceptibility of bacteria to essential oil combinations was determined using a modified Kirby-Bauer disc diffusion assay. Twenty-four Mueller-Hinton agar plates were used to cultivate bacterial cultures; twelve plates were used for each bacteria. Each Mueller-Hinton agar plate was divided into four separate quadrants, labelling the respective quadrant on the bottom of the plate. Two categories of plates were tested: control plates and test plates. Control plates were used to determine the inhibitory potential of each essential oil individually; therefore, no combinations were tested on control plates. Test plates were used to obtain data for combinations of essential oils. For test plates, these quadrants included “Control”, “Oregano and Control”, “Wintergreen and Control”, and “Thyme and Control”. For the purpose of this project, the “Control” solution is tea tree oil. For control plates, these quadrants included “Tea Tree”, “Oregano”, “Wintergreen”, and “Thyme”. Plates were swabbed evenly with bacteria, *E. coli* and *S. aureus* respectively. To do this, a sterile swab was dipped into the bacterial suspension broth and streaked over the surface of the plate.

Disk preparation

Control Plates: Following the swabbing of all agar plates, filter paper discs were prepared. Each quadrant of an agar plate contained one filter paper disc that had absorbed a solution for testing; for discs on control plates, individual oils were absorbed. Each plate

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was comprised of one disc per essential oil: tea tree, oregano, wintergreen, and thyme oil (four total oils were tested, meaning one oil was tested per quadrant); each disc contained 6 μL of solution. Once these discs were prepared, each was placed in its respective quadrant using sterilized forceps. Plates were incubated at 37 $^{\circ}\text{C}$ for 24 hours.

Test Plates: Filter paper discs on the test plates tested combinations of essential oils; these plates were also composed of one quadrant dedicated to a control disc for tea tree oil. For the control quadrant of the test plates, 6 μL of tea tree oil was absorbed by the filter paper discs by directly applying the solution to the disc using a micropipette. Since each disc must contain a total 6 μL of solution total and the combinations were mixed at a 1:1 ratio, combination discs contained 3 μL of tea tree oil (control solution) and 3 μL of a supplementary oil. Once these discs were prepared, each disc was placed in its respective quadrant using sterilized forceps. Plates were incubated at 37 $^{\circ}\text{C}$ for 24 hours.

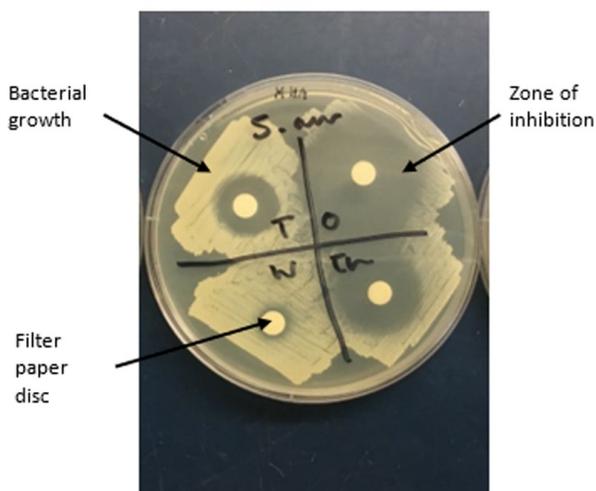


Figure 1: Agar Plate Diagram

This picture provides an example of what a correctly prepared plate will look like after incubation. This plate has four correctly labelled quadrants, each with one filter paper disc containing its respective solution, as described previously.

Data Measurement

After 24 hours of incubation, the zone of inhibition⁶ was measured in millimetres for each quadrant; this data was used to arbitrate the inhibition percentage. The inhibition percentage of the bacterial growth was calculated by utilizing the data obtained from test plates. The three quadrants of the test plate were compared to the control quadrant of that respective plate. The following equation was used:

Inhibition Percentage = $100 (T-C) / C$, where C is the diameter of the zone of inhibition for the control quadrant (tea tree oil individually) and T is the diameter of the zone of inhibition for the test quadrant.

Considering the goal of this research project was to evaluate the antibacterial properties of essential oils, this methodology was an appropriate choice. The modified Kirby-Bauer disk diffusion allowed for data to be obtained for all combinations and individual essential oils; The dependent variable of the Kirby-Bauer disk diffusion (the diameter of the zone of inhibition) corresponded to the goal of this study: measuring the inhibitory potential of essential oil combinations.

Results

Herbs, including essential oils, are defined as any form of a plant product, including leaves, stems, flowers, roots, and seeds (Noller, Kumar, Lajis, & Ali, 2008). When evaluating the possible presence of synergistic interactions between select essential oils as bacterial inhibitors, the data that was obtained confirmed that combining certain essential oils can increase their efficacy in certain cases.

⁶ The area where bacterial growth was inhibited

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TABLE 1: ANOVA SINGLE FACTOR (ESCHERCHIA COLI)

SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	9	142	15.77778	0.694444		
Column 2	9	226	25.11111	3.111111		
Column 3	9	155	17.22222	5.944444		
Column 4	9	187	20.77778	1.444444		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	467.6667	3	155.8889	55.70223	8.43E-13	2.90112
Within Groups	89.55556	32	2.798611			
Total	557.2222	35				

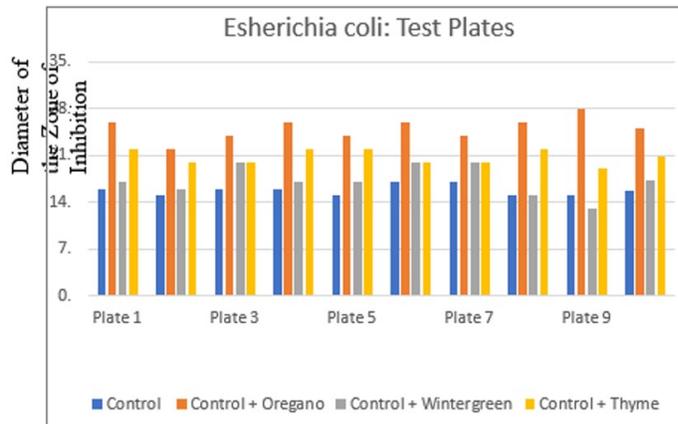
The ANOVA single factor test for E. coli test plates shows a P-value of 8.43×10^{-13} , signifying that the results of this study concerning the inhibition of E. coli growth are statistically significant.

TABLE 2: T TEST: TWO-WAY SAMPLE ASSUMING UNEQUAL VARIANCES (ESHERICHIA COLI)

Key	A (Control)	B (Control + Oregano)	C (Control + Wintergreen)	D (Control + Thyme)		
Combinations	*A-B	A-C	*A-D	*B-C	*B-D	*C-D
T--test P-value	1.8109E-08	0.123522	6.82E-08	1.06E-06	2.79E-05	0.00202
Adjusted P-value	0.008	0.008	0.008	0.008	0.008	0.008

To determine which combinations had a statistical difference, a t test: two-way sample assuming unequal variances was conducted. The P-value was adjusted from .05 to .008 to account for the six tested combinations. The resulting P-values from the t-test demonstrated statistically significant results for all combinations, excluding the tea-tree and wintergreen oil combination (A-C).

FIGURE 1: ESCHERICHIA COLI: TEST PLATES



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TABLE 3: SINGLE FACTOR ANOVA (STAPHYLOCOCCUS AUREUS)

SUMMARY						
Groups	Count	Sum	Average	Variance		
Column 1	9	142	15.77778	0.694444		
Column 2	9	226	25.11111	3.111111		
Column 3	9	155	17.22222	5.944444		
Column 4	9	187	20.77778	1.444444		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	467.6667	3	55.70223	55.70223	8.43E-13	467.6667
Within Groups	89.55556	32	2.798611			
Total	557.2222	35				

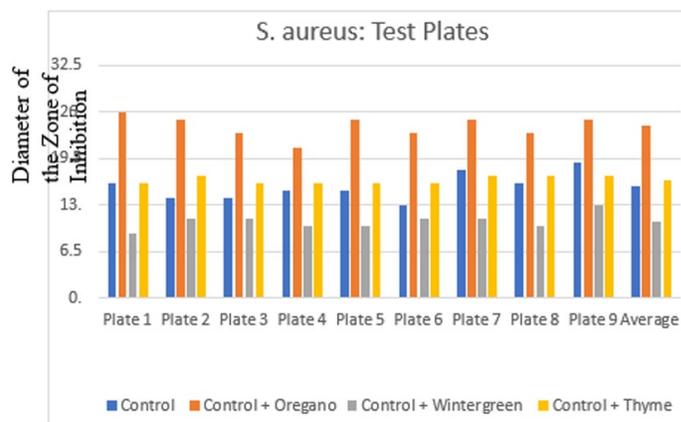
A single factor ANOVA test was conducted to determine if the results concerning the microorganism *Staphylococcus aureus* were statistically significant. With a P-value of 8.43×10^{-13} , the results can be deemed statistically significant.

TABLE 4: T TEST: TWO-WAY SAMPLE ASSUMING UNEQUAL VARIANCES (STAPHYLOCOCCUS AUREUS)

Key A (Control)	B (Control + Oregano)	C (Control + Wintergreen)	D (Control + Thyme)			
Combination	*AB	*AC	AD	*BC	*BD	*CD
T-test P-value	4.32E-08	1.88E-05	0.218088	6.94E-12	8.93E-08	2.31E-08
Adjusted P-value	0.008	0.008	0.008	0.008	0.008	0.008

A t test: two way sample assuming unequal variances was conducted to determine which combinations had statistically significant results concerning their potential synergistic interactions and the inhibition of *Staphylococcus aureus* growth. The P-value was adjusted from .05 to .008 to account for the six combinations. The results show that all combinations proved a statistical difference except for control and thyme oil (tea tree and thyme oil).

FIGURE 2: STAPHYLOCOCCUS AUREUS: TEST PLATES



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TABLE 5: INHIBITON PERCENTAGE (*ESCHERICHIA COLI*) TABLE 6: INHIBITON PERCENTAGE (*STAPHYLOCOCCUS AUREUS*)

Plate number	Control + Oregano	Control + Wintergreen	Control + Thyme
Plate 1	62.50%	6.25%	37.50%
Plate 2	46.67%	6.67%	33.33%
Plate 3	50%	25%	25%
Plate 4	62.50%	6.25%	37.50%
Plate 5	60%	13.33%	46.67%
Plate 6	52.94%	17.65%	17.65%
Plate 7	41.18%	17.65%	17.65%
Plate 8	73.33%	0%	46.67%
Plate 9	86.67%	-13.33%	26.67%
Average	59.53%	8.83%	32.07%

TABLE 6: INHIBITON PERCENTAGE (*STAPHYLOCOCCUS AUREUS*)

Plate number	Control + Oregano	Control + Wintergreen	Control + Thyme
Plate 1	62.50%	-43.75%	0%
Plate 2	78.57%	-21.43%	21.43%
Plate 3	64.29%	-21.43%	14.29%
Plate 4	40%	-33.33%	6.67%
Plate 5	66.67%	-33.33%	6.67%
Plate 6	76.92%	-15.34%	23.08%
Plate 7	38.89%	-63.64%	-5.56%
Plate 8	43.75%	-37.50%	6.25%
Plate 9	31.58%	-31.58%	-10.53%
Average	55.91%	-33.48%	7%

Discussion

The results of this study signify the presence of synergistic interactions between tea tree and oregano oil, making it the combination that is most effective at inhibiting bacterial growth for both *E. coli* and *S. aureus*. These findings supported the initial hypothesis that the combination with the greatest antibacterial efficacy would be tea tree and oregano oil.

When analyzing the statistically significant results and looking at the potentially synergistic combinations, it is apparent that the combination with the highest mean inhibition zone diameter (IZD) is Control-Oregano (tea tree and oregano oil) for both bacteria. This illustrates that the combination with the greatest antibacterial potential is control-oregano (Figure 1 & Figure 2). The inhibition percentages for *E. coli* further support the claim that the control-oreg-

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ano combination has the greatest antibacterial efficacy; the average inhibition percentage for the control-oregano combination was 59.53 percent, meaning that this combination's IZD was 59.53 percent larger than the control groups IZD. Furthermore, this inhibition percentage is considerably larger than that of the other two combinations, further supporting the data demonstrated by the ANOVA, TTEST, and averages IZDs (Table 5). The inhibition percentages for *S. aureus* also demonstrate the antibacterial efficacy of the control-oregano combination; the average inhibition percentage for the control-oregano combination was 55.91 percent. This inhibition percentage is considerably larger than that of the other two combinations. In addition to the synergistic interaction that is demonstrated by this data, one can see how the combination of wintergreen and tea tree oil is antagonistic with an inhibition percentage of -33.48%.

These results gave way to a variety of fascinating findings. For both *E. coli* and *S. aureus*, the tea tree and oregano oil combination had the greatest degree of antibacterial efficacy with an inhibition percentage of 59.53% (*E. coli* – Table 5) and 55.91% (*S. aureus* – Table 6); however, the t-test proved that the same combinations did not elicit the same results for both bacteria. The combinations that proved a statistical difference for *E. coli* were tea tree/oregano oil and tea tree/thyme oil (Table 3) when the values were compared to the control (tea tree oil alone). The combinations that proved a statistical difference for *S. aureus* were tea tree/oregano oil and tea tree/wintergreen oil (Table 4). These results show that combining essential oils does cause a change in efficacy, although this change is not consistent between bacterial species. Further analyzing these results, it is apparent that utilizing essential oils as a medical treatment would require a deeper understanding of how the inhibitory potential of an oil will change in the presence of various bacteria. This assertion is further supported by the work of Ott and Morris (2008), whose study demonstrates that the inhibitory potential of essential oils individually will vary based on the bacterial species being tested.

Furthermore, the results demonstrated that combining essential oils does not always lead to an increase in antibiotic efficacy. For *E. coli*, the combina-

tion of tea tree and wintergreen oil was not proven to have a statistical difference from the control, meaning that the addition of wintergreen oil to tea tree oil did not have any effect on the solutions antibiotic efficacy; however, this combination did prove a statistical difference for *S. aureus*. Unlike the tea tree/oregano oil combination, the tea tree/wintergreen oil combination had antagonistic effects on the growth of *S. aureus*. While the average IZD for tea tree oil alone was 15.6 mm, the average IZD for tea tree and wintergreen oil was 10.7 mm, decreasing the zone of inhibition by 33.5% (Figure 2). This is a significant finding because the tea tree/wintergreen combination was the only tested solution that led to a smaller IZD for either bacteria. From this case, one can conclude that although combining essential oils consistently led to an increase in efficacy, it is possible for a combination to have adverse effects. Should the tea tree/wintergreen solution be used in a medical setting to treat a *S. aureus* infection, the results would have been detrimental to the patient because of the antagonistic relationship between these oils; with this in mind, it is important that one understands that combining essential oils can increase and decrease antibacterial efficacy in certain cases.

Prior studies and literature stress the urgent need to address the increasing growth of antibiotic resistant bacteria, as the increasing use of antibiotics has led to greater antibiotic resistance and there is a need to identify an alternative treatment method. When analyzing the data presented in the study, it is apparent that essential oils do have antibacterial properties and their efficacy can be increased by combining them with supplementary oils; in particular, the combination of tea tree and oregano oil had the greatest increase in antibacterial efficacy; however, the next step, after identifying the particularly effective essential oil solutions, is to implement natural and holistic medicine into treatment regimens. While holistic medicine is unconventional in the United States, it is the main form of medicine in 80% of the currently developing world and was used by ancient civilizations for over 5,000 years; furthermore, plants and plant-based solutions make up 20% of pharmaceuticals in the United States (Noller, Kumar, Lajis, & Ali, 2008). Considering their widespread use, essential oils could be used as

7 Nephropathy- medical term used to denote disease or damage of the kidney, which can eventually result in kidney failure

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a first line of defence for fighting bacterial infections in the United States and ultimately across the world; thus, patients could initially address their infections with natural medicine and utilize conventional antibiotics if the essential oils or herbs were not producing the desired effects. This solution would allow physicians to prescribe fewer antibiotics, limiting the survival of antibiotic resistant bacteria and the toxic side effects of antibiotic usage.

While the results of this study demonstrate an optimistic future for essential oils as an alternative to conventional antibiotics, there are limitations concerning this research project and the use of essential oils as a treatment option. Regarding the execution of this methodology, there was potential for contamination when swabbing the agar plates and transitioning them from the biosafety cabinet to the incubator.

One limitation regarding the use of natural medicine as a treatment is a lack of regulation. According to Stephen Bent (2008), herbs are currently defined by law as dietary supplements, meaning that manufacturers can produce, sell, and market herbs without demonstrating their efficacy or safety. Additionally, in order for a “dietary supplement” (i.e. natural medicine and essential oils) to be removed from the market, it must be deemed unsafe by the FDA, directly contrasting to the regulation of drugs, which must be proven as safe and effective by the FDA before they can be sold (Bent, 2008). This regulatory structure, or lack thereof, limits the reliability of essential oils and natural medicine and their use as a medical treatment. In conjunction with a lack of regulation is the potential for contamination when producing essential oils (Hosihuzzaman & Iqbal Chaudhary, 2008). This contamination, in extreme cases, can lead to the toxicity of essential oils; in the past, contamination has led to reports of nephropathy⁷ caused by commonly used Chinese herbs; of the nephropathy cases, 43 patients developed end-stage renal failure and 39 had prophylactic renal removal (Bent, 2008). These cases have demonstrated how contamination and lack of regulation of essential oils can have implications regarding their medical usage. With potential contamination putting the health of the patient at risk, it is difficult to implement essential oils into treatment regimens. Should more stringent regulations concerning the marketing and production of dietary supplements

(essential oils) be put in place, patients and medical professionals would likely become more confident in the efficacy of essential oils.

The results of this research examined the presence of synergistic interactions between select essential oils, with the combination of tea tree and oregano oil having the greatest antibacterial efficacy. However, this study only addressed four essential oils: tea tree, oregano, wintergreen, and thyme oil; further research could be conducted to look into potential synergistic, or antagonistic interactions, between other essential oils. Prior research shows that other oils that were not tested in this study, such as citrus and curry oil, also exhibited relatively high antibacterial efficacies (Ott & Morris, 2008). Conducting research on these oils could reveal a combination that has a higher degree of efficacy than those in this study. Furthermore, this study only addressed oils in combination with tea tree oil; in the future, combining other oils, such as pure oregano oil with other oils, may provide additional information surrounding medical implications.

Not only could one look into other combinations in the future, but using the tested combinations on other bacteria would allow researchers to gain a deeper understanding of how changing the bacteria affects the efficacy of the essential oils. As discussed earlier, the results for *E. coli* and *S. aureus* were different, showing that the results for one bacteria are not applicable to all. In addition, this study did not compare essential oils directly to antibiotics, but rather compared combinations to individual oils. In the future, a study comparing the combinations with the greatest efficacy to antibiotics directly would not only allow for a greater understanding of how essential oils inhibit bacterial growth but also demonstrate their efficacy in comparison to other treatment options.

Overall, the results of this study demonstrated that synergistic interactions are present between select essential oils and of all the combinations that were tested, tea tree and oregano oil had the greatest inhibitory potential. Using this data, one can identify new, innovative ways to combat bacterial infections, particularly of the bacteria that were studied: *Escherichia coli* and *Staphylococcus aureus*. While antibiotics are commonly used to treat patients with bacterial infections, the results of this study show that essential oils can be an effective treatment option, particularly if combinations with high efficacy are utilized.

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Does CBD Induce Apoptosis in Diffuse Large B Cell Lymphoma?

Sophia Xu

This study assessed the ability of cannabidiol (CBD), a compound derived from cannabis, to induce apoptosis in diffuse large B-cell lymphoma (DLBCL). This experiment consisted of cell viability assays and apoptosis assays, both of which measure important qualities needed in cancer treatment. CBD demonstrated a strong capability in inhibiting cell viability, at concentrations near 50-100 μ M, thereby emphasizing its potential in minimizing relapse. CBD actively induced apoptosis at concentrations above 25 μ M, with apoptosis frequencies reaching nearly 100%. These results reinforce the idea that CBD induces apoptosis throughout many different types of cancers, including DLBCL. Thus, this study demonstrates that CBD has a great potential in cancer treatment by eradicating cancer cells in DLBCL.

Keywords: CBD, DLBCL, Apoptosis, Cell Viability, Cancer Treatment

Introduction

The need to find a solution to the medical world's leading problem of cancer rises each year, as the projected increase of deaths as a result of cancer are expected to grow from 8.2 million in 2012 to 23 million in 2030 ("Cancer Statistics," n.d.). Within that statistic, Non-Hodgkin lymphoma is recognized as one of the most common cancers in the United States, responsible for a predicted 74,680 diagnoses in 2018 ("Key Statistics for Non-Hodgkin Lymphoma," n.d.). This increase in diagnoses emphasizes the importance of developing effective treatments for this cancer. Non-Hodgkin lymphoma primarily affects T lymphocytes and B lymphocytes, which results in a decreased ability to resist pathogens ("American Cancer Society," n.d.). In a very aggressive form of Non-Hodgkin lymphoma, known as diffuse large B-cell lymphoma (DLBCL), B-lymphocytes enlarge and multiply out of control to form tumours. DLBCL accounts for 30-40% of all Non-Hodgkin lymphoma cases and is often fatal, if untreated, suggesting the importance of addressing this disease (Li et al., 2018). Currently, there

are a number of treatments for Non-Hodgkin lymphoma, ranging from chemotherapy and radiation to immunotherapy ("American Cancer Society," n.d.). However, each method of treatment has its own negative aspects that indicates the need for more effective treatment. For example, these treatments are extremely expensive, averaging at 5,871 USD a month, making this option unaffordable to many families and often cutting treatment short, consequently affecting the potential of relapse (Kutikova et al., 2006). Additionally, chemotherapy and radiation are associated with negative side effects such as extreme fatigue, nausea, vomiting, and most notably, hair loss, which can be uncomfortable for patients and should be addressed in the development of new treatments ("American Society of Clinical Oncology," 2017). Most importantly, chemotherapy and radiation are nonspecific techniques, meaning that these treatments will not only target malignant cells, but will also attack the body's own healthy cells ("Chemotherapy to Treat Cancer," 2015). This consequently weakens the immune system and the patient's overall health, resulting in a longer and extremely debilitating recovery process. Thus, there arises a clear need to find better treatment for

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DLBCL that is able to address the problems associated with current treatments.

Recently, studies have pointed towards cannabinoids, compounds derived from cannabis, as a plausible treatment for cancers (Lee, 2012). These cannabinoids have long been known for their therapeutic agents by alleviating pain and nausea, but their relationship in the study of cancer is relatively new (Grotenhermen & Muller-Vahl, 2012). Even so, positive effects of cannabinoids have been noted for certain cancers. In 2008, a team of Harvard scientists demonstrated that the cannabinoid $\Delta 9$ -tetrahydrocannabinol ($\Delta 9$ -THC) significantly reduced the spread of lung cancer (Preet et al., 2007). Similarly, cannabidiol (CBD) activates cell death in aggressive breast cancer cells through the intrinsic apoptotic pathway (Ligresti et al., 2006). These benefits have contributed to the increased interest in cannabinoids and their potential in the realm of cancer treatment. While these cannabinoids have been increasingly researched among different cancers, there is a notable gap in research concerning cannabinoids and their functions in lymphoma, presumably because lymphoma already has effective treatment described above, whereas in other cancers, such as breast cancer, these treatments are not as effective, especially in the latter stages. Yet, as previously indicated, these treatments come with severe consequences and need to be addressed with the development of new cancer treatments. As such, this paper will focus on the therapeutic relations that cannabinoids have with Non-Hodgkin Lymphoma.

There are many different types of cannabinoids, each of which has notable positive effects on the human body. Out of those cannabinoids, $\Delta 9$ -THC and CBD have been most commonly researched in terms of cancer treatment. However, there are certain concerns that arise with the use of cannabinoids as drugs. These cannabinoids are extracted from cannabis, more commonly known as marijuana. While these cannabinoids would be used strictly for its medical applications, cannabis is known for its psychoactive or “high” effect, and this drug can be abused for other reasons. However, it is important to note that the key difference between these two cannabinoids is that $\Delta 9$ -THC demonstrates this psychoactive effect, whereas CBD lacks it (Kohn, 2016). In fact, CBD has been shown to reduce these psychoactive effects, due to the absence of a large binding affinity for CB1 receptors

(Wilcox, 2017). These receptors are responsible for releasing chemical signals, producing the “high” effect of marijuana in the brain (Mackie, 2008). Thus, in order to minimize any ethical concerns, this paper will be limited to CBD rather than the psychoactive cannabis compound $\Delta 9$ -THC.

CBD represents a strong potential candidate for cancer treatment as it addresses many of these problems associated with current treatments. Not only is cannabidiol associated with therapeutic effects such as the alleviation of pain and nausea, thereby countering the negative side effects from current treatment, but CBD has proven to solely target cancer cells, ensuring that the body’s own healthy cells will not be attacked (Massi et al., 2006). Thus, CBD addresses the most important concern with current cancer treatments, in that CBD would result in a stronger recovery process. Furthermore, a CBD drug might be more financially affordable for families. Altogether, CBD could provide a much better option for cancer patients in terms of cost, health, and efficiency. This study will focus on the effect of CBD on DLBCL.

Ultimately, the purpose of cancer treatment is to eradicate cancerous cells that form tumours. In terms of current cancer treatments, radiation uses electromagnetic waves to eradicate these cells. Chemotherapy focuses on the special ability of these cancerous cells to multiply rapidly in order to target and effectively eradicate these cells. In this case, apoptosis would be the mechanism by which CBD would possibly eradicate DLBCL cells. As a result, this paper will evaluate the degree at which CBD induces apoptosis in DLBCL cells as an indicator of its potential as cancer treatment.

Apoptosis refers to programmed cell death in that this type of cell death follows a very systematic approach. There are generally two methods to induce cell death: apoptosis and necrosis. Necrosis involves an external factor, such as a puncture, that induces death. As a result, necrosis causes harm in the human body due to inflammation in the area surrounding the wound. In contrast, apoptosis involves the collapse of the cell and is therefore what this paper will measure since apoptosis would harm the patient less in comparison to necrosis. The process of apoptosis can be conducted in numerous ways. Ultimately, when the cell prepares for its death, proteins known as caspases are activated to break down the cellular components

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that are required for the cell to survive (Elmore, 2007). For example, the activation of caspases initiates the enzymes, DNases, to destroy the nucleus, including the DNA, of the cell, which contributes to its demise (Enari et al., 1998). Once the cell collapses, macrophages are sent to remove these cells, making the process overall very clean, and thus producing no harmful effect parallel to that of necrosis (Fink & Cookson, 2005). The two major pathways involved in apoptosis are the extrinsic pathway or death receptor activated pathway and the intrinsic or mitochondrial pathway (Elmore, 2007). Each of these mechanisms of apoptosis is critical to understanding the processes that CBD may undertake in conducting apoptosis in DLBCL.

This study consists of two experiments: one which assesses the effect of CBD on cell viability and one which assesses the effect of CBD on apoptosis. Both of these measures are important qualities of cancer treatment that needs to be evaluated. Cell viability evaluates the potential for a cell to recover from treatment, thereby determining the extent at which CBD can reduce the potential for relapse in patients. On the other hand, the apoptosis assay measures the ability of CBD to induce cell death in DLBCL and determine the best concentration for a drug.

Literature Review

The research of cannabinoids in the field of cancer has largely pertained to other cancers and is specifically lacking in the realm of lymphoma. Thus, the gap in research is determining how CBD interacts with DLBCL. However, it is also important to understand the mechanisms by which CBD interacts with various cancers to provide information that could be used for this experiment. As such, this paper recognizes the relationship between CBD and breast cancer, glioma, and leukemia, as these studies have proven to yield promising results that are comparable to those of this research.

In breast cancer, CBD was found to effectively inhibit breast cancer cell proliferation through the down regulation of Id-1 expression, which is responsible for tumour proliferation, migration, and invasion when found in high quantities (Ligresti et al., 2006). Thus, CBD has been shown to effectively inhibit the activities of tumours, correlating to a strong response to-

wards breast cancer cells. The study also indicated that among the possible underlying mechanisms involved in this inhibition, CBD directly activated the capsaicin receptor (TrpV1). This receptor functions primarily to detect and regulate body temperature, which could affect these tumour-related activities. Additionally, the activation of TrpV1 may have indirectly activated CB2 receptors via oxidative stress. These CB2 receptors are directly involved in immune suppression, the induction of apoptosis, and the induction of cell migration (Basu et al., 2011). In 2011, another study concluded that CBD induces apoptosis through mitochondrial-mediation in breast cancer (Shrivastava et al., 2011). Thus, CBD alters tumour-related activities in breast cancer and potentially induces apoptosis through various mechanisms including the activation of CB2 receptors and mitochondrial-mediation, as demonstrated by both studies (Ligresti et al., 2006; Shrivastava et al., 2011).

In glioma, numerous mechanisms for apoptosis have been identified, including the release of cytochrome C and the activation of caspase-9 and caspase-8 pathways (Massi et al., 2006). Cytochrome c plays an active role in ATP synthesis in the mitochondria, and its release from the mitochondria to the cytosol is associated with the activation of a caspase cascade, thereby triggering apoptosis (Ow et al., 2008). Additionally, while CBD suppressed proliferation of human glioma cell lines, there was no reported effect of CBD on healthy monocytes (Massi et al., 2006). This suggests that CBD is specific to cancerous cells and solely induces apoptosis in affected, cancerous cells in glioma. This finding is significant since healthy human body cells were unaffected, while cancer cells were destroyed. Furthermore, this result directly addresses issues with chemotherapy and radiation and their inability to differentiate between healthy and cancer cells.

In Leukemia, CBD led to CB2 mediated reduction and activation of caspase-8, caspase-9, and caspase-3 (McKallip et al., 2006). Furthermore, CBD exposure initiated the breakdown of the mitochondrial membrane and release of cytochrome C (McKallip et al., 2006). All of these mechanisms are correlated with the induction of apoptosis. Correspondingly with glioma, CBD had no effect on healthy human monocytes, whereas it induced apoptosis in cancer cells (Gallily et al., 2003). This suggests that this characteristic could be replicated across different types of cancers.

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The similarities and relationships in how CBD induces apoptosis in various cancers suggests a link between intrinsic and extrinsic apoptotic pathways. For example, an increasingly dominant reaction of CBD on cancer cells is the release of cytochrome c, which is associated with the intrinsic pathway in apoptosis, suggesting that this may be a common characteristic of CBD-induced apoptosis. Based upon these studies, CBD seems to conduct apoptosis in a consistent method through the release of cytochrome c and the activation of caspases. On the other hand, it is presently unclear as to how CBD induces apoptosis and the method through which this occurs, specifically in DLBCL. However, based upon the successful conduction of apoptosis in breast cancer, glioma, and leukemia, it is likely that CBD will also induce apoptosis in DLBCL.

Materials and Methods

DLBCL cell lines were established in the laboratory. These cell lines were extracted from a total of 24 patient samples. As a basic growth medium, 1% penicillin/streptomycin (Hyclone, Logan, UT, USA) was used with cell culture of RPMI-1640 medium (Gibco, Rockville, MD, USA) and 15% fetal calf serum (Gibco). All 24 cell lines (CJ, LP, RC, TMD-8, WP, LY-19, MZ, 8LR, HT, MS, Toledo, BJAB, u2392, DS, Pfeiffer, McA, SUDHL-4, HF, SUDHL-6, HBL-1, DB, EJ, Val) were routinely tested for any small parasitic bacteria, also known as mycoplasma, using a MycoSEQ™ Mycoplasma Detection kit (Invitrogen, Carlsbad, CA, USA). This step is particularly important as mycoplasma can induce morphological changes, alter growth rates, and effectively alter the cell viability assays to be conducted (“Mycoplasma Detection and Elimination,” n.d.). These stocks of authenticated cell lines were then stored in liquid nitrogen to guarantee that the cells and growth medium would not evaporate. Two experiments were conducted: the cell viability assay and the apoptosis assay. The cell viability assay measures the ability of the DLBCL cells to recover after exposure to CBD, thereby measuring its ability to relapse. The apoptosis assay was used to measure the degree at which CBD would be able to induce cell death in cancerous cells and is the precursor in determining the efficacy of a drug for treating cancer. For the cell viability assay, concentrations of 0 uM, 1.5

uM, 3.1 uM, 6.25 uM, 12.5 uM, 25 uM, 50 uM, and 100 uM of CBD were used. These concentrations were established by a serial dilution to evaluate cell viability on a greater spectrum. The apoptosis assays used the same concentrations, with the exception of 100 uM. The primary reason for the omission of 100 uM is that an apoptotic trend can be established in lower concentrations. However, in order to consider CBD's implications as a drug, it will be necessary to evaluate the most effective concentration at which CBD inhibits both cell viability and induce apoptosis. Thus, the same concentrations from the cell viability assays were used for the apoptosis assays (with the exception of 100 uM) in order to effectively compare each concentration point and determine possible drug concentrations required for treatment in the future. Cannabidiol was obtained from Tocris Cookson Inc. (Ellisville, MO). For therapeutic compound screenings, CBD was prepared as 1000-fold stock solutions in tissue culture grade dimethyl sulfoxide (DMSO) in order to freeze the cells to prevent the formation of crystals that may damage the cells and produce inaccurate data. The final concentration of DMSO in media was 0.1%, a concentration that has shown no toxicity or effect on the DLBCL cells. For the cell viability assay, cells from representative DLBCL cell lines were plated at 5,000 cells per well, with 20 uL medium and 10% fetal bovine serum to acquire ample data to accurately establish a conclusion. These cells were incubated for 96 hours, as per apoptosis protocol, and DMSO was added. Fetal bovine serum is used primarily for its low level of antibodies and many growth factors. The cell viability assays were performed using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA) by adding CellTiter-Glo Reagent and recording luminescence. For the apoptosis assays, the cells were incubated for 48 hours with the appropriate CBD dilution and DMSO was added. Only the cell line RC was used in the assay in order to minimize time constraints. Unlike the cell viability assays, the induction of apoptosis will not differ per cell line. Thus, a general trend can be concluded and extended from the apoptosis assay, suggesting that one cell line will suffice. The Annexin V/PI staining method, with flow cytometry, was used to assess apoptosis. The assays were performed in duplicate. Statistical analysis consisted of standard deviation in the cell viability assays to determine the precision of these trials.

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Results

Cell Viability Assays

Control- The control for the cell viability assays was established through untreated cells in each cell line, exhibited by the 0 uM column in Figure 1, Figure 2, and Figure 3 below. This information is important because cell viability differs per trial per cell line, as

demonstrated by the various starting points of each trend for the cell lines. Using this control, a comparison can be made to determine the effectiveness of various CBD concentrations by contrasting the number of viable cells left to the control.

Variables- The primary variable that was manipulated in the cell viability assay was the CBD concentration in order to establish both the effectivity of each concentration and address the implications of CBD as a drug. This information will be particularly

Cell Lines 1-8

Table 1. Average Cell Viability count of DLBCL cell lines 1-8 (CJ, LP, RC, TMD-8, WP, LY-3, LY-19, MZ). Averages are taken from three trials of each cell line.

CBD-99 (uM)	(1) CJ Avg	(2) LP Avg	(3) RC Avg	(4) TMD-8 Avg	(5) WP Avg	(6) LY-3 Avg	(7) LY-19 Avg	(8) MZ Avg
0	14.74	20.84	16.34	22.32	19.06	18.71	16.61	19.66
1.5	15.95	21.52	16.47	23.76	21.17	18.37	17.13	18.62
3.1	18.07	19.57	16.40	22.67	20.70	18.26	18.34	19.43
6.25	16.71	19.94	15.42	23.17	21.32	19.72	18.83	17.92
12.5	14.53	17.15	14.30	20.36	20.33	17.72	16.55	13.78
25	9.46	10.28	7.07	14.62	14.17	10.71	10.99	8.19
50	1.51	3.14	0.12	4.49	2.83	1.16	0.22	2.22
100	0.30	0.14	0.26	0.32	1.51	0.90	0.07	4.71

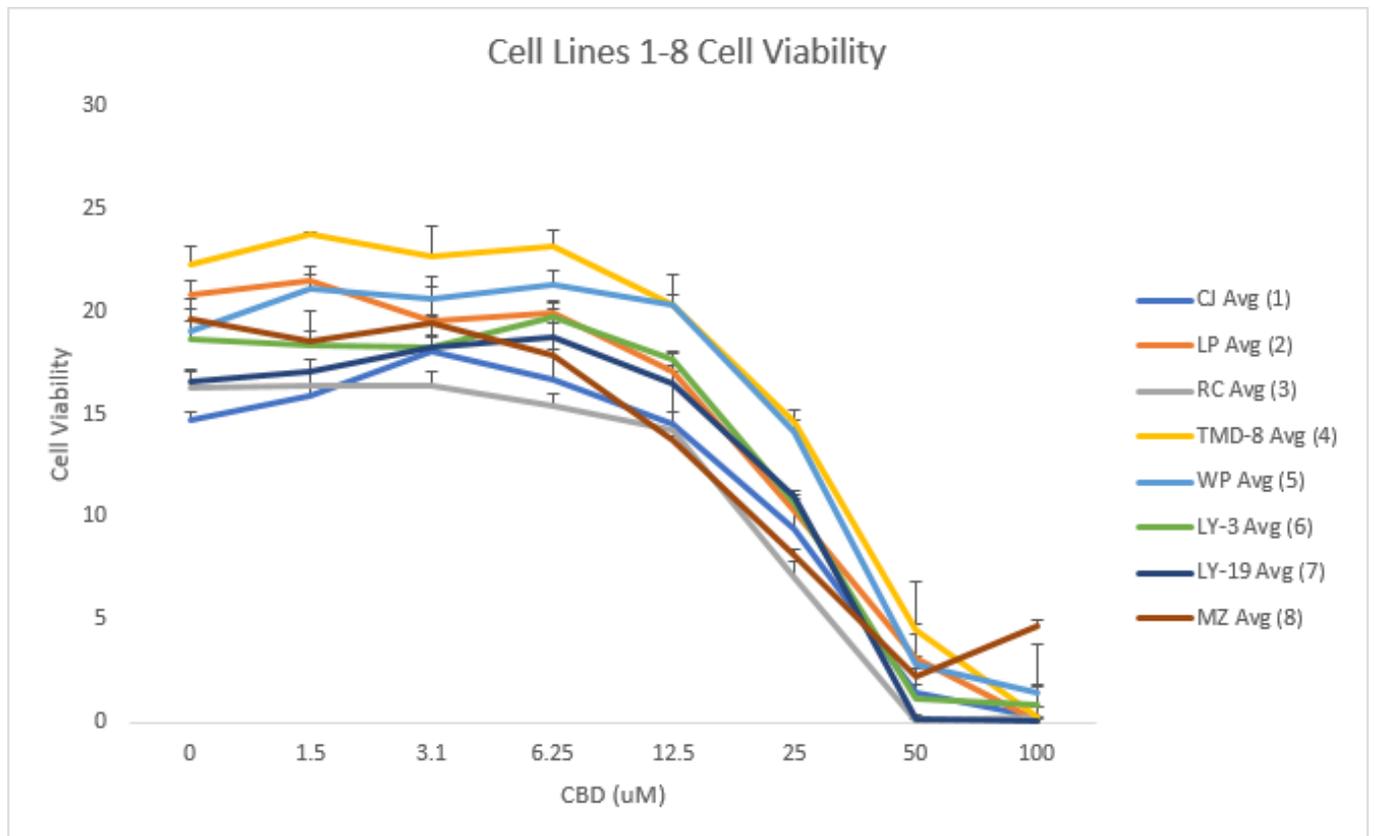


Figure 1. This graph corresponds to the set of values in Table 1. In each cell line, a general, predicted downward trend is established in each cell line. From 50 - 100 uM, each cell line exhibits the lowest cell viability count. Error bars represent standard deviation.

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Cell Lines 9-16

Table 2. Average Cell Viability count of DLBCL cell lines 9-16 (8LR, HT, MS, Toledo, BJAB, u2392, DS, Pheiffer). Averages are taken from three trials of each cell line.

CBD-99 (uM)	(9) LR Avg	(10) HT Avg	(11) MS Avg	(12) Toledo Avg	(13) BJAB Avg	(14) U2932 Avg	(15) DS Avg	(16) Pheiffer Avg
0	14.96	31.93	15.99	23.35	22.17	12.75	20.22	17.99
1.5	12.57	31.58	17.17	26.69	32.91	10.80	25.10	21.20
3.1	16.00	29.41	19.99	28.11	29.47	10.42	23.66	19.84
6.25	13.89	30.45	19.70	29.07	30.02	10.74	25.25	21.46
12.5	14.00	30.27	20.68	25.74	24.53	10.25	24.58	18.06
25	13.68	18.16	15.83	26.93	22.32	11.19	22.33	19.06
50	12.58	5.30	12.40	16.80	18.94	11.89	15.58	17.23
100	3.40	0.16	1.32	0.29	3.61	0.53	0.70	5.57

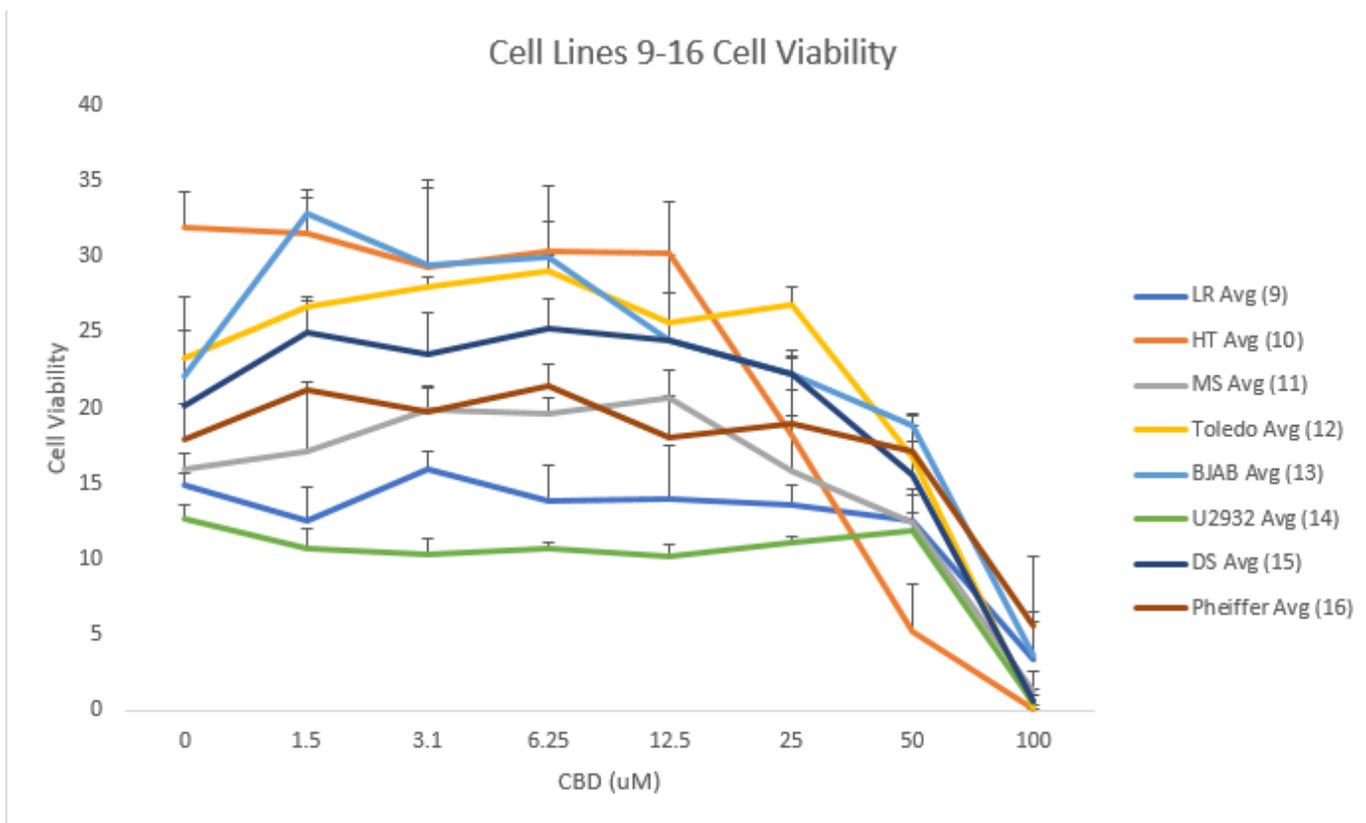


Figure 2. This graph corresponds to the set of values in Table 2. In each cell line, a general, predicted downward trend is established in each cell line. Once again, each cell line exhibits the greatest reduction in cell viability count from 50 -100 uM.

important in understanding the necessary concentrations of CBD for a viable drug treatment. Thus, this experiment is dose-dependent and should produce a dose-dependent trend downwards. A serial dilution was established in which each dosage was half of the previous concentration. Thus, with these concentrations ranging from 0 uM to 100 uM, a wide spectrum is created to examine the ability of CBD to inhibit cell viability at all concentrations, in comparison to a limited scope that may exclude this potential. Ideally, these dose response graphs would depict a relatively

stable decrease in cell viability, from the control to 0 viable cells at 100 uM. Another variable includes the 24 cell lines that were tested. In Figure 1, Figure 2, and Figure 3, the results of the cell lines are divided into three groups of eight cell lines, labelled accordingly. The groupings of these cell lines demonstrate the difference in days in which the assays were performed. The structuring of these experiments into three groups helps to ensure that any possible mistakes that may have occurred would not affect all 24 cell lines.

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Cell Lines 17-24

Table 3. Average Cell Viability count of DLBCL cell lines 17-24 (McA, SUDHL-4, HF, SUDHL-6, HBL-1, DB, EJ, Val). Averages are taken from three trials of each cell line.

CBD-99 (uM)	(17) McA Avg	(18) SUDHL-4 Avg	(19) HF Avg	(20) SUDHL-6 Avg	(21) HBL-1 Avg	(22) DB Avg	(23) EJ Avg	(24) Val Avg
0	17.73	15.31	20.35	14.50	17.54	10.83	17.50	14.87
1.5	18.25	16.45	21.09	12.35	19.41	10.85	19.64	16.81
3.1	17.37	16.64	20.80	11.19	19.25	11.69	20.19	16.85
6.25	16.07	15.20	19.89	13.52	18.61	11.43	18.69	14.89
12.5	13.83	15.97	17.43	11.16	18.68	10.40	17.46	16.13
25	9.42	14.19	16.23	10.18	17.18	8.49	17.31	13.82
50	4.59	5.55	3.32	2.57	8.41	1.28	12.31	12.40
100	1.15	0.05	0.09	0.03	1.00	0.15	6.11	3.58

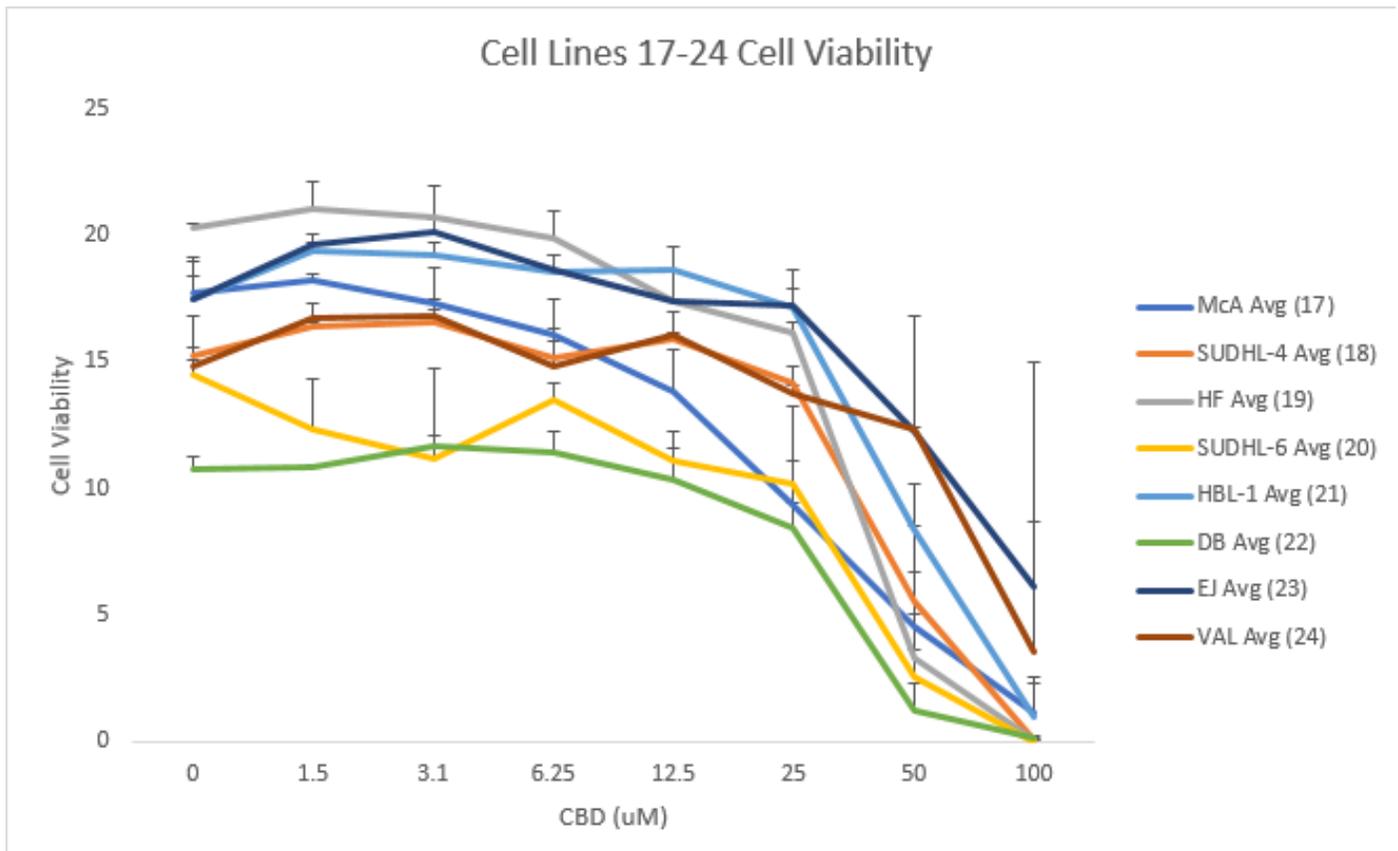


Figure 3. This graph corresponds to the set of values in Table 3. In each cell line, a general, predicted downward trend is established in each cell line. There are minimal increases depending on the cell line. In these trends, the lowest cell viability count and greatest decrease begins at 25 uM and continues until 100 uM.

Results (Figures 1-3). As seen in Figures 1-3, a general downward trend is established in each cell line. This trend fluctuated near the initial cell viability values and remained stagnant until a certain concentration. In Figure 1 and Figure 2, an abrupt decrease is depicted at 50 uM. On the other hand, this same decrease occurs at Figure 3 at 25 uM. Additionally, in Figures 1-3, all graphs demonstrate a significant

reduction in cell viability by 100 uM, suggesting that CBD is effective in inhibiting cell viability. Generally, at 100 uM, cell viability is most effectively inhibited, with the exception of cell line MZ. The tables correspond with the data presented in the graph, in order to concisely show the points used. The cell viability data listed in the table were taken as the average of three trials in order to ensure accuracy.

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Cell Line RC

Table 4. This table indicates the frequency of apoptosis in cell line RC (cell line 3). These concentrations were established from the cell viability assays.

Concentration CBD-99 (uM)	Frequency of Apoptosis
0	27.90%
3.1	22.50%
6.2	34.70%
12.5	66.40%
25	99.00%
50	99.80%

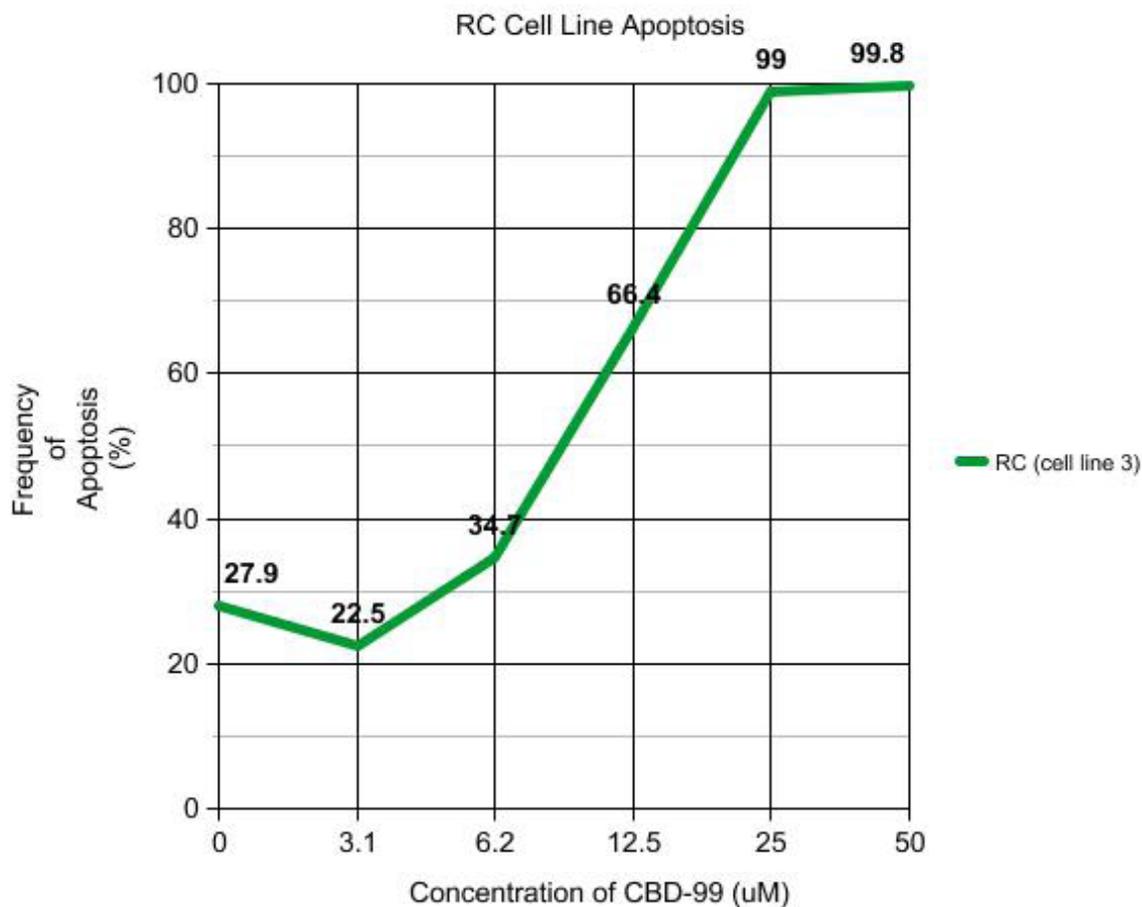


Figure 4. This graph corresponds to the set of values in Table 4.

Apoptosis Assays

Control- In the apoptosis assays, the control was established when CBD had a concentration of 0 uM, as seen in Figure 4. This control installs the basis for the apoptosis assays, allowing for comparison to see if CBD induces apoptosis or inhibits apoptosis. Since the cells can conduct apoptosis themselves, the estab-

lishment of a control at 0 uM allows for an effective comparison to determine the apoptotic frequencies that were induced as a result of CBD, rather than by the cell's own function.

Variable- The primary variable that was manipulated was CBD concentration. While a serial dilution was also utilized, the concentrations ranged from 0 uM to 50 uM as apoptotic frequency as 50 uM suf-

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ficed to indicate a successful conduction of apoptosis in the cell line. The dependent variable measured the frequency of apoptosis.

Results (Figure 4/ Table 4). As seen in Figure 4, an increasing, positive trend is established in the apoptosis assays in cell line RC. Interestingly, the frequency of apoptosis at 3.1 μ M is lower than the control. Generally, a dosage curve for apoptosis should increase exponentially when corresponded with a serial dilution. Between 6.2 μ M and 25 μ M, a steady increase in apoptotic frequency is established. The highest frequency of apoptosis is established at 25 μ M and 50 μ M, at nearly 100%.

Discussion and Analysis

Analysis of Cell Viability Assays (Figure 1-3). The decreasing values for cell viability demonstrate that DLBCL was actively inhibited through the exposure of CBD. On the other hand, if cell viability increased from the control, CBD would have adverse effects by promoting DLBCL. Since cell viability was inhibited, CBD demonstrates a greater potential in cancer treatment. In Figure 1, each line indicates the general trend of decreasing cell viability, affirming the hypothesis that cell viability will significantly decrease as CBD concentration increases. The exception is cell line MZ that increases in cell viability from 50 μ M to 100 μ M. This directly proposes that CBD reduces apoptosis frequencies in high concentrations, refuting the hypothesis that cell viability is inhibited. However, this event can be explained by sources of error. It is likely that the well-received varying amounts of CBD due to air bubbles in the pipet or cells may have been picked up from earlier trials, which will all contribute to inconsistent data readings. Since the other cell lines follow the trend without variations, this is likely a consequence of human error.

In all of the figures, the general trend seems to be a stabilizing effect with minimal changes to cell viability, followed by a large decrease in cell viability. The points at which this extensive reduction occurs will be important in determining adequate concentrations for a potential CBD drug. In both Figure 2 and Figure 3, this reduction occurs at 50 μ M and continues until 100 μ M, whereas in Figure 1, this trend begins at 25 μ M.

Standard deviation varies per cell line per trial suggesting a wide variance in the data. This would suggest that these results are not consistent and can vary thoroughly. However, the calculated coefficient of variation, used to measure relative variability, is less than 100% for the cell lines. Thus, while the standard deviation is large at some concentrations for certain cell lines, this variability is accounted for by the coefficient of variation, thus affirming that these results are plausible.

Analysis of Apoptosis Assay (Figure 4/Table 4). A higher frequency of apoptosis indicates a greater capability of CBD to inhibit cancer cells. Therefore, the expected general trend is a continuous increase in apoptosis frequency as concentration increases. Thus, if this trend is exhibited, it can be deduced that CBD would be effective in inducing apoptosis and can then be considered for cancer treatment. Contrarily, while CBD demonstrated effective inhibition of cell viability, if a negative trend is established in the apoptosis assays, CBD can be ruled out for its implications as a drug. In Figure 4, the positive trend is established where apoptosis frequency increases with concentration. However, the exception is the concentration at 3.1 μ M. Since the control has been established at 27.9%, the expectation is that the subsequent concentrations would yield an apoptosis frequency greater than this value. Nevertheless, at 3.1 μ M, apoptosis frequency decreased from the control, suggesting that CBD may actually reduce apoptosis in DLBCL at that concentration. This deviation can be attributed to human error, similar to the cell viability assays, but should further be looked into for future studies.

From the data presented in Figure 4, the greatest reduction, found by the slope, occurs at 12.5 μ M, with 25-50 μ M having the greatest apoptosis frequency at nearly 100%. This suggests that studies looking into the plausibility of CBD as a drug for DLBCL should focus on the concentration range of 25-50 μ M.

Discussion (Figures 1-4/ Tables 1-4). The purpose of this research is to ultimately determine whether CBD can induce apoptosis in DLBCL, as this represents the essential marker for cancer treatment. The induction of apoptosis in cancerous cells represents the first step in developing cancer treatment, since the primary goal is to eradicate cancerous cells. While apoptosis was the primary focus of this experiment, both cell viability and apoptosis were measured. These cell viability assays represent a measure of the ability

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of the DLBCL cells to recover from CBD treatment, which is a significant factor to consider for treatment. As such, this assay provides insight on the implications of a CBD drug, as an anticancer drug would not only have to induce apoptosis in cancerous cells but also minimize the potential for relapse. Figure 1 and Figure 2 suggest that CBD is capable of inhibiting cell viability, specifically at concentrations around 50 μM to 100 μM , as indicated by the abrupt reduction in cell viability at 50 μM . Similarly, Figure 3 suggests that CBD is capable of inhibiting cell viability at concentrations from 25 μM to 100 μM . Thus, a higher concentration, between 50 μM to 100 μM , should be used to account for all cell lines. However, the key factor that determines whether or not CBD could function as potential cancer treatment lies in its capabilities to eradicate DLBCL through the induction of apoptosis. While cell viability is an important measure to evaluate, if CBD does not induce apoptosis, CBD could be ruled out for treatment altogether. Overall, the apoptosis assays hold greater priority than the cell viability assays as the induction of apoptosis in DLBCL represents the ultimate marker for cancer treatment. From Figure 4, the positive trend established asserts that CBD does induce apoptosis in DLBCL, especially at concentrations greater than 25 μM . This suggests that a concentration point between 50 μM to 100 μM would be successful in inhibiting cell viability and inducing apoptosis. Altogether, these results suggest that CBD induces apoptosis and can be considered as a candidate for cancer treatment, given its benefits in reducing cell viability. Statistical analysis illustrates that these results, while varied, are therefore reliable.

These results overall represent a consensus among the scientific community that CBD does induce apoptosis in cancer. As previously stated, CBD has proven to induce apoptosis through varying, but similar, mechanisms in breast cancer (Ligresti et al., 2006), glioma (Massi et al., 2006), and leukemia (McKallip et al., 2006). This paper has effectively shown that CBD does induce apoptosis in DLBCL, thus indicating that in a larger context, CBD induces apoptosis in multiple cancers.

Limitations

Although the apoptosis assays provided reliable results, the methods of apoptotic inductions by CBD

were not examined. Therefore, the mechanisms of apoptosis could not be compared to previous studies presented in the Literature Review. This remaining lack of knowledge does limit the potential of CBD as a drug for DLBCL and suggests a need for further research in this area. Furthermore, time limitations restrained the number of trials performed for the apoptosis assay, thereby obstructing statistical analysis due to lack of ample data. However, the apoptosis assay demonstrates a significant difference between the control concentration and subsequent concentrations. With nearly a 70% difference in apoptotic frequency, the assertion that CBD induces apoptosis in DLBCL is indisputable. Additionally, since this experiment was conducted *in vitro*, it cannot fully replicate results in the environment of the human body. Thus, the results produced from this experiment cannot be guaranteed in the human body. Nonetheless, since human cell lines were utilized and the induction of apoptosis was ascertained by the assays, this experiment does indicate the ability of CBD to induce apoptosis in human DLBCL cell lines. Given these circumstances, this paper represents a degree to which CBD would induce apoptosis in the human body but is not fully representative. Despite these limitations, the successful conduction of apoptosis in cell line RC and the successful inhibition of all twenty four cell lines in terms of cell viability affirms the possibility of CBD as an anticancer drug.

Conclusion and Future Directions

The results of this study directly suggest that CBD has a strong potential for treating DLBCL. The results concluded from the cell viability assays indicate that CBD is effective in reducing the potential for relapse in cancerous large B lymphocytes. This assay is particularly important in addressing relapse in cancer, in that certain cancerous cells may escape treatment and metastasize to other parts of the body. The apoptosis assays were the main focus of this paper and the results from Figure 4 indicate that CBD is effective in inducing apoptosis in DLBCL. This confirms the hypothesis that CBD induces apoptosis in DLBCL.

The results from this paper indicate that CBD does induce apoptosis in DLBCL, which is the first step in addressing its implications as a drug. However, in

order to fully address this possibility, other factors should be looked into. First, this paper indicates the concentrations of CBD at which it is most effective in reducing cell viability and promoting apoptosis. These concentrations should be regarded in terms of toxicology levels that are acceptable and healthy for the human body. Furthermore, testing should be done to determine any possible side effects that have not previously been accounted for. Other possible considerations include a combination of current treatment techniques with CBD. This combination therapy would be able to minimize the negative limitations of current treatment such as cost, side effects, and weakened immune system while using CBD in moderation (Fisher et al., 2016). Moreover, the administration of CBD as a drug should be considered. Already, certain studies have noted that CBD would be most effective when administered orally due to a high lipid content (Zgair et al., 2017). However, this form of administration could differ depending on the cancer and the site of tumours. Lastly, this paper solely focused on CBD's ability to induce apoptosis but did not look into the different pathways that may have been used, unlike other aforementioned studies. Given that a link between the intrinsic and extrinsic apoptotic pathway in inducing apoptosis was not examined in DLBCL, no conclusions regarding whether this method of apoptosis induction is consistent or similar to other cancers can be made. A further, in-depth analysis of these pathways would be useful in understanding CBD's ability to conduct apoptosis and should be the next focus in the steps leading up to drug development.

Overall, CBD has demonstrated a great capability in targeting DLBCL and substantiates the need for further research in this area to develop treatments. This paper represents the first step in developing a CBD cancer treatment, based on the successful conduction of apoptosis.

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Student Perceptions of the Implementation of Formative Assessment: A Royal St. George's College Case Study

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Formative assessment has been widely implemented for its well-researched positive benefits. Despite this, an absence of research exists when it comes to student evaluations of its implementation. To address this for my school, Royal St. George's College or RSGC, I asked students questions about formative assessment according to the Nicol & Macfarlane-Dick (2007) model in both a survey and an interview. The results revealed that formative assessment allows students to learn about what good performance is, self-assess their own work, and focus on improving, not on their grades. That said, students could learn more if teachers were to make formative assessment more rigorous as well as, providing more feedback. Unfortunately, feedback is often formulaic, and when it is given students are not applying all suggestions or approaching teachers as much as they should. In addition, the results of this study reported that teachers should use feedback more frequently to learn about their students.

Keywords: formative assessment, formative feedback, formative grades, pedagogical practices, student perceptions, teacher feedback

Introduction

Many in the educational field are familiar with formative assessment as it has been implemented around the world for its positive benefits. Haroldson (2012) notes that formative assessment is divided into several categories. Those include the formative use of summative tests, questioning students to spark discussion and learning, and written and oral feedback during class and with class activities. Some of its positive benefits were discussed by Nicol and Macfarlane-Dick (2007) who, when combining pre-existing literature on formative assessment, found evidence that supports its implementation. They discuss how students need opportunities to develop self-regulation in school and

that formative assessment is the best way to do this. Also in that paper, the authors outline seven generally accepted key principles to help teachers implement formative assessment. These are as follows: formative assessment teaches students about what constitutes good performance, it improves a student's ability to self-assess, it teaches students about how they need to improve, it creates situations where students can discuss their learning with teachers, it increases self-esteem and motivation in students, it helps students achieve their academic goals and it allows teachers to tailor teaching around information gained from its implementation. Though the importance of formative assessment has been clearly studied, a gap exists between the idea and its implementation in reality. In-

terestingly, some research reports on student's perception of formative assessments when it has been implemented effectively; however, an absence of research is noted when it comes to student perception of the implementation of formative assessment. That is why I conducted a mixed-method case study at RSGC to address this gap. In the study, I combined a survey with interviews to discover whether the four types of formative assessment mentioned above match the seven key principles of its implementation that were also mentioned above. I also segmented this analysis into specific portions of the population by asking students about their grades and courses. Other schools can examine the RSGC case study as a model for how formative assessment is being used effectively or ineffectively at their respective schools.

Literature Review

Introduction to Formative Assessment

Formative assessment has been widely implemented in schools across North America. The purpose of this assessment is to create teacher feedback that students can learn to improve their understanding of content (Sadler, 1983). Formative assessment, therefore, is implemented through the formative use of summative tests, the questioning of students to spark discussion and learning, and the use of written and oral feedback during class and with class activities. In this way, formative assessment is set of tools that give formative feedback.

Chronology

Formative assessment became widely adopted by education systems across North America as views on teaching shifted. Historically, teaching was very one dimensional with teachers contributing to most if not all of the student's learning, but recent literature has discussed the importance of letting students participate in their learning (Barr & Tagg, 1995). More specifically, student-based learning seeks to encourage active learning, give students more responsibility, autonomy, and accountability, and create student and teacher interdependence (Lea, Stephenson, & Troy, 2003). It also reduces the gap between a teacher's

explanation and a student's understanding (Higgins, Hartley, & Skelton, 2001). This meant that educational experts started to lobby for the implementation of formative assessment in day-to-day schooling in the 1990's and early 2000's (Hutchinson & Hayward, 2005). Presently, formative assessment is still used widely in many education systems (Cotton, 2017). Researchers Nicol and Macfarlane-Dick (2007) went further than this and argued that formative assessment needed to be tailored for more student learning. With a sound basis for student-based learning and a subsequent sound basis for formative assessment, many researchers have found that formative assessment does have positive effects on student learning (Kingston & Nash, 2011)

Deconstruction of Formative Assessment

As stated earlier, researchers Nicol and Macfarlane-Dick (2007) found that student assessment should be tailored to create more student-based learning. They then combined this principle with pre-existing literature to create seven key principles for what formative assessment should do. Those seven principles, summarized below, act as guidelines for the effective implementation of formative assessment that encourages student-based learning and will be used later for the method.

Formative assessment teaches students about what constitutes good performance.

It improves a student's ability to self-assess.

It teaches students about how they need to improve.

It creates situations where students can discuss their learning with teachers.

It increases self-esteem and motivation in students.

It helps students achieve their academic goals.

It allows teachers to tailor teaching around information gained from its implementation.

Although many studies discuss similar attributes of correct implementation of formative assessment (Koh, 2010; Hobson, 1997; Orsmond, Merry, & Callaghan, 2004). I chose the model by Nicol and Macfarlane-Dick (2007) because it effectively combines all the attributes other studies have discussed in a clear and organized manner.

"Helps clarify what good performance is" (Nicol & Macfarlane-Dick, 2007, p. 205). Without formative assessment, students and teachers differ on their

understanding of goals (Norton, 1990). For example, research suggests that teachers cannot express all of the goals on a rubric in a way that a student will understand (Rust, Price, & O'Donovan, 2003; Yorke, 2003). Research has also stressed that students must understand teacher feedback in order for it to be effective (Hounsell, 1997). Formative assessment fills this gap by allowing students to better understand the goals set by the teacher (Nicol & Macfarlane-Dick, 2007).

“Facilitates the development of self-assessment...in learning” (Nicol & Macfarlane-Dick, 2007, p. 205). Students benefit from understanding what good performance is because they can use this as a basis for their own performance (McDonald & Boud, 2003; Taras, 2001; Taras, 2002; Taras, 2003). In order for them to understand what good performance is, they need to be able to assess their work. Through the integration of teacher feedback, formative assessment allows students to practice assessing their own work directly (McDonald & Boud, 2003; Haroldson, 2012).

“Delivers high quality information to students about their learning” (Nicol & Macfarlane-Dick, 2007, p. 205). Through formative assessment activities, students can read and act on teacher feedback. This helps them understand teacher expectations and enables students to apply their teacher's input (Nicol & Macfarlane-Dick, 2007). Teacher feedback should not be authoritative and strictly corrective but instead give the student a sense of what the teacher experiences when reading the work to shift the focus of high quality feedback towards the student (Lunsford, 1997). Teacher feedback should also be given regularly and consistently to maximize the amount of time students spend correcting their own work (Gibbs & Simpson, 2004).

“Encourages teacher and peer dialogue around learning” (Nicol & Macfarlane-Dick, 2007, p. 205). Even when students do get feedback from teachers, it is hard for them to understand what is written when trying to correct their work (Chanock, 2000). More formative assessment creates more opportunities for teachers and students to discuss their learning (Nicol & Macfarlane-Dick, 2007). When these discussions happen, students are better able to understand their expectations, discuss misunderstandings and questions with teachers, and get immediate feedback (Ni-

col & Macfarlane-Dick, p. 210).

“Encourages positive motivational beliefs and self-esteem” (Nicol & Macfarlane-Dick, 2007, p. 205). When assessments are given with grades, students focus more on the grades than on the feedback because of concerns over their grades and their ego. This means they often do not use the feedback to help their learning in the ways that have been listed above (Dweck 2000; Butler 1998; Butler, 1987). This then affects the motivation of students who either work harder to achieve a better grade or who take a low grade as a sign they should not try because they are not good (Nicol & Macfarlane-Dick, 2007). Formative assessment does not have these elements. Instead it focuses on the learning process (Nicol & Macfarlane-Dick, 2007).

“Provides opportunities to close the gap between current and desired performance” (Nicol & Macfarlane-Dick, 2007, p. 205). With many formative assessment tasks, teachers will give students the opportunity to apply the feedback they gave to improve their work. This then allows students to learn through the use of feedback (Nicol & Macfarlane-Dick, 2007).

“Provides information to teachers that can be used to help shape teaching” (Nicol & Macfarlane-Dick, 2007, p. 205). When teachers start providing a multitude of formative feedback they learn about the issues and abilities of each student, which can then be reflected in their feedback; more tailored feedback will then increase the effectiveness of the other principles listed above (Yorke, 2003).

Theory Versus Implementation

A substantial amount of research confirms the importance of formative assessment, a considerable amount of which has been included in this literature review. Now that a basis for the effective implementation of formative assessment has been established the next step is to discuss how the implementation of formative assessment differs from the theory. Differences in implementation often occur due to researchers having different interpretations of formative assessment. (Dorn, 210; Crossouard & Pryor, 2012; Clark, 2012). Given the theory and research has established the rationale and value of formative assessment, the problems mainly stem from an improper use of formative assessment. Understanding how formative assess-

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ment is being implemented is important to ensuring students are obtaining its benefits. In this study, I am focusing on one aspect of implementation, the role of the student in formative assessment.

Method

Research Plan

The goal of this study is to use student perceptions to determine whether or not formative assessment is being implemented correctly at RSGC. Through the review of relevant literature on the topic, we can be confident that formative assessment does help students if implemented correctly. Nicol and Macfarlane-Dick (2007) outlined seven key advantages of formative assessment that occur when it is implemented correctly. Student perception of formative assessment could, therefore, be broken down into student perception of each advantage in the model. In this study, students in the senior school, grades ten through twelve, were given surveys and were asked questions in a recorded interview. This study used stratified-random sampling to select students. The strata were based on grade level in order to ensure a complete representation of education levels, and as such, there were seven grade twelve students, six grade eleven students, and six grade ten students. All participants were male since RSGC is an all-boys school. Before students were asked the questions, they were given a definition of formative assessment and were informed that the study was meant to address their perceptions, not their desires. The questions, which are listed in Appendix A, are based on the seven key principles. They were answered by a yes or a no on the survey and were elaborated on during the discussion. The survey portion of the method gave quantitative data, and the interview gave qualitative data. In this way, the quantitative data gives basic answers to questions, and the discussion added explanations to these basic answers. Students were also asked during the survey portion about the academic path they are pursuing and their grades. This subdivides perceptions into certain segments of the school to more specifically target the weaknesses or strengths of the implementation of formative assessment. The questions are listed in Appendix B and students were given space to write a per-

centage grade, and boxes were given so students could check off the courses they are taking. There may be a separation between student perceptions and actuality, but the results of this study can be extended to explain the correct implementation of formative assessment or problems with this implementation. Other schools can then examine the RSGC case study as a model for how formative assessment is being used effectively or ineffectively at their respective schools. These insights can also then be used as a basis for studying specific strategies employed by RSGC or similar schools. Formative assessment has become an integral part of many schools, and it would be a waste of resources to be employing it ineffectively.

Justification

In this study, I collect both qualitative and quantitative data to make the research more meaningful (Creswell, 2013). By using both, the results are more likely to illustrate whether or not formative assessment is being implemented correctly. The purpose of this study is to focus on student perception because students are the ones who experience the effects of formative assessment. This same logic was used by Vandercruysse, Vandewaetere, Cornillie, and Clarebout (2013) who found that student perception of educational gaming needed to be evaluated to analyze its effectiveness. Additionally, this study used a case study method because this creates the most meaningful findings with limitations of time and money. In this study, I also segment the population based on subject area and on academic grades. The segmentation is based on subjects because the variety of pedagogical practices in specific subjects can create subject-specific perceptions and the segmentation is based on academic performance because on top of measuring intelligence separations in grades represent differences in work ethic and attitudes which that create performance specific perceptions (Ariani, 2016). The questions in this study are based on the study conducted by Nicol and Macfarlane-Dick (2007) and the reasoning for the questions can be found in the literature review. This model is also justified in the literature review. In addition, in this study I use grades ten to twelve because these students would be more familiar with formative assessment in high school.

Findings and Results

The findings and results of this study can be found below. Each category of the Nicol and Macfarlane-Dick (2007) model is listed with the relevant question number in addition to survey and interview data. See Appendix A for a complete list of the questions.

“Helps Clarify What Good Performance Is” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 1 Survey. As you can see in Appendix C, all of the participants stated that formative assessment does help clarify what good performance is.

Question 1 Interview. Many participants, when asked this same question in an interview, discussed that when the teacher provides feedback, their marking or comments provide insight into what they are looking for when summative assessments come around. For example, participant J states that formative assessment allows you to “see the lesson plans and how they’re set out, so everything kinda is laid out in front of you, so you can prepare for several assignments.” Participant C goes further into detail when they state that

“in Math for example, that they want you to just know... oh, at least, uh, specify you, uh, be strict about units, be strict about straight lines, and some may not, so it just makes it easier to tell what the teachers are looking for.”

However, participant N said that “there’s a disconnect, where sometimes they [teachers], uh, expect more than what they have in formative assessments.” This was also mentioned in other interviews.

“Facilitates the Development of Self-Assessment...in Learning” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 2 Survey. As stated in Appendix C, only two out of nineteen participants disagreed that formative assessment helps you develop self-assessment skills.

Question 2 Interview. Those that answered yes stated that formative assessment gives you information about what a teacher is looking for that you can apply when looking over your own work. They also state that it allows you to search for specific things in the future. For example, participant O states that “once I have an idea what the teacher is looking for, I can better assess my own work, and make sure that I’m checking all the boxes for...to get to improve my

mark overall.” Some also stated that formative assessment allows you to improve your knowledge and thus assess your future work. Participant G states that “I’ll learn more. If you see more feedback about your own work, then you’ll understand more and more what’s expected of you.” Participant N also brought up an interesting point when they stated that “I can tell that I got stuff wrong, instead of having it, uh, negatively impact my grade in a course, uh, have more incentive to make sure I get it right.”

“Delivers High Quality Information to Students About Their Learning” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 3 Survey. The first question in this category was whether students received actionable feedback regularly. Appendix C shows that, ten answered yes and, nine answered no. A majority of the science and engineering students in the sample population were concentrated into the yes category whereas those that answered no were from a variety of courses. Additionally, the average summative average of those who answered yes was 89.1% whereas it was 86.1% for those who answered no.

Question 4 Survey. The second question in this category was whether students apply the feedback they are given. Again, as displayed in Appendix C eleven answered yes and, eight answered no. The average summative average of those in the yes category was 89.9% and the average summative average of those in the no category was 86.4%. It is also interesting to note that the distribution of no answers was higher for grade ten students.

Question 3 Interview. Many students who answered no to the first question stated that the feedback they received was formulaic and not applicable. Participant G states that “On formative stuff, a lot of the time, they won’t really give you so much feedback, they’ll just give you numbers.” Students often discussed in other questions that the benefits of formative assessment are not as great because they do not apply all suggestions given to them.

Question 4 Interview. One of the central themes that came up was that participants often disagree with the teacher. Participant H outlines how they would not apply feedback “When I would disagree or I have a strong opinion on why I don’t want to have it or someone else has told me, otherwise.”

“Encourages Teacher and Peer Dialogue Around

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Learning” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 5 Survey. As displayed in Appendix C eleven students said yes an eight said no.

Question 5 Interview. Those students who answered yes said that they could gain more benefits from formative assessment if they talked to teachers. For example, participant P states that “It helps me point out mistakes that I’ve made, and then I can ask the teachers about what I can do or should do.” One of the main reasons participants mentioned for not talking to teachers was that the simplicity of the formative assessments means it is not needed. For example, participant J states that

“for formative learning in general, if it indicates that I need to maybe step it up, or study even more for that certain type of unit, or information, then, yes, for sure, I’ll approach the teacher, and really find out what needs to be done in order for me to achieve that better mark, but then in general, for formative quizzes, when usually maybe one out of one marks, or out of ten marks, and multiple choice, then I don’t think it really matters.”

Some also stated that they already talk to teachers with or without formative assessment.

“Encourages Positive Motivational Beliefs and Self-Esteem” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 6 Survey. The first question under this category was whether students are concerned with their formative grades. The data in Appendix C shows that only three students said yes.

Question 7 Survey. The second question under this category was whether formative grades affected students’ self-esteem. The same people who answered yes on the prior question also answered yes with the addition of three more people. The average academic average of these people was 84.5% compared to 88.8% for those who answered no.

For both questions, the majority of no answers came from people above grade eleven.

Question 6 & 7 Interview. Students did not elaborate beyond a yes or no in the interview.

“Provides Opportunities to Close the Gap Between Current and Desired Performance” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 8 Survey. All participants except for two answered yes to this question.

Question 8 Interview. The reasoning that the ma-

ajority of people gave was that when they applied given feedback or learned from their mistakes, they could improve their work in the future. For example, participant R says that

“I think if, uh, my...my grades do improve after formative assessment, because it gives me an idea of what the teacher is expecting. Um, and then also being able to apply their...their suggestions also, uh, improves, like, my work as well. So, yes.”

“Provides Information to Teachers That Can Be Used to Help Shape Teaching” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 9 Survey. Appendix C shows that for this question eleven people answered no and eight people answered yes. The average summative average of those who answered no was 89% and it was 85.9% for those who answered yes.

Question 9 Interview. One prevailing sentiment expressed by those who said no was that they do not notice teachers adapting their teaching to individual students. Participant M says

“I think they generally cater their teaching to the whole class, so I think it’s rare for someone, for them to change their style of teaching for one person, but I can definitely see it changing if, like, a whole class is, struggles with something.”

However, some said teachers would be happy to talk to you if you approached them directly. Another common sentiment was that teachers could not learn from summative use of formative tests because the marking was not individualized. For example, participant F states that “So, like obviously like I think of multiple choice questions, it’s...it’s really not gonna help them learn about you very much. So, it depends on the assessment I guess.”

Discussion

“Helps Clarify What Good Performance Is” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 1. The literature stresses the importance of students being able to understand goals set by the teacher, and it states that formative assessment does this when appropriately implemented. The fact that all participants agree shows that formative assessment is being implemented correctly in this respect. However, the assertion that formative assessments are easier

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than real assessments contradicts this slightly. If this occurs, students cannot use an easier formative assessment to learn about their performance when that will be lower on the real test.

“Facilitates the Development of Self-Assessment... in Learning” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 2. Again, the literature emphasizes the importance of students being able to assess their own work, and it states that formative assessment does this when implemented correctly. The fact that almost all participants agree shows that formative assessment is being implemented properly in this category.

“Delivers High Quality Information to Students About Their Learning” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 3. The literature states that to reap the benefits of formative assessment it must happen regularly. The fact that some people do not get feedback or that it is formulaic and not useful shows it is not being implemented correctly. As per the literature these students do worse on summative assignments than those that get feedback regularly. If the school were to address this issue, they should focus on and study formative assessments in non-STEM courses in more detail because these were both pointed out by the data and mentioned in interviews.

Question 4. The literature shows that to get the most out of formative assessment students must also apply changes a teacher gives them. This is shown by the fact that students who do use suggestions are better off academically than those who do not. The problem is that students disregard feedback they do not think is right or do not understand instead of approaching the teacher. This last part will be addressed later. Further research could be conducted to find out what types of feedback students disregard and how to stop this or why this is a problem with students in grade ten.

“Encourages Teacher and Peer Dialogue Around Learning” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 5. According to the literature, there is a disconnect between what a teacher writes and what a student understands. To understand entirely, students need to approach teachers with questions and concerns. For example, they could approach teachers with questions about the feedback they disagree with instead of disregarding it. Participants also pointed out in the interview that they do not discuss feed-

back with teachers because it is formulaic. Further researchers or the school could study what would happen if the style of feedback changed.

“Encourages Positive Motivational Beliefs and Self-Esteem” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 6. The literature makes it clear that since formative assessment does not affect academic standing students can focus on feedback, not on their marks. The fact that most students do not care shows that formative assessment is being implemented correctly. Further research could be conducted to determine whether the fact that students disregard comments is connected with a lack of concern.

Question 7. As stated before, the literature indicates that since formative assessment does not affect academic standing students can focus on feedback, not on their marks. Since most students' self-esteem is not affected, it is being implemented correctly. The fact that students with lower grades are more concerned if researched further could prove the literature. Also, if students with low grades are affected by formative assessments, they could actually be lowering their self-esteem. In the last two questions, the students from lower grades were most concerned. This could also be researched further.

“Provides Opportunities to Close the Gap Between Current and Desired Performance” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 8. One of the fundamental principles of formative assessment is that it allows a student to improve a piece of work and learn while doing it. The fact that most agree shows that this is being established at RSGC.

“Provides Information to Teachers That Can Be Used to Help Shape Teaching” (Nicol & Macfarlane-Dick, 2007, p. 205)

Question 9. According to the literature an added benefit of formative assessment is that when a teacher does it, they learn about their students and can tailor their future feedback. The survey showed that eleven people found teachers did not do this and eight did. This shows that to an extent teachers are not learning about their students learning. Furthermore, some people who said yes were then uncertain in the interview. Participants stated that feedback giving is often formulaic. However, this could be a mechanism teachers use to learn about their students. Since students are limited in their perspectives, further research

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could interview teachers at RSGC to determine if they do this. However, one common theme was that because feedback was often not individualized, especially on tests, it would be hard for teachers to tailor their feedback. Further research could also examine if teachers would learn more about their students if more students discussed formative assessment with their teachers.

Limitations

Some limitations of this study include the fact that perceptions of young students are not always accurate, some people had different answers to the survey than what they expressed in the interview, and that it was evident that some participants did not understand questions. It is also possible that a certain type of student was the most likely to agree to participate thus adding bias to the sample population. The small number of people who did agree to participate also restricts this study's ability to make a conclusion. Also, there may be a disconnect between the experiences of male students in this school and female students in other schools thus reducing the ability to extend the results to other schools. In addition, despite being instructed not to, some participants answered based on desires, not experience. Lastly, different participants could have differing ideas on relative topics like what constitutes regular actionable feedback.

Conclusion

The primary goal of this study was to examine the student perceptions of the implementation of formative assessment. By segmenting this according to the Nicol and Macfarlane-Dick (2007) model and by investigating student's perceptions in each of these categories the results of this study could specify implementation perceptions. The results revealed that formative assessment is being implemented in a way that allows students to learn about what good performance is and self-assess their own work. That said, students could learn more if teachers were to make formative assessment harder. This study also revealed that not enough feedback is being given, it is often formulaic and not useful, and when it is given students are not apply-

ing all suggestions or approaching teachers as much as they should be. However, despite a slight concern, formative assessment is allowing students to focus on improving, not on their grades. Students also find that their work does improve when implementing it. In conclusion, teachers should use feedback to learn about their students more often.

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Appendix A

Survey and Interview Questions about Formative Assessment

Principle	Questions
"Helps clarify what good performance is" (Nicol & Macfarlane-Dick, 2007, p. 205).	1. Does feedback given for formative assessment tasks help your understanding of performance goals set by the teacher?
"Facilitates the development of self-assessment...in learning" (Nicol & Macfarlane-Dick, 2007, p. 205).	2. Does formative assessment feedback improve your ability to assess your own work?
"Delivers high quality information to students about their learning" (Nicol & Macfarlane-Dick, 2007, p. 205).	3. Do you receive actionable feedback regularly? 4. Do you apply all suggestions given to you by a teacher?
"Encourages teacher and peer dialogue around learning" (Nicol & Macfarlane-Dick, 2007, p. 205).	5. Do you have more conversations with teachers about your learning when you have been given formative feedback?
"Encourages positive motivational beliefs and self-esteem" (Nicol & Macfarlane-Dick, 2007, p. 205).	6. Are you concerned with your formative grades? 7. Do formative grades affect your self-esteem?
"Provides opportunities to close the gap between current and desired performance" (Nicol & Macfarlane-Dick, 2007, p. 205).	8. Do you believe your work improves after you apply suggestions given during formative assessment?
"Provides information to teachers that can be used to help shape teaching" (Nicol & Macfarlane-Dick, 2007, p. 205).	9. Do you find teachers are able to learn about your learning when they have given you formative feedback?

Appendix B

Personal Survey Questions

Topic	Questions
Academic Performance	<p>10. If you feel comfortable doing so, please record your summative average? If you do not know your average please estimate or extrapolate based on previous performance. Your answers are completely confidential.</p>
Subject Area	<p>11. Please place an X next to the types of courses you are pursuing or plan to pursue. If you are unsure select undecided.</p> <p>Business</p> <p>Fine Arts</p> <p>Humanities/Social Sciences</p> <p>Science/Engineering</p> <p>Undecided</p>

Appendix C

Survey Results

Grade	Participant	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11
10	A	Yes	No	Yes	No	No	No	Yes	Yes	No	80%	Science/Engineering
10	B	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	89%	Science/Engineering
10	C	Yes	Yes	No	No	No	Yes	Yes	No	No	88%	Science/Engineering
10	D	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	78%	Humanities/ Social Sciences, Science/ Engineering
10	E	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes	84%	Fine Arts, Humanities/ Social Sciences, Science/ Engineering
10	F	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	92%	Humanities/ Social Sciences, Science/ Engineering
11	G	Yes	Yes	No	No	Yes	No	No	Yes	No	86%	Humanities/ Social Sciences
11	H	Yes	Yes	No	Yes	No	No	No	Yes	Yes	84%	Business
11	I	Yes	Yes	Yes	No	Yes	No	No	Yes	No	95%	Science/Engineering
11	J	Yes	Yes	No	Yes	Yes	No	No	Yes	Yes	85%	Business, Humanities/ Social Sciences

STUDENT PERCEPTIONS OF THE IMPLEMENTATION OF FORMATIVE ASSESSMENT

11	K	Yes	Yes	Yes	Yes	No	No	No	Yes	No	93%	Science/Engineering
11	L	Yes	Yes	No	No	No	No	No	Yes	No	80%	Business, Humanities/Social Sciences
12	M	Yes	Yes	Yes	No	Yes	No	No	Yes	No	92%	Science/Engineering
12	N	Yes	Yes	No	Yes	No	No	No	Yes	No	89%	Science/Engineering
12	O	Yes	Yes	Yes	Yes	No	No	No	Yes	No	91%	Science/Engineering
12	P	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	86%	Science/Engineering
12	Q	Yes	Yes	No	No	No	No	Yes	Yes	Yes	89%	Science/Engineering
12	R	Yes	No	Yes	Yes	No	No	No	Yes	No	95%	Business, Humanities/Social Sciences
12	S	Yes	Yes	No	Yes	Yes	No	No	Yes	No	90%	Business

A Descriptive Study of Adolescent Perceptions of Rural Versus Urban Kentucky High School Student Mental Health Challenges

Allison Tu

Research has evaluated mental health perspectives of adults, but no studies gather Kentucky students' input on these issues. Understanding youth perceptions is critical to creating effective mental health interventions, as students experience these issues firsthand. Capturing rural and urban divides is also crucial to create programming applicable to both student groups. Therefore, this study aimed to characterize the similarities and differences between the student-reported perceptions of mental health challenges facing urban versus rural students. Data was collected through four focus groups at urban and rural high schools. Thematic analysis through ATLAS.ti showed that students perceived that peers, family, lack of resources, and high stress influenced mental health in both urban and rural areas. Differences between urban and rural perceptions revolved around social media, community culture, and overdose. These results serve as a foundation for future research assessing youth perceptions to identify an effective intervention to improve Kentucky students' mental health.

Keywords: Adolescent; mental health; Kentucky; rural; urban; youth perceptions

Introduction

Youth mental health issues in the United States are skyrocketing. For instance, one in five adolescents suffers from a diagnosable mental health disorder (National Alliance on Mental Illness, n.d.). In Kentucky, the situation is even more dire—according to the Kentucky-specific data from the Youth Risk Behavior Survey, almost one-third of Kentucky youth report they are so sad, anxious, or hopeless they have stopped pursuing activities that they normally enjoy (Kann et al., 2016). The national data from this survey reflects an alarming trend: the percentages of youth who seriously considered attempting suicide and youth who made a suicide plan follow a quadratic pattern, decreasing from 1991-2009 but increasing from 2009-2015 (Kann et al., 2016).

Positive mental health, however, is critical for adolescents in several respects. Untreated mental health challenges significantly decrease quality of life, are detrimental to academic performance, and can lead

to severe issues such as substance abuse or suicide. As Stagman and Cooper (2010) report, “children and youth with mental health problems have lower educational achievement, greater involvement with the criminal justice system, and fewer stable and longer-term placements in the child welfare system,” a finding corroborated by several other studies (Erickson & Abel, 2013; Valdez, Lambert, & Ialongo, 2011). As further evidence that mental health issues jeopardize education and future success, over 40% of students affected by a mental health disorder do not complete secondary school, the highest dropout rate of any disability group (Wagner, Newman, Cameto, & Levine, 2005; National Alliance on Mental Illness, n.d.). Additionally, poor mental health in adolescence has been associated with future anxiety, depression, and suicide (Valdez, Lambert, & Ialongo, 2011).

While mental health is critical for adolescents, few receive appropriate intervention or treatment. The average delay between the onset of symptoms and treatment is 10 years and up to 80% of youth who require treatment do not receive it, indicating that youth are

unable to access suitable services (National Alliance on Mental Illness, n.d.; Stagman & Cooper, 2010). Services themselves also may be ineffective; a meta-analytic review by Farahmand, Grant, Polo, and Duffy (2011) identified only 17% of reviewed school-based mental health programs as effective. Though youth mental health is a consequential concern, few studies have attempted to elucidate adolescent perspectives on mental wellness. Understanding these perceptions, however, is pivotal to the improvement of the issue, as youth are the preeminent stakeholder in their own well-being and have unique insights into drivers of poor mental health.

Literature Review

Barriers to effective mental health prevention and treatment

A lack of mental health education, significant stigma surrounding mental health, and a lack of accessible services are the key factors upon which the literature converges as barriers to effective mental health prevention and treatment. Research suggests that both mental health education and mental health services are inaccessible and inadequate. Story and her colleagues report that “the prevalence of mental illness and death by suicide . . . may be related to limited mental health literacy” (Story et al., 2016). The lack of awareness caused by poor mental health education, in turn, leads to a second major barrier, stigma.

Stigma, often defined as “an actual/inferred attribute that damages the bearer’s reputation and degrades him/her to a socially discredited status,” prevents students from accessing necessary mental health services (Mukolo, Heflinger, & Wallston, 2010). For adolescents, a lack of mental health education is a key perpetuator of stigma, making it one of the most frequently reported reasons for not seeking treatment for mental distress (Jameson & Blank, 2007; Mukolo, Heflinger, & Wallston, 2010; Topkaya, 2015). Stigma increases when incorrect labels and stereotypes are applied to the mentally ill and research has found stigma levels to decrease with appropriate mental health education (Story et al., 2016; Larson & Corrigan, 2010).

Finally, many cannot access effective mental health intervention. A consensus has been established that a

dearth of mental health providers, particularly in rural regions, renders services inaccessible (Jameson & Blank, 2007; Smalley et al., 2010). This shortage is particularly acute in Kentucky—two million Kentuckians reside in these mental health care professional shortage areas and less than 60% of the total need for mental health providers is met in the state (Kaiser Family Foundation, 2016).

Urban versus rural mental health

Mental health culture. Research agrees that residents of rural areas have unique attitudes and culture regarding mental health and mental health interventions that differ from the culture in urban areas. Rural residents have been found to have more significant stigmas surrounding mental illness due to values of self-sufficiency (Story et al., 2016; Bischoff et al., 2014). Focus groups conducted by Bischoff et al. (2014) found that “rural residents often do not ‘even believe in therapists’” and that mental illness is considered particularly shameful in these communities. Bischoff’s findings that rural mental health practices must be sensitive to this culture to be effective are corroborated by other rural health studies (Bischoff et al., 2014; Jameson & Blank, 2007).

Mental health service access. Limited access to mental health services and education in nonmetropolitan areas due to a lack of competent professionals may be a driver of stigma-focused rural mental health culture (Jameson & Blank, 2007; Smalley et al., 2010). Research is inconsistent, however, on whether this leads to increased mental illness rates in rural populations—Story et al. (2016) and Bischoff et al. (2014) found higher rates of mental illness in nonmetropolitan areas while Jameson and Blank (2007) stated that rate of illness was similar across rural-urban divides, but severity is increased in rural areas. Others report that urban youth are at a higher risk of experiencing mental illness than others students due to violence and poverty-related stressors (Farahmand, Grant, Polo, & Duffy, 2011; Valdez, Lambert, & Ialongo, 2011).

Gaps in the current literature

Though recognition and treatment of mental health disorders are critical, there remain significant gaps in the literature surrounding adolescent mental health.

RURAL VERSUS URBAN KENTUCKY HIGH SCHOOL STUDENT MENTAL HEALTH

Few recent studies, and none in Kentucky, have analyzed student perspectives on the issue, which are critical to inform the policies and programs that directly impact youth. Analysis of these perceptions, in turn, allows for the creation of more effective solutions (Aarons et al., 2009; Landeweer, Molewijk, Hem, & Pedersen, 2017). In Kentucky, no studies compare urban versus rural mental health of students, but these geographic distinctions are crucial to developing policies effective for both groups.

Therefore, the present study aims to fill this gap in the body of knowledge by answering the following question: What are the similarities and differences between the student-reported perceptions of mental health challenges facing urban versus rural Kentucky high school students? No hypothesis for this research was created to avoid potential researcher bias that could skew the analysis of the largely qualitative data. Instead, two assumptions were made. It was assumed that mental health is a concern in both rural and urban areas of Kentucky. More specifically, it was also assumed that mental health is a concern at the particular urban and rural schools at which the study was conducted.

Methodology

This mixed descriptive study involved collection of primarily qualitative, and some quantitative, data collected from focus groups. Data was analyzed by conducting a thematic analysis through coding in the qualitative analysis software ATLAS.ti to characterize the similarities and differences of perceptions of mental health among rural and urban Kentucky high schoolers. As opposed to surveys or interviews, focus groups were chosen as the data collection method for this study because, according to Bischoff et al. (2014), “they allow researchers to understand the group dynamics that surround an individual’s perception and processing of the subject matter.” Focus groups provide insight into the general attitude surrounding a topic, which is particularly useful to understand for mental health, which is heavily influenced by an individual’s surrounding community. Additionally, a wide range of perspectives can be discussed during focus

groups, as each participant’s attitudes, culture, and life experience are varied. Group discussion allows participants to comment on others’ ideas, leading to more productive conversations (Bischoff et al., 2014). Neither surveys nor individual interviews would allow the same degree and quality of insights that group conversations do.

Analysis of these rich discussions of youth perceptions elucidated similarities and differences across urban and rural adolescents, filling the gap of understanding student perspectives and answering the research question. This research, though specific to the state of Kentucky, provides a foundation upon which to base future local and far-reaching studies of mental health from a youth point of view.

Four total focus groups were conducted with Kentucky high school students—two at one urban Kentucky high school and two at one rural Kentucky high school. The US Department of Agriculture definition was used to classify schools as either rural or urban. This definition, often used in clinical psychology studies, assigns all counties in the United States a number on a scale of 1 (most urban) to 9 (most rural). Counties between 1 and 3 are considered urban, while counties rated 4-9 are considered rural (US Department of Agriculture, 2016). Though this definition has been criticized for its lack of preciseness on levels more specific than county borders, the majority of studies in this field continue to use this norm; therefore, this research uses the same definition (Jameson & Blank, 2007). The urban groups were conducted in Jefferson County, a 1 on the scale, and the rural groups were conducted in Nelson County, a 6 on the scale.

The principals of Kentucky schools in Jefferson and Nelson Counties were contacted to ask their interest in participating in this study. The first two schools to respond with written agreement of interest and approval for this study were used. For each focus group, 6-10 students were recruited through a convenience sampling method. This sampling method was selected because sampling by randomly selecting students was infeasible—schools were unwilling to release a student roster.

To identify the convenience sample, flyers were distributed within the building and over school announcements. Included on the flyer was information about the incentive for participating (free refreshments), a description of the study, and the contact in-

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formation of the researcher. Interested students were asked to contact the researcher and return a consent form (included in Appendix A) with parental consent and student assent before participating.

As shown in Table 1 and Table 2, a total of 15 students participated in the urban focus groups and a total of 13 students participated in the rural focus groups. Each focus group fell within the target range of 6-10 participants. While the study was open to both female and male students, there were far more female than male participants. Grade levels of the students varied, but urban students were mostly sophomores and rural students were mostly seniors. This is likely due to interested students asking their friends of the same grade level to participate with them.

After enough students volunteered to participate, the researcher coordinated with individual students over text or email to identify a date and time for the focus group that was convenient. In coordination with school staff, a location that ensured students' privacy,

such as a library or closed classroom, was determined for the focus group.

To inform the questions asked during these sessions, a pilot focus group was conducted with six students at the researcher's school. Data from this pilot, the questions for which are included in Appendix B, were only used to modify the procedure for the actual focus groups and were not included in data analysis. Based on the questions that participants seemed to have difficulty answering and whether the data obtained answered the research question of this study, the focus group questions were revised, and the final version is included in Appendix C. The revised questions included largely open-ended qualitative items, though question 9, which asked all participants to rate factors contributing to stigma on a scale of 1-5 (5 being the most impact), was quantitative. This varying data type was included to provide a fuller picture of the emerging themes and to allow all students in the focus group to contribute their thoughts.

Table 1. Total participants in urban focus groups by gender and grade level

Table 1							
<i>Characteristics of Urban Participants</i>							
	Total Participants	Females	Males	Freshmen	Sophomores	Juniors	Seniors
Focus group 1	6	5	1	2	2	2	0
Focus group 2	9	7	2	0	9	0	0
Total	15	12	3	2	11	2	0

Table 2. Total participants in rural focus groups by gender and grade level

Table 2							
<i>Characteristics of Rural Participants</i>							
	Total Participants	Females	Males	Freshmen	Sophomores	Juniors	Seniors
Focus group 1	6	3	3	0	0	2	4
Focus group 2	7	6	1	0	2	0	5
Total	13	9	4	0	2	2	9

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The researcher facilitated the discussion by asking the questions in Appendix C and prompting students with further information or follow-up questions as necessary. Data analysis, informed by the protocol of Fielden, Sillence, and Little (2011), who conducted youth focus groups about obesity, and a thematic analysis guide written by Braun and Clarke (2006), began by reviewing the notes and audio recording of the session immediately after the session ended. This review strengthened the remaining steps of analysis by allowing reflection on how the data collection procedure could be improved (Fielden, Sillence, & Little, 2011). In this study, no improvements were found to be necessary.

After all four focus groups were conducted, each discussion was transcribed verbatim by the researcher, including verbal ticks such as “like” or “um.” Initial ideas about potential themes were recorded, considered an essential step in analysis (Fielden, Sillence, & Little, 2011). Resulting transcriptions and notes were then re-read at least three times to increase the researcher’s familiarity with the data, allowing for accurate coding (Braun & Clarke, 2006). The analysis then moved to ATLAS.ti, a qualitative data analysis software, which was used to facilitate coding of the

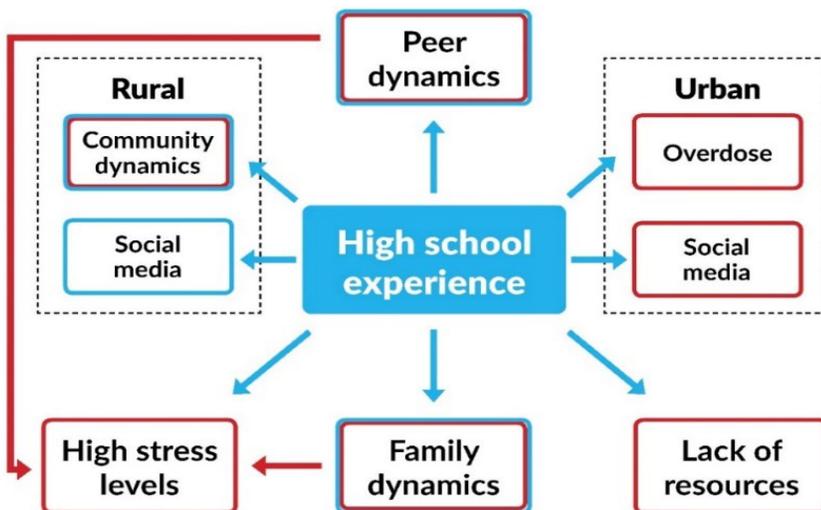
data. The rural focus groups and urban focus groups were separated into different document groups in ATLAS.ti, allowing for separate analysis and later comparison. Using ATLAS.ti, the researcher assigned appropriate codes, which identified concepts in the data that related to the research question, to each statement made by focus group participants (Fielden, Sillence, & Little, 2011). Each initial code was categorized into an overarching theme, and themes that did not have sufficient support were discarded. A list of codes, descriptions, and groupings, exported from ATLAS.ti, is included in Appendix D. As suggested by Braun and Clarke (2006), the relationships among themes were placed into a thematic map for clearer visualization. Finally, the transcriptions were reviewed to ensure nothing was missed. Overall, the thematic analysis allowed for the capturing, characterization, description, and relation among the student-reported themes, answering the research question by revealing similarities and differences between rural and urban youth.

Results and Discussion

Significant themes

Four total focus groups, two in urban settings and two in rural settings, were conducted with Kentucky high school students. Thematic analysis of the four focus groups was informed by Fielden, Sillence, and Little (2011) and Braun and Clarke (2006) and conducted using ATLAS.ti coding software. Analysis revealed several significant similarities and differences between rural and urban settings. Students discussed both positive and negative influences on mental health in relation to two categories: contributors to mental health symptoms or contributors to mental health stigma. Each major theme connected to the broad category of the unique high school experience, further demonstrating the importance of capturing youth perspectives. Three themes—community interactions, overdose, and social media—represented the primary differences between the responses of rural and ur-

Figure 1. Key themes identified through thematic analysis. Each theme related to the unique high school experience; red outlines represent negative influence on mental health, blue outlines represent positive influence, and outlines of both colors indicate both positive and negative influences.



ban students. The following diagram represents major themes that arose, including similarities and differences and relationships among the key themes.

Urban and Rural Similarities

Thematic analysis revealed that several issues—family dynamics, peer interactions, lack of resources, and high stress levels—were reported by both urban and rural students. Students reported stress as a contributor to mental health symptoms, and lack of resources, family relationships, and peer relationships as driving both mental health symptoms and mental health stigma. Each of these themes is further explored.

Family Relationships. Participants in both the rural and urban focus groups stated that family relationships, particularly with parents, generally perpetuated both mental health symptoms and mental health stigma. Students felt pressured to meet their parents' high expectations or please their parents, particularly if they had exceptionally low or high achieving siblings. As one urban male participant stated, "I feel like I have to overcome, like compensate for both of their [his siblings'] mistakes and be even better than that so they [his parents] have a glory in their life." When compared to their siblings, students felt pressure from their parents to fill the shoes of older siblings or pave the way for younger siblings, leading to stress. Rural students stated, "you're supposed to literally pave the way for your younger siblings," and "I have two successful sisters that have gone through college ... And I'm just trying to keep up." Urban and rural students also expressed the feeling that they'd be a burden on their parents if they were to experience or discuss mental health concerns; an urban participant stated "I feel like a lot of people don't even go to anyone [for mental health help] because they don't want to put that on anyone else."

Participants also felt that their parents didn't understand their circumstances; a rural student stated that "parents don't seem to really understand what's going on until you literally have to scream it at their face that this is what is happening." This disconnect prevented students from discussing mental health concerns with parents. Students also reported avoid-

ing talking to their guidance counsellors about mental health for fear that their symptoms would be discussed with their parents.

In terms of mental health stigma, students felt that the generational gap between them and their parents led to misunderstandings and perpetuation of stigma. One student reported "In their [parents'] generation, there wasn't any understanding whenever it comes to mental health issues. It was either you're sane or you're insane." Participants generally felt that family members would be unsupportive or wouldn't understand mental health challenges because the current world presented unique difficulties regarding technology and contemporary political unrest. However, some students, particularly those who were open about personal experience with mental health issues, stated that their parents were supportive. These significant influences of parents and family on mental health, either positively or negatively, align with existing research. Smokowski et al. (2015) found that negative relationships between rural students and parents led to higher rates of anxiety and depression, while positive relationships were associated with lower rates of depression. Oldfield, Humphrey, and Hebron (2016) found a similar relationship, associating insecure parental attachment with emotional difficulties.

Peer interactions. Analysis of quotes about peer interactions demonstrated that similarly to how students felt about family interactions, rural and urban participants associated peers with both positive and negative impacts on mental health. Students felt that their close, trusted friends would be supportive if they were told that a peer was experiencing mental health symptoms. An urban participant stated, "If I came up to [friend's name] and was talking about how I had mental health symptoms, I think she'd be shocked at first, but she'd be very supportive."

However, both rural and urban students believed that friends with whom they were less close would deny the issue, make jokes at the expense of the student experiencing mental health challenges, or not take the issue seriously. For example, a rural student said, "One thing that's very prominent is the fact that whenever you tell somebody that you have anxiety or depression, they're like 'but you don't really, do you?'" Participants mentioned that these reactions, many of which occur through social media, may be due to a lack of awareness or understanding of mental health.

Students believed that a general culture of not taking mental health seriously—for example, an urban student stated “saying phrases like ‘I’m gonna kill myself’ is just so common that you just don’t think it’s true”—made it difficult to tell when students were truly struggling and when students were being facetious.

Additionally, urban and rural students both feared that if they were to discuss a mental health challenge with a peer, they would be judged and treated as an outcast. One participant said, “I’m scared, I don’t want them to think differently of me, like I know judgment is a very big deal in our society” to explain why she was hesitant to start a conversation about mental health. This fear may drive mental health symptoms; a participant said, “a lot of people don’t want to be judged so they keep it to themselves and it builds up and builds up just because they don’t want other people to see them in a bad way,” reflecting that students often hide their symptoms until they reach a breaking point.

This significant influence, both positive and negative, of peers on mental health is corroborated by current literature. Mackrell and Lavender (2004) examined the impact of peer relationships in the context of mental health, finding that peers are significant sources of support in crisis, while Jones et al. (2011) found that increased loneliness among youth led to a higher incidence of depression and suicide. Oldfield, Humphrey, and Hebron (2016) established that positive peer relationships are a strong predictor of well-being. Current research also demonstrates the negative influence of peers, particularly significant stigmatization and teasing of peers struggling with mental health challenges (Mackrell & Lavender, 2004; O’Driscoll, Heary, Hennessy, & Mckeague, 2012).

Lack of resources. Throughout the focus groups, students discussed a dearth of mental health resources as a perpetuating factor of mental health stigma and symptoms. Students cited a lack of awareness, a result of poor mental health education, as a major factor in stereotyping. For example, a rural male participant stated “A lot of people go through and see people who are depressed or anxious as just generally crazy ... That’s why people are probably so afraid to be able to be open about it too.” While school counsellors and staff, hotlines, parents, therapy, medication, and friends were mentioned when students were asked what mental health resources were available, no other community resources or evidence-based programs were discussed.

One significant theme among both urban and rural students was the weaknesses of school counsellors in providing mental health guidance. Students reported being told that counsellors were an available resource, but they didn’t feel as though counsellors would be helpful in mental health concerns. Urban students felt that counsellors were inaccessible, documenting a long appointment process before one could meet with a counsellor and feeling that their counsellors were too busy with other students or tasks to help. Rural students felt that their counsellors “probably wouldn’t do jack about it,” meaning that they would likely not do anything to address the concern. This is likely driven by Kentucky’s lack of school counsellors; the student-to-counsellor ratio in 2014-15 was 453:1, close to double the recommended ratio of 250:1 (National Association for College Admission Counseling & American School Counsellor Association, n.d.). This lack of counsellors may contribute to their inaccessibility.

High stress levels. Students reported that balancing school with other obligations, such as work, maintaining a social life, standardized testing, applying for college, and feeling pressured to meet family expectations, led to high levels of stress. A rural student reported feeling overwhelmed, saying “It’s hard to juggle it as a teenager because there’s just so many things.” Additionally, identify conflicts, a hallmark of adolescents’ development, were reported by students as drivers of youth mental health symptoms. Students discussed struggling with determining who they are and what they’d like to pursue after high school. This pressure of feeling forced to plan the rest of their lives as teenagers compounded other stressors, such as school and social pressure. For example, one urban student stated, “I don’t have that clear image of what I’m gonna do so I don’t know what to do from here on out, it’s confusing.” Current literature supports this finding; teens report stress levels that are higher than those of adults and 30% feel depressed or sad because of stress (Bethune, 2014). Studies of both urban and rural adolescent populations found significant links between high stress levels and depressive symptoms (Carleton, Esparza, Thaxter, & Grant, 2008; Young & Chau, 2016).

Urban and Rural Differences

Thematic analysis of rural groups and urban groups showed three primary differences in mental health perceptions. These variations revolved around community interactions, social media, and overdose.

Community interactions. As opposed to urban students, rural students reported both positive and negative influences of community culture, while urban students believed that community culture had little impact on mental health. Rural students felt a strong sense of connection and comfort with their peers—rural focus group participants discussed feeling very open about broaching the topic of mental health with close friends. Students stated that “I know us friends are always here for each other” and “there’s this mutual respect that we all have for each other,” demonstrating their close bonds.

Simultaneously, however, rural participants felt that community culture had significantly more impact on stigma than urban students. Students believed that this stigma was a result of ingrained do-it-yourself attitudes and immense focus on religion. One participant said “My dad always taught me that if you can’t do it yourself, then there’s no point in trying at all,” while another stated “they’re [church members] like

‘God’s always with you, you should never be sad.’ And I was like, ‘well why am I sad? I must be upset about something.’” This finding is consistent with Bischoff et al. (2014)’s landmark study, which reported the importance of considering unique cultural factors when providing mental health care to rural patients.

Social media. Urban students considered social media to be largely detrimental to mental health, stating “Let’s say I just post a picture ... somebody can screenshot that and post it on their page and make fun of me,” and “media is one of the biggest factors [that contributes to stigma], just because ... people joke about depression and all that a lot.”

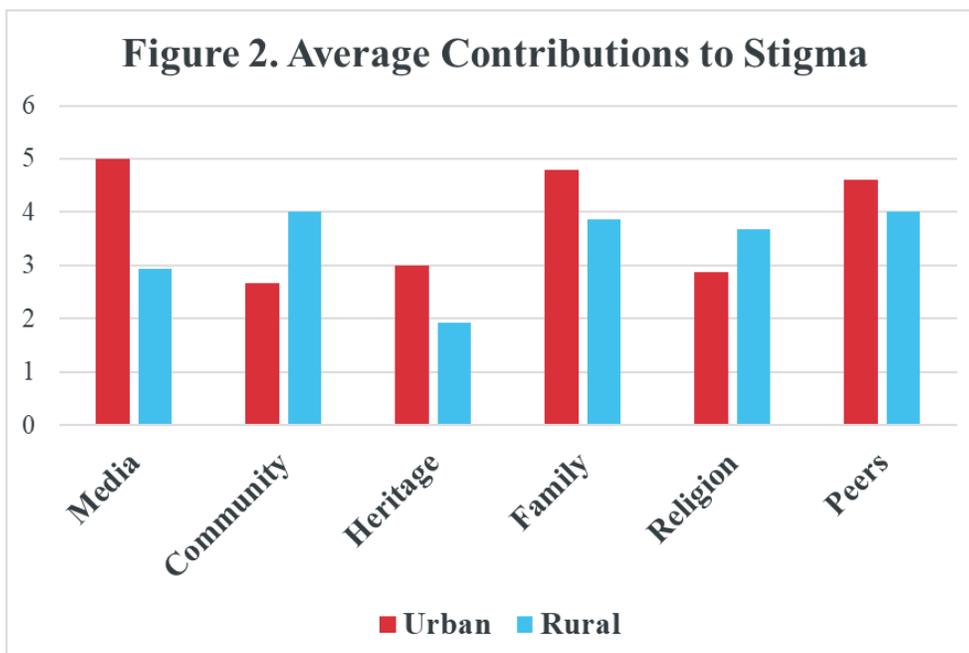
However, rural students believed that social media can be beneficial to mental health, citing various websites as ways to cope with sadness. Students in one focus group agreed that watching inspirational or funny videos on YouTube allows them to relieve stress and feel better. Participants also found comfort in seeing others’ mental health journeys on social media, which helped them feel less isolated.

Prior research has noted that social media may have negative impacts on mental health, including symptoms driven by cyberbullying (Brown & Bobkowski, 2011). Other studies, however, have found that students who spend more time on social media report lessened anxiety, possibly because they are using communication tools in a constructive manner (George, Russell, Piontak, & Odgers, 2017). Little research investigates differential influences of social media on urban versus rural adolescents, and more investigation

is necessary to characterize relationships between youth social media use and community environment.

Overdose. Overdose of family members, friends, or peers as a contributor to mental health symptoms was reported solely by urban students. Both urban and rural students discussed drug use among adolescents as a coping mechanism, but no rural students mentioned overdose. This contrasts with national statistics—the nonmetro overdose death

Figure 2. Average contributions of six factors to stigma, as reported by urban and rural students.



rate was slightly higher in 2015 than the metro overdose rate, at 17.0 cases per 100,000 people compared to 16.2 urban cases per 100,000 (Mack, Jones, & Ballesteros, 2017). This finding may be a result of the specific communities in which the study was conducted having a different relationship to drug overdose, but more investigation is necessary.

Quantitative data: contributions of factors to stigma

One quantitative item was included during the focus groups to evaluate the degree to which students believed different factors contributed to stigma. The mean values for each of these factors are included in Figure 2.

As this study was primarily qualitative, the sample size was relatively small, including a total of 28 students. A more comprehensive quantitative study with a larger sample size would be necessary to glean more insights from this data. However, this data does support the qualitative findings. The largest differences between urban and rural students were with media, with a difference of 2.07, and community culture, with a difference of 1.33, which were two of the three thematic differences between urban and rural areas.

Overall, analysis of quantitative and qualitative data revealed that the influence of family dynamics, peer interactions, lack of resources, and high stress levels were similarities across urban and rural students, while the roles of community culture, social media, and overdose represented differences. This finding summarizes adolescent perspectives to fill the preexisting gap of a lack of youth voice about mental health.

Limitations & Implications

The four focus groups conducted to gather data for this study were likely able to capture a representative and complete picture of student perceptions of mental health in the individual rural and urban high schools—Greg Guest and colleagues found that 90% of themes were able to be discovered after three to six focus groups (Guest, Namey, & Mckenna, 2017). However, the sample size of one rural and one urban high school limited this study in that the findings are not fully generalizable to urban or rural Kentucky high school students as a whole. While many of the

same themes would likely arise in other urban and rural schools, more focus groups would need to be conducted with students in other communities to generalize findings to all Kentucky high schoolers.

Additionally, the convenience sampling method used to recruit participants may have resulted in a sample of students who were more understanding of or comfortable discussing mental health. This may have biased the data to reflect more awareness of and less stigma or stereotyping around the issue. However, the themes relating to driving factors of mental health challenges and stigma around mental health challenges, which represent the bulk of the data collected, are likely the same across populations with or without interest in mental health.

Though this study had some limitations, the data generated is still valuable in understanding the unique factors that drive mental health symptoms and stigma among Kentucky high schoolers. This represented a foundational study in Kentucky student mental health—no previous research has evaluated adolescents' perceptions of the issue. Understanding these perspectives is critically important; students have a unique understanding of their own mental health concerns and the policies and programs that intend to address their well-being. If effective ways to improve youth mental health are to be developed, students' perspectives must be understood. This research provides a base upon which to further explore youth mental health through additional focus groups and targeted surveys.

Conclusion and Future Directions

To allow for generalization of these themes to all Kentucky students, a further study will involve additional focus groups across more urban and rural schools, geographic locations, and communities in the state. A quantitative survey will be developed based on the results of these additional sessions. Targeting the survey to certain themes that arose from this research and that will arise in further studies will allow the survey to focus on the most important themes. Eventually, a policy or program recommendation to improve youth mental health that applies to urban and rural Kentucky high schoolers will be developed from this work. A recommendation informed

by student perspectives will be the most effective in improving the well-being of Kentucky youth.

The emerging themes identified in these focus groups, supplemented by quantitative data, represent new insights about mental health through the lens of adolescents. While research has previously been conducted regarding adult perspectives on mental health issues, evaluating student perspectives revealed mental health factors that revolve around the high school experience (Story et al., 2016; Bischoff et al., 2014; DeRigne, Porterfield, & Metz, 2009; Farahmand, Grant, Polo, & Duffy, 2011). Investigation and comparison of rural and urban perspectives showed that the influence of family, peers, high stress levels, and lack of resources were similar between both groups, while overdose, social media, and community culture were different.

These results indicate that several factors, particularly social media and overdose, need further investigation. Results from this work will provide a foundation for further research investigating youth perspectives of mental health and an eventual policy or program recommendation to improve adolescent well-being.

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Appendix A

The consent form on the following page is the form, including both parent or guardian consent and youth assent, that all participants returned to the researcher prior to the focus group sessions.

Human Informed Consent Form

Instructions to the Student Researcher(s): An informed consent/assent/permission form should be developed in consultation with the Adult Sponsor, Designated Supervisor or Qualified Scientist. This form is used to provide information to the research participant (or parent/guardian) and to document written informed consent, minor assent, and/or parental permission.

- When written documentation is required, the researcher keeps the original, signed form.
- Students may use this sample form or may copy ALL elements of it into a new document.

If the form is serving to document parental permission, a copy of any survey or questionnaire must be attached.

Student Researcher(s): _____

Title of Project: A Descriptive Study of Adolescent Perceptions of Rural Versus Urban Kentucky High School Student Mental Health Challenges

I am asking for your voluntary participation in my science fair project. Please read the following information about the project. If you would like to participate, please sign in the appropriate area below.

Purpose of the project: **The purpose of my project is to determine the differences in the student-reported perceptions of mental health challenges facing rural versus urban Kentucky high schoolers.**

If you participate, you will be asked to: You will be asked to participate in a focus group during which I will ask questions about mental health at your school, mental health services and resources offered at your school or in your community, and the best ways to address student mental health challenges.

Time required for participation: Focus groups will last between 1-1.5 hours.

Potential Risks of Study: **We will be discussing mental health, which can be a sensitive topic.**

Benefits: **These focus groups will give you the opportunity to participate in valuable research that may permanently change the way mental health is addressed in Kentucky schools. Additionally, refreshments will be provided.**

How confidentiality will be maintained: Participants will be fully anonymous and names will not be collected. Any names mentioned during the focus groups will be redacted or made anonymous in the final paper and presentation.

If you have any questions about this study, feel free to contact:

Adult Sponsor/QS/DS: _____

Phone/email: _____

Voluntary Participation:

Participation in this study is completely voluntary. If you decide not to participate there will not be any negative consequences. Please be aware that if you decide to participate, you may stop participating at any time and you may decide not to answer any specific question.

By signing this form I am attesting that I have read and understand the information above and I freely give my consent/assent to participate or permission for my child to participate.

Adult Informed Consent or Minor Assent

Date Reviewed & Signed: _____

Research Participant Printed Name: _____

Signature: _____

Parental/Guardian Permission (if applicable)

Date Reviewed & Signed: _____

Parent/Guardian Printed Name: _____

Signature: _____

Appendix B

Pilot focus group questions:

What does mental health mean to you?

Do you associate a stigma with “mental health?”
Why?

How do you think the stigma surrounding mental health is influenced by cultural factors?

What do you think contributes the most to development of mental health challenges among high schoolers?

What do you think is the most significant barrier for high school students to accessing mental health treatment?

How does your school address mental health? Is it effective? Why or why not?

Are you aware of any good resources you could use if you thought you were experiencing a mental health challenge? Why do you like those resources?

What would help you better discuss mental health topics with your family or peers?

What do you think is the best way to prevent and treat mental health challenges?

Is there anything else you'd like to tell the group?

Appendix C

Finalized focus group questions:

Tell me your name and your favorite flavor of ice cream. (Icebreaker)

What do you think of when you hear the phrase “mental health”?

When you hear about someone who is experiencing mental health symptoms, what are your first thoughts?

Follow-up question: How do you imagine your friends would respond to this news?

Follow-up question: To what extent do you think there's a stigma attached to people experiencing mental health symptoms? Stigma is defined as an attribute that an actual or inferred attribute that damages the bearer's reputation and degrades him or her to a socially discredited status.

How can you tell when you or your peers are experiencing mental health symptoms?

What do you think are some of the factors that contribute to high school students experiencing mental health symptoms?

What do you or your peers do to deal with mental health symptoms?

Are you aware of any good resources within or outside of school you could use if you thought you were experiencing a mental health challenge?

Follow-up question: What's good about those resources?

What gets in the way of more students using those resources?

I'm going to read a list of factors and ask you about how strongly they contribute to the stigma surrounding mental health. Please hold up fingers from 1-5, with five being the strongest impact on stigma. Factors included community culture, religious culture, heritage, family, peers, and media.

What stands out most to you about how these different factors contribute to the stigma around mental health?

If you were experiencing more mental health symptoms, what would you want people in your life to do for you?

Is there anything else you'd like to tell the group?

Appendix D

This appendix includes a list of codes, grouped by the thematic grouping in ATLAS.ti. The “theme” grouping of codes, at the end of the appendix, indicates codes used to classify quotes into broad categories, which facilitated analysis. Themes for the thematic analysis were then identified by examining relationships among individual codes and code groups.

Project: AP Research

Report created by Allison on 4/20/2018

Code Report - Grouped by: Code Groups

All (79) codes

Groupless

13 Codes:

○ environment

Comment: by Allison

When students discuss the impact of environmental factors on mental health

○ good-quote

Comment: by Allison

Notable or good quotes

○ honesty

Comment: by Allison

Honesty or getting to the point when it comes to interactions about mental health

○ isolation

Comment: by Allison

When students discuss isolation, feeling isolated, etc

○ listen

Comment: by Allison

Listening to someone with mental health concerns

○ medication

Comment: by Allison

Medication in reference to

treating mental health disorders

○ personality

Comment: by Allison

When participants discuss the role of personality in mental health, whether it's negative, positive, or neutral statement of fact

○ spamaccount

Comment: by Allison

For discussion about spam accounts on social media

○ support

Comment: by Allison

Discussing wanting mental health support/discussing support in general

○ treatnormally

Comment: by Allison

Discussing wanting to be treated normally

○ trust

Comment: by Allison

Theme of being able to trust or connect with or feel close with someone or a group

○ understand

Comment: by Allison

When students discuss wanting to be understood or the role of someone else understanding what the person is going through

○ unexpected

Comment: by Allison

When participants discuss how mental health symptoms can be unexpected

Negative influence

36 Codes:

○ n-awareness

Comment: by Allison

When participants discuss lack of awareness/how lack of awareness is bad

○ n-balance

Comment: by Allison

Negative effects or influence of

having to balance so many things at once

○ n-beingdifferent

Comment: by Allison

Not wanting to stand out or be different

○ n-burden

Comment: by Allison

Negative influence of feeling like a burden

○ n-community

Comment: by Allison

Negative influence of community and community culture

○ n-coping

Comment: by Allison

When participants discuss negative coping mechanisms.

○ n-counsellor

Comment: by Allison

Negative or unhelpful school counsellors

○ n-cyberbullying

Comment: by Allison

For discussion about cyberbullying

○ n-denial

Comment: by Allison

When participants discuss denial of symptoms

○ n-drugs

Comment: by Allison

When participants discuss drugs and negative influences on mental health

○ n-expectations

Comment: by Allison

Negative influence of expectations, from parents, self, or anyone

○ n-failure

Comment: by Allison

Negative influence of fear of failure or of making mistakes

○ n-family

Comment: by Allison

Negative influence of family on mental health

○ n-future

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Comment: by Allison
Not knowing about the future is stressful

n-gender

Comment: by Allison
Negative gender biases, stereotypes, influence

n-generationalgap

Comment: by Allison
Discussing a generational gap/discussing the differences in the “times” when students’ relatives were young, etc

n-heritage

Comment: by Allison
Negative influence of heritage, culture of ethnic background

n-hidesymptoms

Comment: by Allison
When participants discuss hiding mental health symptoms

n-identity

Comment: by Allison
Identify conflict or negative influence of identity

n-internalthought

Comment: by Allison
Negative internal thoughts/cycles of spiraling, etc

n-job

Comment: by Allison
Negative effects of having a job or balancing work with other things

n-joking

Comment: by Allison
This code is for when people discuss joking about mental health or not taking mental health seriously in a negative way.

n-judgement

Comment: by Allison
Negative influence of judgement

n-media

Comment: by Allison
Negative influence of media on mental health

n-money

Comment: by Allison
Negative influence of money or worrying about finances on mental health

n-noresources

Comment: by Allison
Not knowing about resources or not knowing if/what resources are available

n-overdose

Comment: by Allison
Negative influence of peer or family overdose

n-peers

Comment: by Allison
Negative peer influences

n-school

Comment: by Allison
Negative impact of school/standardized testing, often in terms of causing mental health symptoms

n-selfpressure

Comment: by Allison
High self-expectations or self pressure

n-sleep

Comment: by Allison
Not getting enough sleep

n-solvealone

Comment: by Allison
When people discuss trying to resolve mental health symptoms on their own

n-sports

Comment: by Allison
Negative influence of sports on mental health

n-stereotype

Comment: by Allison
When participants seem to have a negative stereotype around mental health or mention a negative stereotype

n-stress

Comment: by Allison
Negative influence of stress

n-treateddifferently

Comment: by Allison

When people discuss being treated differently/treating others differently

Positive influence

11 Codes:

p-club

Comment: by Allison
Positive influence of a club

p-community

Comment: by Allison
When students describe a close community/positive influences of community

p-coping

Comment: by Allison
Positive coping mechanisms

p-exercise

Comment: by Allison
Exercising and positive influences on mental health

p-family

Comment: by Allison
Positive influence of family on mental health

p-music

Comment: by Allison
Positive effect of music on mental health

p-outlet

Comment: by Allison
Positive coping through outlets

p-peers

Comment: by Allison
Positive influence of peers

p-religion

Comment: by Allison
Positive influence of religion or church

p-school

Comment: by Allison
Positive influence of school, including teachers, on mental health

p-sleep

Comment: by Allison
Positive effects of sleep on men-

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tal health

Speakers-Rural

4 Codes:

B-Rural

Comment: by Allison

This code is for when boys are speaking during the rural focus groups.

Group-Rural

Comment: by Allison

This code is for when the group is speaking during rural focus groups.

G-Rural

Comment: by Allison

This code is for when girls are speaking during rural focus groups.

Moderator-AT

Comment: by Allison

This code is for the moderator speaking in both the rural and urban focus groups.

Speakers-Urban

4 Codes:

B-Urban

Comment: by Allison

This code is for when urban boys are speaking during focus groups.

Group-Urban

Comment: by Allison

This code is for when the group is speaking during urban focus groups.

G-Urban

Comment: by Allison

This code is for when urban girls are speaking.

Moderator-AT

Comment: by Allison

This code is for the moderator speaking in both the rural and urban focus groups.

Symptoms

7 Codes:

s-anxiety

Comment: by Allison

When anxiety is discussed

s-actdifferently

Comment: by Allison

Symptom of acting differently, commonly seen in question 4

s-apathy

Comment: by Allison

When students discuss the symptom of apathy

s-eating

Comment: by Allison

Symptom of changing eating habits

s-overwhelmed

Comment: by Allison

Feeling overwhelmed

s-sleep

Comment: by Allison

Changing sleep habits as a symptom

s-smallchange

Comment: by Allison

When students discuss small changes in behavior

Themes

5 Codes:

t-coping

Comment: by Allison

Theme of coping mechanisms

t-interaction

Comment: by Allison

When participants are discussing an interaction between them and someone else about mental health, negative, positive, or neutral

t-resources

Comment: by Allison

Anything relating to mental health resources, education, treatment, or other

t-stigma

Comment: by Allison

Any time participants are referring to stigma, negative, positive, or neutral

t-symptoms

Comment: by Allison

Anything relating to mental health symptoms, perpetuating them or causing them

LED Colour Temperature and its Effect on the Growth of Hydroponic Lettuce Seedlings

Justin Shaw

Previous research has shown that differences in light quality can have profound effects on the growth of hydroponic lettuce. This experiment attempts to determine the correlation between the colour temperature of LED grow lights and the growth of hydroponic lettuce. Initially, this study exposed a total of 252 Butterhead lettuce seedlings to various temperatures of white LED lights to determine if there was any correlation between the warmth of light emitted and the growth of lettuce seedlings. Growth was measured in four variables: height, number of leaves, wet mass, and dry mass. When harvested at 28 days, the results suggest that there is a statistically significant difference between plants grown under 3,000K and 6,000K conditions, with results showing as much as a 232% increase in growth for plants grown under 6,000K lights.

Keywords: Colour Temperature, LED, Hydroponics, Butterhead Lettuce

Introduction

By the year 2050, the United Nations projects that the global population will exceed 9.7 billion people, the majority of whom will be moving into cities, taking up a more Western diet (United Nations, 2017). Current agricultural outputs would thus be insufficient to feed the world without an estimated 60% increase in global food production by 2050 (Alexandratos and Bruinsma, 2012; Despommier 2009; Godfrey et al., 2010; Ray et al., 2013). To put this in perspective, meeting those projections would require growing more food in the next ten years than has ever been grown in the course of human history (Bourne, 2015). Historically, there are two main ways to increase the total agricultural output (Thornton, 2012; Tilman et al., 2001):

- develop more new land, or
- increase the efficiency of current agricultural practices.

To meet these goals by exclusively developing more new land would require a land mass the size of Brazil (Despommier, 2012). According to a NASA-funded Stanford researcher, the vast majority of new agricultural land developed in the past decade came from the unsustainable deforestation of rainforests in developing countries (Gibbs et al., 2010). Even without the harmful environmental side-effects of deforestation, current agricultural development is nowhere close to the scale that would be necessary to support a 60% increase in global food production. Therefore, the first method of increasing agricultural output by developing more farmland is unfeasible. Unfortunately, various environmental factors (such as global warming and water scarcity) have restricted the efficiency of agricultural production and already pose harmful environmental side effects, making the second method unlikely to be effective (Alexandratos and Bruinsma, 2012; Godfrey et al., 2010; Ray et al., 2013). For instance, according to a paper published in the journal

Science, it is projected that over the next decade pesticide usage will increase threefold, which would push many coastal marine zones past the point of no return (Tilman et al., 2001). This is because agricultural pesticide runoff creates an abundance of nitrogen in rivers and streams, feeding algae which consume all of the dissolved oxygen in an area. These areas are called a dead-zones because there is no dissolved oxygen in the water, making it ecologically 'dead' or incapable of supporting complex life. The use of chemical pesticides along the Mississippi River has created a large dead-zone in the Gulf of Mexico the size of a small state (Diaz and Rosenberg, 2008; Tilman et al., 2001). Given the increasing frequency of droughts, water usage is also a large concern given that agriculture accounts for about 70% of all freshwater usage in the United States (Barbosa et al., 2015; Winter et al. 2017). For these reasons, increasing the efficiency of current agricultural practices is becoming more and more difficult. Some argue that these kinds of Malthusian doomsday predictions have always been predicted and have never come true; however, the methods that were effective for farmers yesterday are ineffective today (Godfrey et al., 2010; Thornton, 2012; Tilman et al., 2001). To feed the growing global population without sacrificing the health of our planet, a radically new way of growing food is necessary.

Literature Review

Hydroponics

The use of hydroponic farming has the potential to radically change the way food is grown. Hydroponic technology was first deployed in World War II to provide the US Armed Forces with fresh fruit and vegetables in the desolate, and often barren islands of the Pacific. The technology sustained American troops in their victorious island-hopping campaign across the Pacific as it could grow large quantities of food without taking up a large footprint on each island (Jones Jr., 1982).

Hydroponic farming is an efficient way of producing enough food to meet the growing needs of growing global populations. Hydroponic systems do not need soil, can use up to 11 times less water for the

same crop, and can be grown year-round (Barbosa et al., 2015; Winter et al., 2017). Most soil-based growers harvest their crops at most three times per year, but hydroponic plant factories can harvest up to 22 times per year in a fraction of the space (Despommier, 2013; Fischetti, 2008). In a hydroponics system, plants are cultivated in a solution that contains all the nutrients needed for plant growth instead of soil. This means they can be grown indoors which effectively eliminates the need for chemical pesticides, herbicides, or fungicides that are used in traditional outdoor agriculture. Additionally, this allows the farmer to stack multiple tiers of plants, which increases the density of plants that can be grown in a given area. There are many different types of hydroponic systems, each being specialized to a specific set of objectives and constraints. Regardless of the system used, the most common way to germinate seeds is with an expanded rock fiber called rockwool. This report will focus on the colour temperature of LED lights and how they affect the germination of seeds grown in rockwool. Because the nursery conditions are generally the same for each of the different types of systems described below, the results of this paper can easily be applied to all systems described below (Both, 1998).

Nutrient Film Technique. The Nutrient Film Technique (NFT) cycles nutrients past exposed roots via a pump (Figure 1). NFT systems are generally the least expensive hydroponic systems to purchase and operate because when the water is running continuously, it oxygenates itself. In other systems, where the water is not cycled, the use of an air pump must be implemented to oxygenate the solution. Lack of proper oxygenation can lead to negative crop yields (Both, 1998). The NFT system is also ideal for urban areas that have footprint constraints. The end of one tube can be connected to the beginning of another and the whole system can be stacked several layers high, creating a dense agricultural environment that decreases the farm's footprint and saves money (Both, 1998; de-Anda and Shear, 2017).

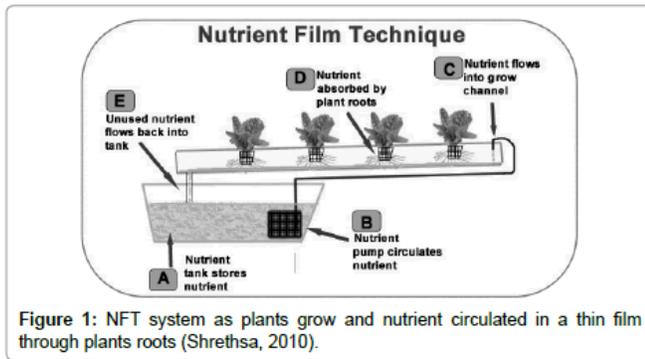


Figure 1: NFT systems cycle nutrients past exposed roots via a pump (Mchunu et al., 2017).

Deep Water Culture. Another type of system, called Deep Water Culture (DWC), uses a nutrient solution in which roots are submerged. As the water level decreases, due to plant metabolism and evaporation, more of the roots are exposed to the air which increases oxygenation to the roots as they mature. However, the use of this system still requires an oxygenator for best results. This system is best for areas with ample amounts of natural sunlight and open space as it would be difficult to scale up this setup in an urban environment without the ability to stack multiple layers (Both, 1998; deAnda and Shear, 2017).

Floating Raft Culture. A variation on the DWC method, Floating Raft Culture (FRC) uses a floating raft which holds the plants above the reservoir (Figure 2). This method is very common in commercial hydroponic systems where space and light are not issues.

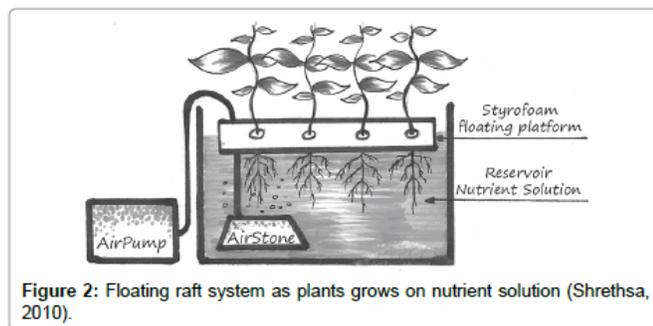


Figure 2: FRC uses a raft which holds the plants above the reservoir (Mchunu et al., 2017).

Aquaponics. Unlike the other methods, an Aquaponics system is not about the structural design of the farm; rather it is about the nutrients that are delivered to the plant. In aquaponics, plant nutrients are pro-

vided by fish (Figure 3). The result is an artificial micro-ecosystem: the fish produce organic compounds that are vital to plant growth and the plants absorb organic compounds which also cleans the water for the fish. In all of these systems, it is not uncommon to see the nutrient solution be substituted for an aquaponics system.

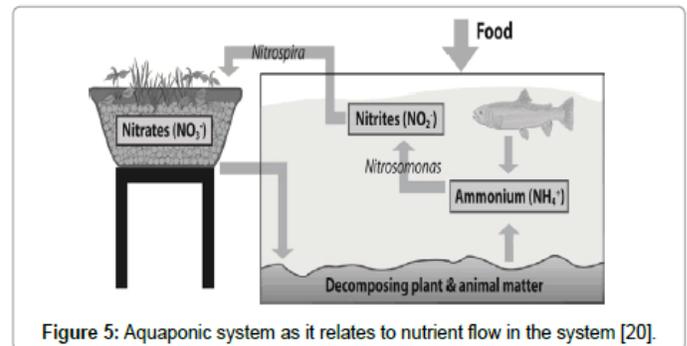


Figure 3: In Aquaponics, plant nutrients are provided by fish (FAO, 2014).

Aeroponics. In addition to hydroponics, many other systems have been developed as an alternative to traditional agriculture. One example is aeroponics where nutrients are taken from a reservoir and sprayed out of a fine nozzle at exposed roots. Aeroponics systems are great for high density urban areas because they can produce a lot of fresh fruit and vegetables in a small space. This misting method can be stacked on top of itself, which increases food production density similar to the NFT method. In many plant factories, this method is common because everything can be automated. Additionally, the quality of crops coming out of these high-tech plant factories has the potential to be greater than traditional agriculture because farmers can customize each aspect of the internal environment (like CO_2 content or humidity) to specific tastes or preferences.

Research Objective

In response to the growing dangers of industrial agriculture, hydroponics offers a viable alternative that is capable of meeting targets for future demands at a fraction of the cost (Barbosa et al., 2015; deAnda and Shear, 2017; Winter et al., 2017). However, the

efficiency of current hydroponic technology is not able to meet those targets. Therefore, the objective of this experiment is to examine the effects of colour temperature on the growth of Butterhead lettuce seedlings in a simulated hydroponic environment.

Photobiology

It is generally accepted that plants grow best when the majority of emitted light is in the red and blue (RB) spectrum (Folta and Maruhnich, 2007; Lee et al., 2014; Su et al., 2014; Wang et al., 2015). Small quantities of other wavelengths have also been proven beneficial for plant metabolism (Smith et al., 2017). This is because the photoreceptors (a cell protein that absorbs light) absorb red and blue photons (particles of light) more efficiently than other wavelengths. Competing studies have also contested whether or not the use of green light is effective for plant growth (Folta and Maruhnich, 2007; Kim et al., 2004; Smith et al., 2017). For instance, new research has shown that green light may also play a role in the creation of specific plant proteins that aid in growth (Kim et al., 2004; Smith et al., 2017). Other research shows that this light might not correlate into measurable plant development (Su et al., 2014). In general, using red and blue Light Emitting Diodes (LEDs) has been shown to be more efficient than only using white (Lee et al., 2014). Additional studies show that the use of far-red (the region between red and infra-red) lighting can enhance plant flowering (Deitzer et al., 1979).

This experiment used three different colour temperatures of light (3,000K through 6,000K) that vary in the proportion of red light to blue light. Each of the lights is technically 'white' so it contains all wavelengths of colours, but in general the higher the colour temperature the bluer the light. Therefore, the 3,000K light has the highest proportion of red photons and the 6,000K light has the highest proportion of blue photons.

In addition to the colour of light, the type of bulb is also important in horticultural research. Recent studies with new LEDs have shown that LEDs can be more energy efficient than the industry-standard Philips T5 High Output Compact Fluorescent Light (Reineke et al., 2009). Organic LEDs (OLEDs) are even more ef-

ficient but have not been tested on plant growth.

Plants perceive light differently than humans. While the range of light plants can absorb is roughly the same as the visible spectrum in humans, the efficiency (the ratio of the quantity absorbed by the cell to the quantity of light that hits the cell) at which they absorb each wavelength is vastly different. The spectrum of light available for plants is called photosynthetically active radiation (PAR). This describes the range of wavelengths of light from about 400nm to 700nm. There are two common ways of measuring the brightness of light in the PAR spectrum, either by 'weighing' all photons in the spectrum equally, called photosynthetic photon flux (PPF), or by giving a weight to certain photons based on the plant's ability to absorb that wavelength, called yield photon flux (YPF). Generally speaking, red photons are easier to absorb, due to their lower energy, thus resulting in about 20% to 30% more photosynthetic activity than a blue photon (McCree, 1971). Because you have to know the specific frequency of the light source before you can weight the wavelengths, YPF is harder to measure than PPF.

Another difference between plant and human light perception is how each perceives brightness. The human retina uses a logarithmic scale when sensing light. This means going from one to two light sources would have the same effect as from 50 to 100 light sources. However, plants are linear in their perception of light; the difference between one light and two is equivalent to the difference between 50 and 51. This means that controlling the brightness level in lights is crucial. To measure brightness, Biologists describe the density of photons hitting a given surface area every second, photosynthetic photon flux density (PPFD). PPFD will be discussed more in relation to Photoperiodism (section 3.3).

According to Dr. Toyoki Kozai, Professor Emeritus and Chief Director of the Japan Plant Factory Association Center for Environment, Health, and Field Sciences at Chiba University, due to an insufficient research base on the topic, "there should be more room for improving the [lighting use efficiency] of [closed plant production systems]" (Kozai, 2013). While there is a surplus of knowledge about the differences in efficiencies between growing methods and solution contents, and wavelength of various lighting systems, more research needs to be done on the colour temper-

ature of indoor hydroponic lighting systems (Kozai, 2013; Reineke et al., 2009). “Lighting Use Efficiency” is one of the most vital, yet underdeveloped aspects of plant factories. This paper aims to fill that gap by evaluating the varying ‘efficiencies’ of different colour temperatures of LED lights.

Butterhead Lettuce

Butterhead lettuce is the most widely used crop in research-based hydroponics as it grows relatively quickly (Barbosa et al., 2015; Brechner and Both, 2013; Jones, 2005; Ryder, 1999; Tyson et al., 2013). Lettuce grows best in cooler climates and is well documented in research-related studies of hydroponics (Dufault et al., 2009; Fischetti, 2008). Butterhead lettuce can be sold at a local farmer’s market for a profit to help subsidize the initial costs of building a small-scale “Closed Plant Production System” (CPPS), although these systems are generally inexpensive (Becraft, 2017; Brechner and Both, 2013; deAnda and Shear, 2017; Jones, 2005).

Methods

In order to determine if various colour temperatures of LED lights will have any effect on the growth of lettuce, this experiment developed a quantitative experimental approach that closely modelled common commercial hydroponic germination techniques to measure the growth of lettuce seedlings in a hydroponic simulation. This study germinated a total of 252 Butterhead lettuce seeds in hyper-absorbent expanded rock-fiber cubes (Rockwool) in 3 different chambers each with a different colour temperature of light. Rockwool is the most common growth medium for both commercial and laboratory-oriented growers as it closely models traditional soil-based growing while absorbing a large volume of water for its size. Two seeds per rockwool cube were planted in each of the three chambers, grown over the course of two growing periods. A scaled down version of a Closed Plant Production System (CPPS), and utilized a standard 11- by 22-inch gardening tray and humidity dome, all enclosed by identical rigid white containers. The humidity dome is a standard-fit transparent dome which serves two functions:

- it increases the humidity inside the enclosure which aids the growth of lettuce seedlings,
- it protects the LED lights from water damage.

The interior of each chamber was painted white to reflect as much light as possible, which increases the efficiency of the LEDs dramatically. In each of the two growing periods, 21 rockwool cubes (42 seeds) were soaked in a nutrient-rich bath for 2 hours and then placed in each of the three chambers. Rockwool cubes can hold water for a matter of weeks before drying out, but nutrient levels may vary over that time. Excessive watering can lead to nutrient buildup and can harm vital plant functions, so nutrients were replenished by spraying each chamber with a nutrient solution every three days. After the standard growth period of 28 days (after planting), various measurements were taken (described in section 3.6).

Crop Selection

This paper uses Butterhead lettuce as a test crop as it grows relatively fast, is compact, and is supported by a large base of past research. The large base of literature surrounding Butterhead lettuce means this paper has direct and pragmatic implications for both commercial hydroponic growers, and academic researchers alike as they incubate their own lettuce seedlings and are questioning the relationship between colour temperature and plant growth as a means of improving the efficiency of plant production.

Nutrients

In hydroponic conditions, all of the nutrients needed for plant growth are dissolved into a solution to which the roots are exposed. This type of setup is advantageous because the nutrients available to the plant can be more accurately controlled. In general, plants need two categories of nutrients: micronutrients and macronutrients. This paper utilizes a discipline-standard mix of Masterblend™ 4-18-38, Calcium Nitrate, and Magnesium Sulfate. The numbers 4-18-38 in the fertilizer naming system stand for the relative abundance of Nitrogen, Phosphorous, and Potassium (NPK). For example, this experiment used a formula with 4% Nitrogen, 18% Phosphorous, and 38% Potassium by volume. The combination of these ingredients sufficiently satisfies both the macro- and micro-

nutrient requirements of the plants. The specific ratio between these chemicals is very important as a lack of nutrients may lead to visual deficiencies. However, a more in-depth justification for each of the nutrients is beyond the scope of this research paper.

Photoperiodism

Past research has shown that plants need to spend specific amounts of time in both light and darkness. The photoperiod of this experiment is tuned precisely with that of developing lettuce seedlings; 18 hours of light and 6 hours of dark. The use of a digital 24-hour timer ensures that all of the trials get the same amount of light. Although this model attempts to equalize the amount of light that hits each plant's leaves, differences in the PPF output of the lights themselves may also limit the consistency of the light absorption of the plants. This is to say that some lights may be fractionally 'brighter' than others which may influence data. This can not be corrected for as the difference in PPF between colour temperatures is an intrinsic property of each light, and should be considered as such when evaluating the data processes.

LED Strips

Due to both cost and space constraints, the use of a high density and relatively low-cost lighting was essential. For this reason, LED light strips were the best choice because they output a lot of light relative to their size, were easily attachable to the interior of each box, and could be wired together and supplied by a standard computer power supply unit (PSU) which was repurposed for this experiment.

Colour Temperature

The manufacturer of the LED light strips does not provide strips in the 4,000 Kelvin (K) range. This is most likely because the 4,000 K phosphor is too expensive to be profitable for the company. While this limitation is unfortunate, having three sample points at 3000K, 5000K, and 6000K was sufficient to demonstrate a correlation between colour temperature and the various methods of measuring growth (described in section 3.6, below).

Dimensions of 'Growth'

In general, the metrics for measuring plant growth vary widely based on the objective of the research. However, it is typical to include at least three different quantitative measures so as to get a wide breadth of data about each plant in order to tell a complete story. While no individual measurement fully explains the status of the plant, a combination of these three perspectives can give a more detailed picture of how each plant grew under the various conditions. The following three measurements were the most common way of measuring plant growth in academic biological research.

Height. Height (the distance from the base of the plant at the surface of the rockwool to the tip of the longest leaf) can have various implications in biological terms. In general, a large height value can imply that the plant grew fully and prospered in the given conditions. From an evolutionary standpoint, a taller plant is favoured over a small plant as tall plants have easier access to sunlight than do plants of smaller stature. However, that is not always the case. 'Bolting' is a term used to describe a plant that does not have adequate light and thus grows thin and tall in an attempt to reach light. This is a potential drawback of this method of measurement however, in combination with other measures, the height of a plant is an integral aspect of its overall growth.

Number of Leaves. The number of leaves can have multiple implications with respect to plant growth. It is widely agreed that the more leaves a plant has, the healthier its growth is. Growing a new leaf requires a lot of energy that the plant must spend and thus is a good indicator of its overall stage of growth. However, the number of leaves does not describe the health or size of those leaves, so while a plant may have a large number of leaves, they might all be small, discoloured, or misshapen.

Biomass. This paper utilizes two ways of finding the mass of each plant: wet mass and dry mass. Whereas the wet mass is the mass of the plant as grown in the chamber (including the water inside the plant), the dry mass is the mass of the plant devoid of water (by evaporating water out of the plant). This paper used a standard convection oven set to 100 degrees Fahrenheit to dry the plants over a period of 4 hours, after which the plants were cooled down and their mass

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was taken. For both the wet and dry mass, only the average mass was recorded because the scale was inaccurate at lower masses. The mass of all plants was taken simultaneously and the data (Table 3 and 4) represent the average. The dry mass shows the actual mass of organic matter that was grown in the process of creating the plant. This is important because it serves to show how much 'growth' the plant ended up doing via the net change in organic matter that was created by the plant. Additionally, wet mass is an important statistic as it shows the absorption of water (and therefore nutrients) by a plant. If a plant's wet mass is not significantly greater than its dry mass, the plant failed to absorb water from the rockwool which would lead to negative growth yields. In hydroponics, where all the nutrients are dissolved in a solution, different wet mass values may indicate different absorption rates as a result of the colour temperature. Therefore, analysis of both the wet and dry masses will provide a more complete picture of the true growth of the plant, especially when such plant is grown in hydroponic conditions.

Results

Height (cm)	3000K	5000K	6000K
Average	9.4	13.1	13.2
Standard Deviation	4.2	4.2	4.7

Table I. Height of seedlings after 28 days of growth in a controlled-environment chamber under various colour temperatures of LED lights.

Note: This table shows the height (in centimetres) of each seedling grown under three different colour temperatures of LED strip lights; 3000K, 5000K, and 6000K. Notably, there is nearly no difference between the heights of 5000K and 6000K plants. Both the 5000K and 6000K chambers had plants that grew very tall and sturdy (13.1 and 13.2cm, respectively), compared to 9.4cm for 3000K. Overall, the data is strongly correlated with a linear fit as the Pearson correlation coefficient ($R^2 = 0.901$).

# of leaves	3,000 K	5,000 K	6,000 K
Average	8.7	10.8	12.2
Standard Deviation	2.3	2.8	4.0

Table II. Number of leaves after 28 days of growth in a controlled-environment chamber under various colour temperatures of LED lights.

Note: The data in this table shows the average number of leaves on each plant under each of the three different colour temperatures of LED lights (3,000, 5000K, and 6,000 Kelvin). In general the average number of leaves increases as the colour temperature increases. There is a notable gap between the 3000K and 5000K plant average (8.7 versus 10.8 leaves). This difference is less notable between 5000K and 6000K (10.8 versus 12.2 leaves). This relationship is almost perfectly linear with a remarkably high Pearson correlation coefficient ($R^2 = 0.996$).

Average Mass (g)	3,000 K	5,000 K	6,000 K
Wet Mass	3.1	5.7	18.9
Dry Mass	3.0	4.9	12.3

Table III. Wet and dry masses of seedlings after 28 days of growth in a controlled-environment chamber under various colour temperatures of LED lights.

Note: This table describes the wet mass and dry mass of each plant 28 DAP. As shown above, both the average wet mass and dry mass of the plants increases with increasing colour temperature. There is a notable lack of change between the wet and dry masses of the 3000K chamber (3.1g wet versus 3.0g dry) because the plants grown in this chamber had trouble absorbing water. The rockwool was constantly dry but for the sake of reliability no extra water was given. The correlation was moderately strong (Wet Mass $R^2 = 0.718$, Dry Mass $R^2 = 0.754$).

Discussion

Germination Rates

Throughout the course of this paper, the germination rates between different colour temperatures varied substantially. For example, of the seedlings initially fertilized in the 3000K chamber (two seeds per Rockwool cube), less than 30% germinated. Contrastingly, a larger percentage of the seeds in the 6000K chamber germinated (67% germination rate). This has two main implications. First, the sample size representing 3,000 K plants is smaller than the other two which may vary the validity of the data slightly. Second, the difference in germination rates may be correlated with the difference in colour temperature. However, further research must be done in this area in order to determine the underlying factor causing this significant discrepancy of germination rates between colour temperatures as the results from this experiment are insufficient to describe this relationship with any certainty.

Experimental Constraints

While there was enough time to replicate the initial trial, some accuracy was lost due to the time and space constraints of this experiment. A lack of room inside the chamber meant there was a smaller-than-optimal carrying capacity for each enclosure. The larger plants dominated the enclosures and blocked light from reaching the smaller plants. This was nearly made up for by repeated trials; however, further research may find it best to use larger chambers for conducting this type of research to prevent crowding. The culmination of these issues was that each trial was stopped short of a full growing period (40-50 DAP) to allow room for the next trial, which slightly decreased the validity of the data. This decrease in growth duration ended up being inconsequential as after 28 days of growing there were significant and measurable differences between groups. Future research should look to optimize the length of the growth period of each trial with oversized enclosures.

Conclusion

Results

The results of this paper have profound implications as to the viability of hydroponics to act as an alternative to traditional agricultural practices. Traditional hydroponic intuition would point toward the low colour temperature light as having the best output as they have the greatest red-to-blue light ratio. However, the results of this paper point in the opposite direction, that the cooler light (highest colour temperature) was best for plant growth.

This experiment found that colour temperature was strongly correlated (R^2 of 0.901) with the height of a plant grown in hydroponic conditions (ranging from an average of 9.4cm to 13.2cm in height). This trend was consistent across all temperatures of light and was justified by multiple trials of over 250 individual seeds.

Additionally, the number of leaves on each plant was very strongly correlated (R^2 of 0.996) to an increase in colour temperature (averaging 8.7 to 12.2 leaves). Although the standard deviation increased dramatically for the 6000K enclosure, this can be attributed solely to the lack of space in the enclosure. The large plants completely dominated the 6000K enclosure which meant that the smaller plants were too small to thrive.

Perhaps most impressive, the average wet mass of the plants grown under 6000K LEDs was 232% higher than that of the 5000K plants and the dry mass saw similar improvements. While there is only a moderate linear correlation between a plant's biomass and colour temperature, it is possible that a quadratic or even exponential fit may be a more appropriate fit for the data.

This research orients itself in opposition to traditional hydroponic intuition, creating a new understanding of the relationship between the colour of light and horticultural growth. Our results suggest that higher colour temperatures are conducive to greater growth in plants.

Implications

The objective of this paper was to find and optimize the best growing conditions for Butterhead lettuce using only differences in the colour temperature of LED lights. Against conventional intuition, the results of this paper suggest that a higher colour temperature (cooler light) is strongly correlated with both the number of leaves (R^2 was 0.996) and height (R^2 was 0.901) and moderately correlated with the wet (R^2 was 0.718) and dry masses (R^2 was 0.754). This has tremendous implications for hydroponic researchers and commercial growers alike as the results of this paper can be directly translated into optimizing the lighting conditions for commercial hydroponic farms, especially in states like California where Butterhead lettuce is commonly grown hydroponically. To that end, hydroponic growers of all backgrounds, whether growing in a high-tech lab or in an abandoned warehouse in the inner city, should look to use higher colour temperature lights to grow their lettuce.

Looking Forward

While past research has a primary focus on the biochemical properties of hydroponics, this paper examines the difference that the colour temperature of LED lights can have on the overall growth of hydroponically-grown Butterhead lettuce. Even among photo-biological research, there is a strong need for more research on the biological application of new LED technology including colour temperature, colour-programmable, and even organic-LED (OLED) lighting technology.

Acknowledgements

I would like to thank my mentor, Dr. Toyoki Kozai for his expert guidance in this field and for giving me words of encouragement throughout this journey. I would also like to thank the Gig Harbor High School English and Science departments for helping guide my research process, specifically Mrs. Hupper and Mrs. Marten for their constant support and endless generosity.

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Contributors

Hector Cure was born in Bogota, Colombia on September 11 of 1999. He is currently a Senior at Colegio Nueva Granada. For the past two years, he has participated in two research studies in the medical field, such as PET Scans for pancreatic cancer detection, the experience of the Clínica General del Norte with GIST. This year he was able to conduct his own study that united both psychology and medicine by trying to find if cognitive behavioral therapy was effective for treating depression in stage II pancreatic cancer patients. Hector is going next fall to the University of Pennsylvania to study molecular and cellular biology and psychology on the pre-med track. Additionally, at school, he is the president of the tutoring committee of the National Honor Society, and he gives tutoring sessions to children from impoverished communities as well as his classmates. Aside from his academics, he is a black belt in karate and mentors lower belts and occasionally swim. Finally, Hector loves to travel around the world and he would like to travel to several Asian countries, including China, Korea, and Thailand. Last summer, Hector traveled to Africa to do social service in Tanzania with an organization called Projects Abroad, and two summers ago he visited Tokyo and Dubai. He would love to continue learning about new cultures and traveling to amazing places with a strong cultural heritage.

Rachael Dickenson is a 12th grader whose lifelong interest in plants and guidance from stellar mentors led her to research involving invasive species. She looks forward to doing more research in the future and hopes to become an environmental engineer.

Steven Du is a grade 12 student who attends St. Andrew's College. He will be studying software engineering next year but takes a keen interest into the social sciences.

Lida Ehteshami is a junior at DeBakey High School in Houston, Texas. She is interested in international relations, economics, and issues involving global politics. In the future, she hopes to become an international lawyer.

Isaac Gallogly conducted this research while a senior at Hampton High School, in the suburbs of Pittsburgh, Pennsylvania. He plans to attend Vassar College in the fall of 2018.

William Howard-Waddingham is a high school student in his senior year. He will be attending Yale University for Political Science as a member of the Class of 2022. He is passionate for social justice reform, prompting the topic of inquiry.

Megan Leinenbach is a junior at Lake Howell High School. Within the last year, she has conducted microbiological research about the antibacterial efficacy of essential oils. In her school, Megan is involved in student government, national honors society, science honors society, math honors society, the academic team, and softball.

Ella Moxley is a senior at Norman North High School in Norman, Oklahoma. She is involved in Speech and Debate and National Honors Society. Next year she will attend Oberlin College and study environmental policy. She would like her teacher, Janice Mullan, and her research consultant, Ann Beutel.

Andrew Pyper currently attends Royal St. George's College, and after his graduation, he will be attending The University of Chicago to pursue his passion for economics.

Noor Said is currently a senior high school student enrolled in AP Research. In her free time, Noor enjoys reading, painting, and traveling with her family.

Justin Shaw is a student at Gig Harbor High School located in Gig Harbor, Washington. He is passionate about finding new ways to use technology to solve practical problems in the world. He is also an armature kite-flier, programmer and soccer player.

Allison Tu is a student at duPont Manual High School in Louisville, Kentucky. She is passionate about student advocacy and is a member of the Prichard Committee Student Voice Team and an ambassador to the separate organization Student Voice. She is dedicated to elevating the principles of student-driven movements to her own work, and founded and serves

as Executive Director of STEM Y, a nonprofit organization aiming to break gender, racial, and socioeconomic barriers through student-driven STEM education. She is also passionate about adolescent mental health, and founded a student alliance to improve the wellbeing of Kentucky students.

Sophia Xu is a junior student at Michael E. DeBakey High School for Health Professions. She is particularly interested in the field of medical science and is currently working towards becoming a non-invasive cardiologist. She is currently the District J Representative for the Houston Mayor's Youth Council and co-founded Future Business Leaders of America (FBLA). She is also working in MD Anderson to further her research. In her free time, she teaches piano to students, after acquiring her teaching certificate by taking theory, basic harmony, intermediate harmony, level 10, and music history. In piano, she has won 1st division, 3rd place at Blinn's Piano Competition and 1st place in the Independent Music Association competition. In other awards, her team won 3rd place in Community Awareness for raising Parkinson's Disease and 1st place in Medical Innovations in Health Occupations Students of America (HOSA).

Faculty and Advisors to Contributors

Ernesto Carriazo teaches at Colegio Nueva Granada in Columbia.

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Hongyan Li is a teacher at the DeBakey High School for Health Professions at Houston, TX. She teaches AP Research, Biology, and Forensic Science.

Her primary goal is to help young people to achieve their dreams. She received her M.D. from Hebei Medical University in China in 1997, and her Ph.D. in Neuroscience from University of Arkansas in 2007. Then for eight years, she has focused on researching the molecular mechanisms in visual transduction in University of Texas Health Science Center at Houston. During those years working as a physician and a scientist, she also enjoyed training students and helping them with their career goals. Therefore, in 2015 she became a High School Science teacher. Personally, she enjoys sewing, biking, and playing badminton.

Stacey Marten is in her fourth year as the Librarian and Tech Lead at Gig Harbor High School. Teaming up with the AP Research teacher, Jessica Hupper, was the perfect partnership for a librarian that emphasizes strong research skills for students. Before obtaining her certification in Library/Media studies she taught social studies and history classes for 14 years at Sedgwick Junior High in South Kitsap, Washington. She is National Board certified in Social Studies & History/Adolescent & Young Adulthood.

Janice Mullan, a teacher at Norman North High School in Norman, Oklahoma, leads the Norman North High School's AP Capstone program and also teaches English 4. She has taught for 20 years. She was and continues to be deeply involved in the development and implementation of the AP Capstone program within the school district. She earned a Bachelor's degree in Journalism (Professional Writing) from the University of Oklahoma. She served 12 years in the Air Force and spent three of those years teaching journalism to fledgling Armed Forces journalists. After her time in the service, she became a student again and earned her Masters in Education (English) from the University of Central Oklahoma, and began teaching English including AP English Literature and AP English Language. Four years ago, her principal approached her to undertake a new curriculum, AP Capstone, which has proven to be a truly invigorating program that develops invaluable confidence in the students as they hone their academic writing and presentation skills. Her students each year have been a delight as they enthusiastically pursue a wide range of research interests and topics using inquiry-based and cooperative learning strategies, renewing her own

passion for not only teaching and aiding students, but also the joys of academic research.

Brian O'Connor teaches at Colegio Nueva Granada in Columbia.

Shannon Roos is an English and AP Capstone teacher and an instructional coach at Hampton High School in Pennsylvania. She holds a bachelor's degree in English and secondary education from Carlow University and her master's degree in instructional technology from Wilkes University. Through her teaching and coaching she strives to help students see themselves as problem solvers who can face challenges with curiosity and confidence. She looks forward to continuing to support these ideas in the AP Research course.

Michelle Satchwell is an English teacher at Carmel High School in Carmel, Indiana. She teaches AP Research in the AP Capstone program as well as dual-credit and honors classes. She has been an AP College Board Consultant for AP Capstone since 2014. Co-author of Reading Lists for College-Bound Students and STACS: Strategies to Acquire Composition Skills, Mrs. Satchwell was the Carmel Clay school corporation's Teacher of the Year in 2004 and is an Armstrong Teacher Educator at Indiana University. Mrs. Satchwell is a devoted educator who delights in helping her students reach their potentials.

Samantha Scheepers is an Upper School English and AP Research teacher at St. Andrew's College. She is also the chair of school's Standing Committee for Excellence in Teaching and Learning. She holds a Master's of Teaching, specializing in metacognition and reading comprehension, as well as a BA (Hons.) with high distinction in English and Renaissance Studies from the University of Toronto. She has recently completed a teaching fellowship focused on leadership in independent schools at Columbia University and has presented her research on cooperative learning through the International Boys' School Coalition.

Tonya Sukhu teaches at the Michael E. DeBaakey High School for Health Professions in Houston, Texas.

Alesia Williams has a background in the humanities. She has a BA in art history from Berea College and has completed coursework for a MA from the University of Cincinnati. She has a MAT with a focus on English from Spalding University. She has been teaching in the public school system in Louisville, KY for sixteen years. Thirteen of those years have been at duPont Manual High School which has five magnets and is typically ranked as the best high school in Kentucky. She has been teaching AP English Language and Composition for ten years and AP Research for one year. She is focused on helping her students achieve at the highest levels especially in regards to academic writing. She encourages her students to think of themselves as writers and to pursue writing competitions and publication. Her students have been recognized with top honors from the NCTE and KCTE writing competitions, the Scholastic Art and Writing competition, and the MIT INSPIRE research competition.

Edison Yagoubian is a graduate of UCF college of sciences. His degree focus is on molecular & microbiology. He has postgraduate research experience in malaria prevention and clean water maintenance. He has been teaching AP level biology/physics since 2010, and he has been a research advisor for two years. His focus as an advisor is always on maintaining a high standard for the student projects and guiding young researchers to ask deeper questions, approach a problem from many perspectives, and to remember that research is the cornerstone of innovation.

Editors of the Journal

- Suzanne Conklin Akbari
BA, MA, MPhil, PhD
- Suzanne Conklin Akbari is professor of English and Medieval Studies at the University of Toronto, and was educated at Johns Hopkins and Columbia. Her research focuses on the intersection of English and Comparative Literature with intellectual history and philosophy, ranging from neo-platonism and science in the twelfth century to national identity and religious conflict in the fifteenth century. Akbari's books are on optics and allegory (*Seeing Through the Veil*), European views of Islam and the Orient (*Idols in the East*), and travel literature (*Marco Polo*); she is currently at work on *Small Change: Metaphor and Metamorphosis in Chaucer and Christine de Pizan*. She is volume editor for the *Norton Anthology of World Literature* (Volume B: 100-1500), co-editor of the *Norton Anthology of Western Literature*, and editor of *The Oxford Handbook to Chaucer*. She has begun a new research project called *The Shape of Time*, contrasting the temporal breaks found in medieval chronicle traditions with poetic narrations of the historical past. Akbari is cross-appointed to the following units at the University of Toronto: Centre for Medieval Studies; Centre for Comparative Literature; Centre for Jewish Studies; Department of Near and Middle Eastern Civilizations; Centre for Reformation and Renaissance Studies.
- Barrie Bennett
BPE, MEd, PhD
- Barrie Bennett is professor emeritus at the Ontario Institute for Studies in Education at the University of Toronto (OISE/UT). His research work focuses primarily on the design of powerful learning environments for students and teachers through the process of systemic change. He is currently working in districts in three countries on long-term projects related to instructional intelligence and systemic change (Australia, Ireland and Canada). Instructional intelligence involves intersecting the current research on curriculum, assessment, and instruction guided by what is known about how students and teachers learn. That intersection being driven by what is known about change and systemic change. He also assists teachers, schools, and districts with issues related to classroom management and school wide-discipline. Barrie has taught at the elementary and secondary levels, as well as, having worked in group homes, prisons, and security units for juvenile offenders. He has written six books: *Cooperative Learning: Where Heart Meet Mind*; *Classroom Management: A Thinking and Caring Approach*; *Beyond Monet: The Artful Science of Instructional Integration*; *Graphic Intelligence: Playing With Possibilities* and most recently *Power Plays*. Currently he is just finishing a text titled, *Effective Group Work: Beyond Cooperative Learning*.

- Anthony Campbell
BA, MA, PhD
- Anthony Campbell established Grow for Good Urban Teaching Farm in 2013 as a business model innovation laboratory and learning centre for young entrepreneurs. He spent time working throughout North America, Europe, Australia, Asia and now resides in his hometown of Toronto. Examples of Anthony's work are documented in *The Innovator's Field Guide* (2014), co-authored by David Crosswhite and Peter Skarzynski, as well as multiple Harvard Business School and Corporate Executive Board case studies chronicling the innovation and capability-building efforts of companies such as Samsung, Whirlpool, Best Buy and McDonald's. Previously, Anthony taught Film Studies, Writing and English Literature at The University of Western Ontario.
- Jeremy B. Caplan
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- Jeremy Caplan is an Associate Professor in Psychology Department at the University of Alberta, where he is also the Principal Investigator at the University of Alberta Computational Memory Lab. The lab is focused on human verbal memory behaviour and its basis in cognitive and neural processes. The team takes several approaches towards research, including mathematical modeling, measures of behaviour in the cognitive psychology tradition, and measures of brain activity using electroencephalography (event-related potentials and oscillations) and functional magnetic resonance imaging. He has been a referee for 38 academic journals.
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- Dr. Clarke is the director of Pain Services and the medical director of the Pain Research Unit at the Toronto General Hospital. He is appointed to the Institute of Medical Sciences at the University of Toronto and is a graduate of the Royal College Clinician Scientist Program. His research interests include identifying novel acute pain treatments following major surgery, identifying the factors involved in the transition of acute postsurgical pain to chronic pain, studying the genetics of acute and chronic pain after surgery, and identifying risk factors associated with continued opioid use and poor health related quality of life after major surgery as well as the efficacy of hyperbaric medicine. Over the past five years he has authored 47 peer reviewed manuscripts.
- Will Fripp
BA, MA
- Will Fripp is a public affairs and political risk analyst for Canadian and international clients. A B.A. in History and Political Science from Victoria University at the University of Toronto and an M.A. in Intelligence and International Relations from the University of Salford in Manchester, England, he is a historian specializing in intelligence and espionage, and its modern influences. Will anchored www.spiesintheshadows.com, a web based curriculum outlining Canadian foreign intelligence history and its impacts on Canada's national development. An occasional lecturer, Will's writings and review articles appear in peer-reviewed academic journals like *Intelligence and National Security*, and elsewhere.

- Michael Gemar
BSc, BA, PhD
- Michael Gemar received undergraduate degrees in Psychology and Philosophy from Rice University, and a PhD in experimental psychology from the University of Toronto. He has worked as a researcher at the Centre for Addiction and Mental Health, examining the cognitive and neural correlates of mood disorders, and was involved in a landmark study demonstrating the efficacy of mindfulness meditation to prevent depressive relapse. He has co-authored numerous journal articles, and taught for over a decade at U of T. More recently, he has worked in the area of health policy, and is currently at a Canadian non-profit.
- Jennifer Goldberg
BA, BEd, MA
- Jennifer Goldberg holds an M.A. in History from the University of Toronto. Her graduate studies focused on teacher misconduct in 19th century Ontario, and her research is published in *Historical Studies in Education*. She currently teaches at Havergal College, where she has also served as Chair of Teaching and Learning. In this capacity, she has explored the role of feedback in student learning, and has presented on this work at the National Coalition of Girls' Schools and Conference of Independent Teachers of English.
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- Margaret Herridge is a Professor of Medicine and Senior Scientist at the University of Toronto. She is also a senior clinician in Critical Care and Respiratory medicine at University Health Network. Her research focus is on long-term outcomes after critical illness for patients and families and specifically on functional, neuropsychological, healthcare utilization and quality of life metrics. Her graduate studies were in Cell and Molecular Biology at Queen's University where she subsequently obtained her degree in Medicine. After completing her clinical training in Internal Medicine/Respirology and Critical Care at the University of Toronto, she obtained her Master of Public Health in Epidemiology and Statistics from the Harvard School of Public Health.
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- Tim Hutton is a teacher-librarian at Royal St. George's College. He has a BA in History and American Studies from the University of Toronto and a Masters in Library and Information Science from San Jose State University. At the secondary level, he has taught courses in the social sciences, humanities and communications technology, including a locally designed interdisciplinary course in urban studies.

Ira Jacobs

Dip Phys Ed,
MHK, DrMedSc

Professor Ira Jacobs became dean of the Faculty of Kinesiology & Physical Education at the University of Toronto on July 1, 2010, and was re-appointed to his current second decanal term. Before assuming this role, Jacobs was chair of York University's School of Kinesiology and Health Science from 2007 until 2010, and a federal government scientist from 1982 until 2007.

Jacobs earned his doctorate in clinical physiology from Sweden's Karolinska Institute, where he specialized in skeletal muscle metabolism. For the next 25 years, he did extensive exercise physiology research in Canada's human sciences laboratory, operated by the Department of National Defence. There, Jacobs rose to the position of chief scientist and led a unique international research group that helped to enhance the performance of military special operations units through their research into physiological, nutritional and pharmacological strategies.

He is a past president of the Canadian Society for Exercise Physiology and the Canadian Council of University Physical Education and Kinesiology Administrators. He is a fellow of the American College of Sports Medicine, an international fellow of the US National Academy of Kinesiology, and in 2016, he was named a Fellow of the Canadian Academy of Health Sciences.

Jacobs' research has led to the publication of more than 200 scientific articles, reports and book chapters about his research interests that include the physiological responses to physical exertion in environmental extremes, performance enhancement through pharmacological and nutritional manipulation of metabolism, and exercise pharmacology.

During his term as dean, the Faculty of Kinesiology & Physical Education has been rated as among the top academic programs in the world for kinesiology, physical education, sport and exercise sciences.

John Lambersky

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John Lambersky is a teacher and head of the Canadian and World Studies department at Royal St. George's College in Toronto, where he leads the AP Capstone program. He has presented his work on teaching practice at the conferences of the International Boys' School Coalition, the National Association of Independent Schools, and the Canadian Accredited Independent Schools. His academic research is focused on school culture as a mechanism for school improvement. His work has been featured in *Leadership and Policy in Schools*, *The Dalhousie Review*, and *The Nashawaak Review*.

Lori Loeb
BA, MA, PhD

Lori Loeb is Associate Professor of Modern British history at the University of Toronto. She has a Masters in Museum Studies and a PhD in History. A specialist in the Victorian period, she is the author of *Consuming Angels: Advertising and Victorian Women*. Generally, she writes about things in nineteenth-century Britain. A past Deputy Chair and Associate Chair (Graduate) of the History Department, she is currently MA Coordinator. She teaches courses in nineteenth and twentieth-century British history, Victorian material culture and the English country house.

Jaime Malic
BA (Hons), MA,
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Jaime Malic recently completed her PhD in Educational Leadership and Policy at the Ontario Institute for Studies in Education at the University of Toronto. Her research focused on leadership values and practices in independent schools in Ontario. Jaime has more than ten years of experience as an educator in both independent and public schools. She currently teaches AP Capstone Seminar and senior English courses at St. Clement's School. Jaime has served as both a Reader for AP Capstone Seminar and a writer on the Item-Writing Committee for the Ontario Secondary School Literacy Test. She has written for *Independent Teacher* and presented on various topics at the Conference of Independent Teachers of English Annual Conference, the Ontario Advanced Placement Administration Conference, and the Advanced Placement Annual Conference.

William J.
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BASc, MEng,
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William McCausland is an associate professor of economics at the Université de Montréal. His research applies Bayesian statistical methods in two main areas. The first is discrete choice, at the interface of economics and psychology, where researchers study how people make choices from a small menu of available options. The second is time series modelling in economics, which has many applications in macroeconomics and financial economics. His undergraduate studies were in Engineering and he received his Ph.D. degree in economics from the University of Minnesota.

Michael
Simmonds
BPE, M.A., Dip.
Ed., MEd, EdD

Michael Simmonds has worked in independent schools for over two decades. He taught science, biology, chemistry, physics, and math before becoming an administrator and Head of School. He earned graduate degrees from both McGill and Columbia universities respectively before receiving his doctorate from the University of British Columbia in Educational Policy & Leadership. His work on accountability synopticism is published in the peer-reviewed, *The International Education Journal: Comparative Perspectives*. He currently works at Havergal College as the VP School Life, Operations & Student Wellness.

Guidelines for Contributors

The Young Researcher is a peer-reviewed journal dedicated to publishing the best original research from secondary school students.

The journal's mission is to provide a larger audience for the original academic research of ambitious secondary students, provide a forum for peer-review, and create a community of young researchers. In addition, the journal strives to advance the quality of academic writing in secondary schools.

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The journal encourages submissions of original research (including relevant replication studies) from a wide range of academic disciplines within the social sciences, humanities, and sciences.

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Conclusion and Future Directions

References

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- All units of measurement should be in metric wherever possible

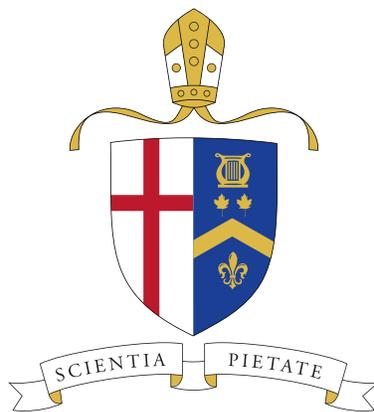
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