

ACKNOWLEDGEMENTS

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LETTER FROM THE EDITOR

One of the world's largest and oldest organisms is called Pando. Located in Utah's Fishlake National Forest, it's a grove of aspen trees that covers 107 acres. But while we look at Pando and see 47,000 trees, they are all actually related—in fact, they are the same tree. They share a root system, and most new trees sprout directly from these roots rather than from seeds, so each tree is basically a clone of the surrounding ones. This grove is widely studied as scientists try to determine how it has lived so long and grown so large, withstanding the onslaught of time, wildfire, drought, disease, and climate change.

While society may not exactly be a single living organism, we face many of the same challenges that Pando does. How we approach those challenges tells a great deal about how we will succeed. When we point out differences and assign blame, we push solutions further down the road. Luckily, we live in a time where interdisciplinary collaboration is valued more than ever before. We draw on what came before to make new connections today. This ever-expanding body of knowledge is the root system of our society, providing stability, nourishment, and growth.

I'm not a statistician or an economist; I've never examined the issues surrounding community gardens, calculated the components of a vibrant economy, or probed the causes of chronic illness. But I benefit from those who do. We are strongest when we remember that we stand together. As academics, scientists, and citizens, each contributes to the success of the whole.

I'd like to thank Dr. John Cavitt and Erin Kendall from the Office of Undergraduate Research, for making this project possible; Tess Sawaya, for stepping in at the last moment for an outstanding design and layout; and especially our faculty reviewers, who provide the expert advice and mentorship so vital to this endeavor.

And finally, here's to the scholars featured in the 13th edition of *Ergo*, whose work today will become the foundation of tomorrow's progress.

All the best,



Hillary Barton
Editor



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Telitha E. Lindquist College of Arts & Humanities



Pocahontas And Semiotics: An Explanation Of Semiotics Using Disney's 1995 Film *Pocahontas*

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Abstract

This study undertakes a rhetorical textual analysis of the Disney film *Pocahontas* in order to make an argument that the theory of semiotics can and should be productively applied to help understand how social patterns of meaning can foster intercultural miscommunication. From this case, the researcher develops lessons for foregrounding cultural symbols and for promoting effective cross-cultural interaction.

Introduction

The Walt Disney Company was established in Los Angeles California in October of 1923. Many children have grown up watching films produced by this company. One such film, *Pocahontas*, purports to explain one early interaction between English settlers and native peoples before the founding of the United States. Although the film is not historically accurate, it does demonstrate the historical truth that much of the conflict between the two groups was caused by miscommunication and misinterpretation.

Semiotics, or the study of signs, explains that society shapes the meaning behind an object, symbol, or word. Two completely different societies can create different meanings for the same literal thing. This concept has been especially interesting to me as I have studied three languages other than English—Portuguese, Spanish, and French. Words often do not directly translate from one language to another. Not only in the denotation of the word, but also in the connotation. This diversity of meaning is a result of the differences in each society. We will use semiotics to analyze *Pocahontas* as a case study to understand how social patterns of meaning can foster intercultural miscommunication. First, the theory of semiotics will be briefly explained. Next, we will review a

brief summary of *Pocahontas*. Then we will move onto the methods of research. Finally, we will analyze *Pocahontas* using semiotics and draw conclusions.

Review Of Literature On The Theory Of Semiotics

At its core, “Semiotics is the science of signs, a sign being anything that can be used to stand for something else” (Berger, 2013, p. 1). Expounding on that statement, Berger cites Maya Pines: “Everything we do sends messages about us in a variety of codes [. . .] Yet we seldom realize that we have received such messages, and would have trouble explaining the rules under which they operate” (Berger, 2013, p. 1). Essentially, semiotics asks us to explicitly analyze how we encode and decode messages in ways that seem natural or invisible to us.

For instance, imagine for a moment walking into your house and smelling the cookies your mom is making, your grandmother’s recipe. This might mean several things to you—anticipation of a delicious treat, the comfort of a warm home, or sorrow from missing your late grandmother. Just that smell sends a meaningful message.

This example can further explain the core concepts of this theory. “Saussure divided signs into two parts: every sign is made of sound-images or *signifiers* and the concepts generated by the signifiers, *signifieds*” (Berger, 2013, p. 1). A sign has two parts: signifier + signified = sign. The signifier is “the physical form of the sign as we perceive it through our sense; an image” (Griffin et al., 2015, p. 328). In our example, the signifier is the smell of grandma’s cookies. The signified is “the meaning we associate with the sign” (Griffin et al., 2015, p. 328). The feeling of missing grandma is the signified.

Harrison (2003) elaborates, “[A] well-known painting by Rene Magritte [demonstrates] this relationship in a striking and explicit manner. By putting the sentence *This is not a pipe* below a highly realistic representation of a pipe, Magritte reminds viewers that the image is not reality but artifice—in other words, a representation or sign” (p. 47).

Each sign has a denotation and a connotation. The myth is “the connotative meaning that signs carry wherever they go; myth makes what is cultural seem natural” (Griffin et al., 2015, p. 328). This

is where we start to get the influence of society because the group decides the meanings. “Because every community is different, the sign used by one community may be different from those used by another. For example, the color red indicates mourning for people in Ivory Coast, whereas, it represents procreation and life for people in India” (Harrison, 2003, p. 48). The interesting thing about the meaning we give to something is that it can change over time. Berger found that “semiotics teaches us not only about how to find the meaning of signs but also that these meanings are based on society and its codes; society creates meaning in signs and these meanings can change” (Berger, 2013, p. 26). Berger gives the humorous example of hairstyle to show a change: “long hair in men used to signify ‘artistic’ but now long hair has lost that meaning; it can mean anything nowadays: poets, truck drivers and baseball pitchers” (Berger, 2013, p. 22).

Method

Artifact: Pocahontas

The film takes place in the 1600s in what is now the United States. A group of men from Europe arrive and begin the colonization of Jamestown. They are led by Governor Ratcliffe, a greedy man in search of gold, and the adventurous and ambitious Captain John Smith. Soon after arriving in the new land John meets Pocahontas, daughter of Chief Powhatan. She is a curious and rambunctious young woman who is betrothed to Kocoum, the stern warrior. Pocahontas and John learn of one another’s cultures and attempt to stop the fighting between their peoples (Walt Disney, 1995).

Data Collection and Textual Analysis

In order to apply semiotics theory to the movie *Pocahontas*, I performed a rhetorical textual analysis (Keyton, 2014). I generated a list of key theoretical terms from semiotics theory that served as my code book for the textual analysis. Although I have seen this movie dozens of times, I re-watched it with the codebook. I looked for examples of the key terms and jotted down the scene and situation for each. I specifically took notes of which characters were in the scene, dialog, facial expressions, attitudes, etc. I noted when there was a difference between the two societies, such as having the same signifier but opposing signified in their signs. After watching the film, I went

over a few of the concepts with my husband as a form of qualitative data-conferencing. Although he is not a communications major, he confirmed my analysis of examples from the movie based on the vocabulary definitions.

Analysis

While watching the film I observed the differences of each character's reaction to certain objects, sounds and words. The characters' cultures seemed to impact their ideology, their values, and their perception of the other civilization.

Signs, Signifiers, Signified

In the film, I saw many examples of signs. One such example was music. Pocahontas and John hear the Indian drums in the distance and Pocahontas says, "they mean trouble." The signifier is the sound of the drums and the signified is trouble.

An object can also be a sign. Chief Powhatan arranges a marriage between his daughter, Pocahontas, and Kocoum, the warrior. He tells her to be strong and consistent like the river. To him, the signifier is the river, and the signified means strength and obedience. To Pocahontas the signifier is still the river, but the twists and rapids change the signified to freedom and adventure. Before she meets John, Pocahontas sees what she calls "strange clouds." She tries to give meaning to the signifier but does not fully understand the sign. We later discover that those "clouds" are actually the sails of the ship carrying the settlers. The settlers believe that the Indians are hiding their gold. When Pocahontas asks what gold is, John says "it's yellow, comes out of the ground, really valuable." Thinking she understands, Pocahontas, holding out an ear of corn says, "Oh here, we have lots of it!" In this scenario the signified meaning is similar, but John and Pocahontas are referring to different signifiers, gold and corn.

Gestures are also a form of a sign. In one of the first encounters of Pocahontas and John, they share how each culture says hello. John Smith puts his hand out and says, "It's called a handshake." Pocahontas stares at his hand and says, "It's not doing anything." To John, signifier is the handshake and its meaning is greeting. For Pocahontas, the sign is not complete because she does not understand

the connection between the signifier and the signified. Once the connection is made, she shows John how her people say hello: a wave.

One of the most powerful scenes in this movie is when John explains his purpose in the New World. He tells Pocahontas that they want to teach the Indians how to be civilized. Pocahontas becomes offended and starts to leave. John runs after her and says, “Wait, but there is so much we can teach you. We have improved the lives of savages all over the world.” Pocahontas replies, “SAVAGES?!” Surprised by her response John says, “Not that you’re a savage . . .” Pocahontas replies, “Just my *people*?” John tries to explain what he meant to say, but struggles to find his words. Pocahontas then says to him, “What you mean is, not like you.” To John, the signifier is the word “savage,” and the meaning is the native peoples of the lands he had helped develop. To Pocahontas the signifier “savage” has a very different signified; to her, it is a demeaning expression.

Ideology

In semiotics, ideology is “Knowledge presented as common sense or natural, especially when its social construction is ignored or suppressed” (Griffin et al., 2015, p. 333). Societies can have different meanings for things. In the movie, the settlers are in search of gold to establish themselves in society. To them, gold represents value and power. John Smith is shocked to find out that the Indians do not have gold. Pocahontas doesn’t even seem interested in having gold in the form of currency. When John inquires about gold, Pocahontas shows him a freshly picked ear of corn. Initially, John does not understand why Pocahontas is uninterested in his gold coins. The ideology of his culture values power, wealth, conquering, building, etc. She does not share his same ideology; her culture places more value in nature, growth, safety, and family pride.

Denotative and Connotative sign systems

A *denotative sign system* is “a descriptive sign without ideological content,” and a *connotative sign system* is “a mythic sign that has lost its historical referent; form without substance” (Griffin et al., 2015, p. 331–332). The denotative system involves the original sign: the image/word with an associated meaning. The connotative system is when that sign becomes the new signifier and acquires a different signified (meaning), creating a new sign.

The word *Indian* reflects the shifting value system of signs. In *Pocahontas*, the settlers refer to the people of the new land as “savages,” or “injuns.” In this portrayal of early encounters with the natives, we could say the signifier of this sign is the word *Indian* and the signified is the natives of the land. Now, when referring to that specific group of people, we generally say “Native American,” in part for political correctness but also because when we say “Indian” we think of people from the country India.

Discussion

In conclusion, we find that there is much that the theory of semiotics can teach us. Signs can be anything: objects, places, words, gestures, music, foods, etc. They are made from the connection of a signifier to a signified. As Ferdinand Saussure said, “Language is a system of signs that express ideas” (Saussure, 1966, p. 16). A strong message can be sent with very few to no words. How we interpret those messages is largely affected by our upbringing. Society and culture can have an impact on the ideology and myth of signs. We each grow up in cultures where we are exposed to certain ideas, beliefs, traditions, morals, and values. Our languages (verbal and nonverbal/symbolic) are shaped by those systems of meaning in which we were raised. We should be mindful of these cultural differences and be considerate of the fact that our signs might not be universal. In *Pocahontas*, a war almost breaks out between the two societies due to a lack of understanding. I firmly believe that many problems we face in our modern world can be avoided if we take the time to learn about some of the signs of other cultures.

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Two Weeks Notice:

A Face-Negotiation Theory Analysis

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Abstract

This study undertakes a rhetorical content analysis of the romantic comedy *Two Weeks Notice* to argue that Ting-Toomey's face-negotiation theory should be extended to analyze communication in interpersonal romantic relationships. If culture is widely understood as a product of gender, socio-economics, family structure, and other factors, then interpersonal romantic relationships often involve people with different self-construals and cultural backgrounds. Thus, using face negotiation to study interpersonal romantic communication may help better explain conflict and provide a path towards resolving those misunderstandings.

Introduction

Stella Ting-Toomey's face-negotiation theory assumes that "people of every culture are always negotiating face" (Griffin, 2012, 408). Our lives are a constant balancing act to maintain the face, or projected image, that we create. Each of us controls this face through our verbal and nonverbal communications with the world around us. Face is directly impacted by our culture. Ting-Toomey's theory was originally written to focus on intercultural and international communication. However, while it has not been fully studied in interpersonal communication, I argue that face negotiation is an important process in interpersonal romantic relationships. This research paper performs a content analysis of the popular romantic comedy *Two Weeks Notice* [sic] (Berman & McLaglan, 2002) in order to demonstrate face-negotiation theory's utility in explaining interpersonal conflict and providing a path towards resolving those misunderstandings. This perspective is crucial to interpersonal relationships because of distinct cultures previously excluded from the assumptions of the face-negotiation theory. As we evaluate the cultures of those with whom we communicate we can gain resolution, which serves as a powerful communication tool.

Review of Literature on Ting-Toomey's Face-Negotiation Theory

Face-negotiation theory explains that the main motivation of our communication is to negotiate face. "Face is an individual's claimed sense of favorable image in the context of social and relational networks" (Zhang, Ting-Toomey & Oetzel, 2014). In order to control the self that others perceive us to be, people use differing techniques based on the culture of the communicator.

Culture changes how we choose to portray ourselves to others because in different cultures different characteristics are valued. Ting-Toomey argued that collectivistic cultures attribute success to the "we" whereas individualistic cultures value "I" successes as most important. This attribution to cultural collectivism-individualism is vital to understanding the face that others put up because our self-construal is influenced by our cultural environment. "Cultural collectivism-individualism also had indirect effects on conflict styles mediated through self-construal and face concerns consecutively" (Oetzel & Ting-Toomey, 2003).

Another key component of face negotiation is an individual's conflict management style. Ting-Toomey's theory argues that people choose conflict management tactics according to what fosters and preserves face in ways most relevant to their self-construal and cultural background. For instance, people in individualistic cultures might be more likely to pick conflict styles like dominating and integrating. Dominating is viewing the conflict as if it were a competition; one side of the conflict wants to "win" or conquer over the other opinion. Integrating is a management style in which pursues "problem solving through open discussion; collaborating for a win-win resolution of conflict." (Griffin, 2012, p.408)

On the other hand, people in more collectivistic cultures are more likely to use management styles like avoiding and obliging communication. Avoiding is when a person chooses not to respond or to intentionally withdraw from the potential conflict to save either their own face or the face of another. Obliging is "accommodating or giving in to the wishes of another in a conflict situation." (Griffin, 2012, p.408)

While Ting-Toomey's theory focuses deeply on how face is negotiated in an intercultural or international environment, she spends less time attending to how face might be negotiated in other communication contexts.

Method

Artifact: Two Weeks Notice

Lucy Kelson and George Wade are the main characters of *Two Weeks Notice* (Berman & McLaglen, 2002). Lucy is an environmental activist and lawyer while George is a rich and famous businessman from New York. Lucy accepts a job with George's company Wade Corporation despite her better judgment and find herself consumed with constant calls, texts, meetings, and questions from George. When Lucy decides to quit her job, she and George have to navigate the face negotiation within their interpersonal romantic relationship.

Data Collection and Textual Analysis

In order to apply Ting-Toomey's face-negotiation theory to the movie *Two Weeks Notice*, I performed a rhetorical content analysis (Keyton, 2014). I generated a list of key theoretical terms from face-negotiation theory that served as my code book for the content analysis. I then watched the full movie a first time with a second person viewing it with me. As we watched, we both used the code book to analyze the movie scene by scene and coded specific scenes and dialogue based on the vocabulary terms in my code book. After the completion of the movie we each chose the key scenes and terms being represented in the movie and discussed to resolve differences, creating a qualitative form of intercoder reliability.

Analysis

Face and Collectivistic-Individualistic Culture

Two Weeks Notice illustrates that cultural differences in communication and face saving are not only present in international/intercultural encounters but apply to interpersonal relationships as well. Lucy Kelson and George Wade battle with the negotiation of their face within the context of their gender culture and their wealth cultures.

Gender Culture

The film presents main characters Lucy and George as from different gender cultures—positioning Lucy's femininity as collectivistic and communal and George's masculinity as individualistic. Lucy is not the

typical submissive sensitive type; in fact, she can be a bit brash at times. She does, however, show many of the communication patterns typically associated with cooperative feminine communication (Griffin, 2016). For instance, at the start of the film, we see Lucy wearing a flowing patterned skirt, with frizzy unkempt hair, and sensible flats. However, when she meets George, she changes to a fitted pantsuit, with perfectly placed hair and makeup. The underlying tone of this dramatic change is that Lucy becomes more feminine as she changes her preferred appearance to fit with George's communication expectations/background. However, we see that the opposite is true for George. In the first scene he is filled with confidence. He oozes popularity; just moments after meeting Lucy he is openly discussing how he may enjoy having sex with her at some point. These are typical communication patterns associated with individualistic, masculine communication (Griffin, 2016)

A second example of the impact of these gender cultures is portrayed through the scene in which Lucy tries to give her two weeks' notice. Lucy says "Why don't we just call it quits. Please consider this my two weeks' notice." George replies, "I find you ungrateful; if you don't want to be disturbed why do you keep your phone on? You like emergencies; you crave the excitement." At that point, Lucy gives in and begins to display her collectivistic communication approach, saying, "Alright, George, you are completely right; this has nothing to do with you. This is all my fault." This conversation clearly illustrates the different culture lenses through which George and Lucy are viewing their experiences. George is unwilling to take accountability for causing conflict in Lucy's life, and Lucy, instead of pressing forward with an individualistic lens, quickly transitions to her female collectivistic resolutions. As she chooses to take accountability for the dysfunction of their relationship, she assumes this role in order to remove the conflict within the relationship. This means that Lucy prioritizes saving face for George, instead of negotiating her own face. As a result, the two characters are clearly engaged in "intercultural" face negotiation because of their different gender backgrounds.

Wealth Culture

The film also presents Lucy and George as from different wealth cultures—positioning Lucy's lower-middle-class background as collectivistic and George's upper-class urban background as

individualistic. Lucy and her family are depicted as lower middle-class because they live in a small apartment in a more communal area. Lucy implies this has given her a collective cultural mindset. During her first interaction with George, he asks, “What’s your background?” Lucy responds, “I work for the Coalition for the Homeless, legal defense . . .” “That can’t pay very much,” George responds, to which Lucy replies, “Well, I’m not very interested in money.” Lucy takes the job at Wade Corporation not for the money, but to save her beloved community center. A community center that, as she explains to George, “turns strangers into neighbors.”

George lives in a hotel that he owns. He wants for nothing, and can manipulate and coerce people because of his money. In the film, George is a protagonist of the story and is portrayed as very likeable. He is clearly positioned as having a dominant individualistic/competitive outlook because of this wealth culture in which he exists.

Overall, Lucy comes from a more collective culture. Her gender and wealth culture focuses on rapport, connection with other people, and standing for the greater good. In juxtaposition, George, both in his wealth culture and his gender culture, lives in a world of individualism. Because face-negotiation theory explains how face is negotiated in intercultural communication, it should be expanded from its primary focus on international communication to interpersonal romantic communication.

Face Negotiation’s Influence on Conflict Management

Because Lucy and George come from different communication cultures, the conflicts in the movie can be analyzed through face-negotiation theory’s predictions for conflict resolution approaches. For instance, when Lucy accepts the job at Wade Corporation, George promises Lucy that he will preserve the community center in her hometown. However, after she submits her two weeks’ notice, he goes back on his word. Romantic tension is also building; Lucy has feelings for George and is hurt when she sees George in his apartment (undressed) with another woman. The culmination of these two situations is the following scene in which both characters attempt to preserve face:

Lucy (to George): “You promised me a community center.”

George, now yelling: “YES, I PROMISED! I PROMISED! I’m sorry, I can’t control the economy, I can’t control my brother, I did promise and I did let you down. I’m sorry, but you know what? I’m human and I think you will find a lot of people are.”

Lucy interrupts: “I am human. I am human, too.”

George, completely exasperated: “Are you? Because you are too perfect, you’re too wonderful and none of us can keep up with you . . . You know, that’s probably why all of those other guys bolted as fast as their Birkenstocks could take them. ‘Cause you’re intolerable; no one wants to be preached to, no one wants to live with a saint; saints are boring.”

In this scene, Lucy is using the avoiding approach to conflict management while George is using the dominating approach, which reflects their cultural backgrounds. Lucy engages in avoidance conflict management as she attempts to change the subject from George’s sex life to the community center, a pertinent but less emotional matter, but George is unwilling to compromise. George attempts to use dominating styles to solve the conflict by yelling and insisting that he has no control over the solution. This conversation is a painful example of face negotiation. Both are attempting to save the image they have lost. Lucy engages in face restoration as she tries to avoid the hurt she feels from her experience; she is trying to seem tough and uninterested. Similarly, George attempts to respond to the face threat by abdicating responsibility for the failed project (so he didn’t really “lose”) and blaming Lucy for causing him to not follow through.

Conclusion

In considering the application of face-negotiation theory to this next dimension of relational communication there is a significant application in avenues not previously considered. If culture is widely understood as a product of gender, socio-economics, family structure, and other factors, then interpersonal romantic relationships often involve people with different self-construals and cultural backgrounds. This analysis brings to the surface the underlying implications of

both class issues and gendered speech communities, and their impact on our communication tendencies. The collectivistic-individualistic cultural groups highlighted in face-negotiation theory are influenced by both gender and social class, and those factors may overlap in their construction of our approach to face negotiation. Considering the influence that these two specific lenses bring into interpersonal romantic communication, the impact of such cultures becomes far reaching within the context of every relationship and conflict management experience.

If George and Lucy were able to evaluate the cultural communication lenses causing their different approaches to conflict, resolution would be possible. Without this perspective they fall into the painful trap of misunderstanding and incompatible face negotiation. Thus, using face negotiation to study interpersonal romantic communication may help better explain conflict and provide a path towards resolving those misunderstandings. Future application of face-negotiation theory to the broader spectrum of cultural backgrounds could be key to understanding and resolving conflict on a micro-communication level. In both intercultural and interpersonal communication, understanding others' cultural contribution to their face can help us to more effectively manage conflict.

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Heterosexuality As A Measure Of Success In Arthurian Literature

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Abstract

This paper analyzes *Sir Gawain and the Green Knight* and Marie De France's *Lanval* in order to demonstrate how heterosexuality is viewed as a measure of success in medieval literature. These pieces of literature primarily use women as objects in order to condemn homosexuality and to solidify the supposed importance of heterosexuality to society as a whole. This paper also makes a comparison between the aforementioned Arthurian tales and more modern adaptations in order to demonstrate how these traditional values can either be upheld or subverted.

Introduction

In medieval literature, female characters generally fall into two main categories: the seductress and the sex object. Both of these archetypes revolve around sex in relation to a male character. Thus, whether the man is in favor of the proceedings or not, the woman acts as a conduit to achieve and maintain the staunchly heterosexual ideals of the time period. This is something that I noted especially within the Arthurian tales. In these tales, heterosexuality is viewed as representative of success. The women in these stories either help or hurt men in achieving that success. I will be looking at the homophobia present within Marie De France's *Lanval* and the undertones of homosexual desire within *Sir Gawain and the Green Knight* in order to demonstrate the connection between women and the heteronormativity maintained by these stories. I will also bring in some more modern stories in order to make a comparison between the Arthurian legends of the middle ages and the modern age.

Lanval

In *Lanval*, there are two women of major import: Guinevere and Lanval's fairy lover. The fairy acts as the proof of Lanval's heterosexuality throughout the poem, and thus also as the conduit for his success. At the beginning of the poem, Lanval is not held in high regard by Arthur or his fellow knights. It says, "he had/spent all his money and his good,/for Arthur gave him not a thing" (De France, 2018, pp. 29–31). Another measure of success at this time is money and respect, of which Lanval has neither. Without these successes, Lanval is effectively not performing the expected standards of masculinity set forward at this time. It is only after he meets and consummates his relationship with the fairy that he receives that desired wealth and respect (De France, 2018). By establishing material and worldly success as intrinsically entwined with heterosexual behavior, Marie De France is effectively placing heterosexuality as part of that ultimate success. It is important to note that while this success cannot be achieved without the fairy, she is never named, thus reducing her to a faceless conduit through which Lanval's heterosexuality and success can be established.

Guinevere plays the role of the seductress in this poem. However, while she does not act as the proof of Lanval's heterosexuality and thus his success as the fairy does, she still ultimately causes Lanval's successes to be further broadcasted. When Guinevere tries to seduce Lanval and gets rejected, she says to him, "it is often rumored, sire,/for women you have no desire!" (De France, 2018, pp. 279–280). This is the first outright mention of homosexuality in this poem, and it is certainly not a positive one. Thus heterosexuality is established as the only acceptable option. The accusation is a threat to Lanval's status, his success, and so he responds that he cannot possibly favor men because he has a female lover far more beautiful than Guinevere (De France, 2018). Guinevere then becomes angry and claims that Lanval said this in the context of trying to seduce her and being rebuffed (De France, 2018). Lanval is put on trial for his supposed actions, but since he broke his promise to the fairy to never speak to anyone of her, it seems that he will lose the case. Guinevere's actions have invalidated Lanval's heterosexuality and lost him his lover, the physical manifestation of his heterosexuality, and as a consequence he may lose his other successes as well.

Lanval is set free, however, because the fairy shows up to his trial and pleads for him. Once everyone can see that Lanval was not lying, once

his heterosexuality has been reestablished, he is freed and rides off with his fairy lover. In this case, the fairy is once again acting as the proof of Lanval's heterosexuality, and thus is also acting as his savior. However, despite being his savior, the power still lies with Lanval. In an essay discussing the roles of women in medieval literature, Michelle Sweeney (2014) stated, "the fairy's power to save Lanval [. . .] is her body: she is the essence of beauty and sexuality [. . .] Lanval is [. . .] more powerful in his own right for having spoken the truth and having a relationship with such a beautiful body" (p. 169). The fairy is still a faceless object that enhances Lanval's success by acting as proof of his heterosexuality. As mentioned earlier, despite the fact that Guinevere's actions initially invalidated Lanval's heterosexuality and put him in danger, these same actions ultimately were to Lanval's benefit. If these events had not taken place, Lanval would have had to continue to keep his lover secret forever, and his lack of open heterosexuality may have eventually been questioned anyway. Now, however, Lanval's heterosexual successes are out in the open for everyone to appreciate. In medieval literature, even when women attempt to invalidate men's successes, they often will only help to further establish them.

Sir Gawain and the Green Knight

In *Sir Gawain and the Green Knight*, Gawain's heterosexuality is never established clearly as it is in Lanval. In fact, Gawain outright rejects the only advances levelled towards him by a woman. It may seem strange, then, that I chose to include this poem in a paper discussing how heterosexuality is representative of success within the context of medieval Arthurian works. However, while Lanval does not directly establish his heterosexuality, he does work to disestablish his homosexuality. In a paper discussing the interconnectivity of homophobia and misogyny in this poem, David L. Boyd explains this concept in regards to the game that Gawain and Bertilak establish. For this game, Bertilak suggests, "what I win in the woods will be yours,/and what you gain while I'm gone you will give to me" (*Gawain*, 2018, pp. 1106–1107). Boyd explains that because of this game, the apparently heterosexual attempted seduction of Gawain by Bertilak's wife takes on a new meaning. He says, "If Gawain had intercourse with Lady Bertilak, who would serve as his receptacle for sexual activity (his gain), he would be required to give to Bertilak what he had received" meaning that according to the rules of the game, Gawain would be

required to have intercourse with Sir Bertilak (Boyd, 1998, p. 79). This assertion is further proved by the fact that after the first day, Gawain “kissed him [Bertilak] in the comeliest way he could” in order to give Bertilak the kiss that he had gained from his wife (*Gawain*, 2018, p. 1389). By seemingly rejecting heterosexuality, Gawain is in fact rejecting the homosexual implications that his actions might have, and during the first two days, he comes out with Bertilak’s hard-earned killings. Therefore, his rejection of homosexuality leads to his success.

Boyd also identifies how the symbolic nature of Gawain’s clothes makes it all the more important for him to resist the trap that Bertilak has set. On his shield, Gawain has a pentangle, or a five-pointed star. It is meant to represent many things, but Boyd (1998) focuses on how it “represents the interdependent moral, religious, and martial codes through which masculine identity is formed [. . .] According to the significance of the pentangle's fifth point, he must practice sexual and spiritual [. . .] chastity or purity” (p. 82). Masculine identity at the time was inextricably linked to heterosexuality; therefore, even though Gawain also practices sexual purity, that purity partially represents his masculine identity, which also includes heterosexuality as a default. If Gawain were to act in an unchaste manner with Sir Bertilak’s wife, he would not only be acting in a manner that leads to homosexual activities, but he would also in the process lose his manhood.

Gawain’s success in rejecting impurity and homosexuality only last so long, however. While Gawain has spurned all of Bertilak’s wife’s physical advances, he is not able to resist taking her girdle when she says that anyone who wears it “will be safe against anyone who seeks to strike him” (*Gawain*, 2018, p. 1853). Since Gawain’s main purpose in taking the girdle is to keep him safe, he does not give it to Sir Bertilak as he should have according to the rules of their game. Boyd (1998) explains that wearing this ill-gotten woman’s garment and kneeling before the Green Knight feminizes Gawain, thus introducing elements of homosexuality that Gawain has fallen prey to. Once it has been revealed to him that the Green Knight orchestrated the entire situation, Gawain expresses his remorse at his actions. He says of the girdle, “the symbol of sin, for which my neck bears the scar;/a sign of my fault and offence and failure,/of the cowardice and covetousness I came to commit” (*Gawain*, 2018, pp. 2506–2508). Gawain does not walk away from his temptations unscathed as Lanval did. The difference

here is that while Lanval was accused of homosexuality, he never actually committed any sexual offence. Gawain, however, while not acting on homosexuality directly, is feminized by his actions, thus losing some of his masculine identity, and his ultimate success is tainted.

The women in this story serve similar roles to the ones in *Lanval*. Bertilak's wife, much like the fairy, does not have a name. She is known only in relation to her husband, and is used by him as a tool to test Gawain's moral fortitude, or his masculine identity. She is not acting of her own volition, but rather being used as an object of corruption by her husband. She is set in the role of a temptress, but she is also set as the means by which Gawain can prove himself to be truly successful. Another woman in this story is Morgan le Fay. She is named at the end of the poem as the originator behind this elaborate trick (*Gawain*, 2018). In an article about Morgan's role in this poem, Edith Whitehurst Williams (1985) said, "however malevolent her initial intent may have been, it has an ultimately salutary effect on Gawain because it presses him into the discovery of his own humanity" (p. 39). In other words, despite the potentially harmful effects of her actions, just like Guinevere, the situation ultimately benefits Gawain because he is given the opportunity to prove his manhood and heterosexuality to the world. Despite his mistakes, Gawain still comes out of the situation alive and with a deeper understanding of the limitations to his manhood.

Modern Adaptations

More recent adaptations of the Arthurian legends have similar themes. In the BBC's *Merlin*, the storyline eventually comes to the union of Arthur and Guinevere. In this story, they are about to be married, and everything seems to be going according to plan. Morgana, as she is called here, wants to sabotage their plans. To do this, she brings in Lancelot, who had previously left the show, and convinces him to try and seduce Guinevere. It does not work at first, but Morgana puts a spell on her that causes her to give in. Arthur finds out and sends Guinevere away, effectively calling off the wedding (Watkins, 2011). In later episodes, Morgana's meddling is revealed and Guinevere is allowed to come back and marry Arthur (McBrien, 2011).

At first glance, this seems like a reversal of the trope seen in the previous works, and in some ways, it is. Instead of a woman trying to

seduce a man and getting said man in trouble, it is Guinevere who is unwillingly seduced and wrongfully accused. However, despite these reversals, this storyline is not so much about Guinevere's perseverance and ultimate establishment of her heterosexuality, although that is a factor; it is about the effect that these events have on Arthur. By having Guinevere and Arthur get married, this establishes Arthur's heterosexuality. Morgana's attempts to call off the wedding call Arthur's heterosexuality into question, leaving her to act as the temptress in the context of this story. When Guinevere ultimately marries Arthur, she acts as the object that fully cements Arthur's heterosexuality. This highlights Arthur's overall success as a king.

While it may seem that heteronormativity and the use of women as objects to maintain that structure may be woven into the fabric of the Arthurian tales, even when made for modern audiences, that is not always the case. In Douglas Clegg's novel, *Mordred, Bastard Son*, the situation is different. In this book, Mordred is the offspring of Morgan le Fay, who was unwillingly impregnated by Arthur. In most versions of the Arthurian tales, Morgana has a varied and complicated relationship with Arthur. In this situation, their relationship is not a positive one. Already, the assertion of heterosexuality is cast in a negative light. Later on, Mordred is encouraged to not engage in sexual intercourse, as doing so will inhibit his magical growth. This, in a classical telling of this tale, would introduce the terms by which Mordred's moral test happens. Instead of the temptation being orchestrated by a woman, however, Mordred is faced with Lancelot. He finds Lancelot attractive, and eventually engages in sexual intercourse with him. Instead of this being viewed as a failure of his moral test in the book as a whole, it instead acts as an assertion of Mordred's homosexuality in connection with the idea that Mordred can also be successful. This is an intentional deviation from the typical structure of a morality tale. Instead of using women to assert male heterosexuality and success, homosexuality is asserted as the more desirable outcome.

Conclusion

Traditionally, women in Arthurian tales take on the role of the temptress or the savior. Women in both roles serve to cement the male character's heterosexuality. The temptresses do this by calling the man's heterosexuality into question, which highlights how important

heterosexuality is, allowing for the absolute assertion of the man's heterosexuality and success later on. The saviors are the women who act only as sexual objects that prove the man's heterosexuality by nature of their existence. It is important to keep this in mind when approaching any piece of Arthurian literature, medieval or modern. Unless a piece of literature is intentionally subverting these tropes as in *Mordred, Bastard Son*, it is likely that heterosexuality is being established as the only valid option with women acting as the agents of proving that heterosexuality. This has an effect on how these works are analyzed. How important the assertion of heterosexuality is to a piece will allow for greater insight into what commentaries are being made about the rest of society. Even for more modern pieces, whether these norms are being upheld or subverted will demonstrate certain attitudes present in the time that they are written. If any broader claims are to be made about an Arthurian-based work, how attitudes towards heterosexuality and homosexuality are presented should be carefully considered.

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***Faustus*—Marlowe’s Undercover Agent**

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Abstract

Christopher Marlowe’s *The Tragical History of Dr. Faustus* is a scathing commentary on the incorrigibility of Elizabethan spies. Dr. Faustus, a learned man who prides himself on his intellectual superiority, is representative of Elizabethan humanists and syllogists. However, Marlowe uses a character who is obviously reprobate to demonstrate that, like Elizabethan spies, Faustus’ horrifying end is well-deserved. Marlowe’s Lucifer, whose peripheral role in the work highlights a spymaster’s absence in the recruitment of intelligence candidates, which duty is relegated to lesser beings like Mephistopheles, is reminiscent of Sir Francis Walsingham, spymaster to a large network of spies. Ultimately, Marlowe’s play issues an unambiguous warning against using logic to justify choices that are clear violations of decency and integrity.

Faustus—Marlowe’s Undercover Agent

To maintain her authority and for protection during religious and political turmoil, Queen Elizabeth I used a network of intelligencers, or spies, to ferret out her enemies, especially those loyal to Catholicism. Sir Francis Walsingham was one of her spymasters whose overzealous perception of hostilities to the crown bred brutality (Chapman, 1881). As England sought to expand power, Walsingham sought intelligencers to help enlarge his network of spies, which parallels the reasons Christopher Marlowe’s Lucifer in *The Tragical History of Dr. Faustus* seeks souls—the first of many similarities between Faustus and the Elizabethan spy world (5.40). Walsingham and Lucifer maintained a well-ordered hierarchy of familiars or spirits, terms used colloquially to refer to spies, which network, whose recruitment ranged from within the State Church to universities, evoked a sense of paranoia and skepticism among the English in the same way Lucifer’s spirits evoked fear in the pious (Chapman, 1881). That paranoia was well-placed. Chapman discusses the “unusual

barbarity” Elizabethan spies used on religious deviants (i.e., Catholics and recusants)—sometimes without any real evidence of a crime (pp. 24, cf. Riggs, 2004). Moreover, the Privy Council, for whom spies worked, was known for scapegoating its intelligencers (Riggs, 2004).

Besides the political and religious turmoil, Elizabethans were moving towards an independent introspection that allowed them to marry traditional religious notions of humanity with syllogistic ones. *Faustus* is rife with the tension between these two processes of creating self, but it is hard to overlook the fact that Faustus never abandons his accountability to God despite the implied superiority of syllogism. This unbreakable bond argues with the assertion that this play is a commentary on atheism (Ornstein, 1968), but it also compels Faustus, Marlowe, and, ultimately, the audience, to admit that syllogism does not supersede religious ideals, and, therefore, cannot justify one’s choice to become a spy. Perhaps Marlowe’s recognition of humanity’s responsibility to God is why he chose Faustus—who violated God’s commandments so rebelliously that the legends about him insist his fate was deserved—to deliver a morality play whose central message can be interpreted as a warning against enlisting in Walsingham’s spy network.

There have been many attempts at compiling a cogent and accurate biography of Christopher Marlowe that suggest he was a spy or, at least, peripherally involved with spies, but these attempts almost always end with an equivocation that the evidence is circumstantial at best. However, I assert that Marlowe’s treatment of Dr. Faustus implies an intimate and first-hand knowledge of not only the emotional turmoil and constant threat spies lived under but of the irrevocable and loathsome commitment to which they were bound. Further, the nature of Marlowe’s murder and the subsequent release of his murderers and his friendship with Sir Francis Walsingham’s nephew, Thomas Walsingham, who was an intermediary, upholds the theory that he was, at the very least, acquainted with the spy community (Riggs, 2004).

John Faustus was a college graduate, educated in the fine art of syllogism and “glutted” with “learning’s golden gifts,” but being full of “sweet *Analytics*” was not enough for Faustus (Prologue 24; 1.6). For all of his intelligence and learning, and despite his many episodes of regret and uncertainty, Faustus refuses to acknowledge

the horror that awaits him. His theological acquaintance breeds uncertainty—the Good and Bad Angels pulling his conscience in different directions—but he stays his course, rejecting the redemption that repentance freely offers for the superior rewards of syllogism (5.201–208). Faustus’ choice to join Lucifer is an obviously bad one, but I disagree that Faustus “makes a mistake which ninety-nine out of a hundred people would not be stupid enough to commit” (Hopkins, 2000, p. 68). Marlowe’s Faustus is less nefarious than the Faust of legend because Marlowe wanted his audience to see something of themselves in Faustus so that they could recognize their own errors of rationalizing apparently bad decisions. Marlowe’s message is clear: do not rationalize the choice to serve Walsingham but choose God—or goodness—instead. Though syllogism may breed promises of greatness, Marlowe never lets Faustus accomplish that, and Faustus is in the end only as much as he was in the beginning.

While contemplating his choices, Faustus refers to the Latin phrase “*on kai me on*,” which is translated as “being and not being” (1.12; Greenblatt, 2018, p. 681). That phrase defines an intelligencer. An intelligencer sets aside his true self to become the character who can complete the task of gathering intelligence. He does not, however, completely abandon his natural persona, returning to it when the mission is complete, therefore existing as two people. The duality causes Faustus to reflect upon Justinian law, which says that two people cannot possess the one thing to which they are entitled, and one person must be satisfied with something else equal to the first (Marlowe 1.27; Greenblatt, 2018). So, according to Justinian law, both personas of a spy are equally entitled. However, Faustus recognizes that the law is “paltry” because the dichotomy of a spy’s personas creates an unresolvable conflict (1.30). It is not possible to be a scholar and a spy because it is not possible to achieve equal success in each endeavor as one will inevitably devour the other. The time spent undercover would infringe on time for discovering “some wondrous cure,” and the reputation of spying for the Queen would overcome the reputation of a good and trustworthy physician (1.14–20).

Faustus’ involvement with spirits—working for them, with them, and becoming one of them—is an extended metaphor for his involvement with the intelligence community (5.96–105). Mephistopheles, the middle man between Lucifer and Faustus, is a version of Thomas

Walsingham who was “an intermediary between Sir Francis and field agents” (Riggs, 2018, p. 144). In addition to acting as the intermediary, Mephistopheles is a recruiter who enlists for Lucifer’s kingdom the “glorious soul” who “rack[s] the name of” Lucifer’s enemy (3.49). In Elizabethan society, recruiters often visited universities in search of candidates—a fact that parallels Faustus in that one could argue that he was not far removed from university when he considers whether to begin his career as a doctor or a necromancer, the implication being that he was introduced to necromancy while at university (Riggs, 2018; Marlowe 1.1–63). Once candidates were identified, they were often lured into the profession with promises of “great rewards,” and Mephistopheles’ promises are great, indeed (Hammill, 2008, p. 291). He promises to never withhold information (except as it relates to God, the enemy), give all that Faustus asks (except a wife), and be all that Faustus wants him to be (5.97–100). Moreover, Mephistopheles agrees to help him obtain riches (which Faustus never obtains), protect him from his enemies (but not his colleagues), and help his friends (3.92–96). With respect to riches, Riggs (2004) notes one recorded account of a spy complaining of never receiving a cent for his work, and Marlowe, one could argue, was murdered by colleagues from the intelligence community.

Nevertheless, a spy’s loyalty had to be absolute. In Faustus’ world, this loyalty is as much vocal as it is physical. He was placed under a strict censorship order, forbidden to invoke the name of God or seek redemption through him, and even Mephistopheles is prohibited from answering certain questions because it required him to speak “against [his] kingdom” (5.254). Vocal expressions of loyalty are not enough, though, and Mephistopheles requires a binding contract to enforce Faustus’ commitment (5.36). Once the agreement is made, there is simply no escape from it. Spies sought to gather information at all costs, and information is a commodity Faustus craves (1.20; 5.244). Once acquired, however, spies inadvertently made their physical bodies the property of the throne because they stored the information they gathered within themselves. England owned them. Faustus clearly understands this and reminds himself throughout the play that he cannot undo his choice—he is beyond redemption (5.106–109).

Faustus’ and Mephistopheles’ encounter with the Pope contradicts earlier enforcements of censorship. Typically, when Faustus invokes

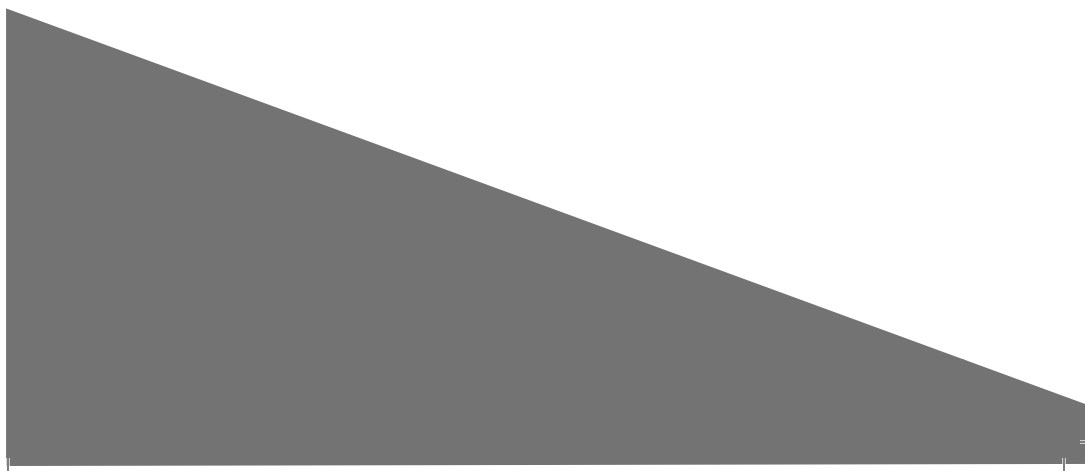
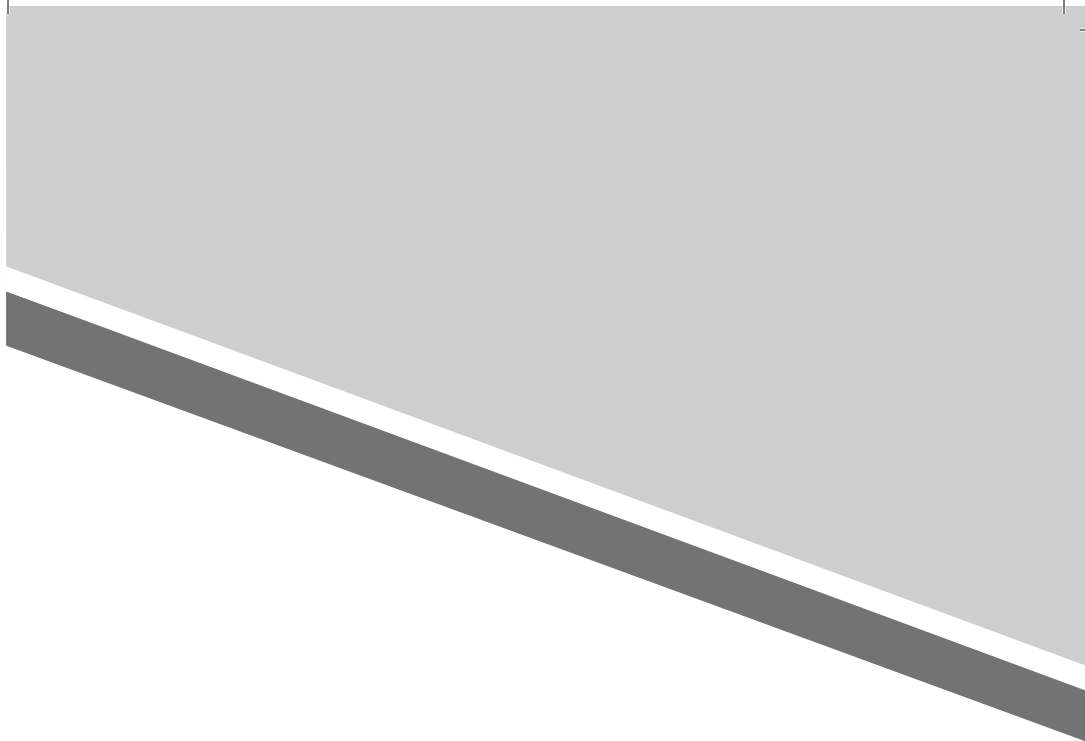
the name of God, Mephistopheles complains and threatens. However, when the two visit the Pope, there is no reaction to the Pope's prayers or signs of the cross (7.75–98). This scene is an indication that Marlowe felt the English possessed religious superiority (or felt compelled by censorship to say so), which reinforces the claim that Marlowe's criticism is of the spy industry and not necessarily of theology. Mephistopheles refuses to recognize the righteousness of Catholics, and their calls upon deity generate no reaction from him, suggesting that he refuses to acknowledge their membership in God's kingdom—an attitude similar to that of Elizabethan spies. This scene is also a commentary on the war the spies were waging against Catholicism, demonstrating that Elizabeth's network of spies really had little impact on leaders of the Catholic church. Faustus' jabs at the Pope are juvenile and half-hearted and have little effect on him, suggesting that the intelligence community was not hindering the propagation of Catholicism at all, a fact easily seen today. Further, the presence of the seven deadly sins represented by fellow spirits, prior to this scene, as entertainment is an illustration of the hypocrisy of Walsingham's army (5.281–327). Spies were employed to spy on recusants and Catholics as if their Protestant affiliation imbued them with moral superiority. Yet, Lucifer and Faustus find the sins of pride, gluttony, covetousness, wrath, lechery, sloth, and envy highly entertaining.

The fact that Faustus' life is shortened because of his contract with Lucifer highlights the danger of living the life of a spy. Though they were often protected for their work (cf. Greenblatt, 2018), spies were dispensable tools of the Queen whose handlers were quick to scapegoat and/or murder them (Riggs, 2004). Intelligencers were aware of their mortality, living under the constant danger of capture, torture, and death either by the governments on which they spied or the council for which they worked. Faustus is clearly aware of his end at the onset of his contract, which seems to contradict the idea of employing syllogism in his decision-making process. Perhaps, this perspicuous detail is Marlowe's way of driving home the gross fallibility of weighing logic against decency. Spies give up so much to be spies—safety, recognition, family, self—and their lives parallel Mephistopheles' whose hell follows him to wherever he is, which hell signifies to the audience the futility and incorrigibility of becoming a spy (3.76).

Riggs (2004) believes that Marlowe “projected his predicament onto his protagonist” (p. 249). Hopkins (2000) asserts that Marlowe’s plays show that “the external landscape is always coloured by the landscape of the mind” (p. ix). Hammill (2008) argues that Marlowe “thinks through the contemporary political terms by which the inner workings of political crises can be grasped, and he displays those inner workings in literary form” (p. 292). Though they were not arguing that *The Tragical History of Doctor Faustus* was a criticism of the Elizabethan spy regime, I agree that Marlowe’s personal acquaintance with and awareness of “political crises” “coloured” his perception of the intelligence community and compelled him to “project [that] predicament” onto Dr. Faustus. The intense emotion of the final soliloquy represents his acute understanding of what it felt like to embark upon a questionable and irredeemable course in life. Marlowe’s treatment of Dr. Faustus demonstrates an unambiguous statement of the indefensible nature of recklessly abandoning one’s soul to the employment of intelligence.

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The Effect of Purchasing Textbooks on Student Performance

Author

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Abstract

This research analyzes the relationship between student textbook purchases and final grades earned. The study surveys students in English 2010, Economics 2010, and Physics 2210. The class Physics 2210 will act as a control for this study as this class requires the use of Open Educational Resources.

Students were surveyed at Weber State University and were asked if they had purchased the textbook for their class. This data was then sent to the Institutional Research Office on campus where additional data about the student was then tied to their responses. This data included the student's grade in the class, the semester the student took the class, and the student's overall GPA. The impact of having the class textbook on course grades is dependent on whether the analysis included GPA. See Results section for additional information.

Introduction

Prices of required class materials could be acting as a barrier to entry for college education, causing students to not enroll in classes that require costly materials. The U.S. Government Accountability Office (GAO) released a report in 2013 that showed real textbook costs rose 82% from 2002 to 2012, alongside an 89% increase in tuition and fees during that period (GAO, 2013).

It is important to note that Open Educational Resources (OER) can serve as a substitute to required textbooks. OER are educational materials, either available online for free or in print for a reduced price. OER have gained enough attention to warrant federal legislation be drafted to address the topic in The Affordable College Textbook Act H.R. 3840/S. 1864 (SPARC, 2018). The purpose of this legislation is to create a grant that encourages higher education to pursue and invest in

creating and promoting OER on college campuses.

This research analyzes the relationship between student textbook purchases and class grades. The study surveys students in English 2010, Economics 2010, and Physics 2210. The class Physics 2210 will act as a control for this study as this class requires the use of Open Educational Resources.

Literature Review

While the financial impact of textbooks is what makes this research relevant, the focus is primarily on what educational impact textbooks have on students. Brigham Young University (BYU) also recently published research that examined the reception of OER, focusing on faculty perceptions. Most of the faculty were willing to look into the possibility of the material but were skeptical if the material would be sufficient. This research also found that 20.6% of students reported registering for fewer classes because of the cost of materials, and that 33.28% of students reported delaying enrollment in courses for the same reason (Martin, Belikov, Hilton, Wiley, & Fischer, 2017).

BYU's research is significant to this study as a reference paper. Textbook costs have increased significantly which will impact how many students purchase textbooks for classes. Weber State University's (WSU) student body is 56% non-traditional students, according to Weber State's student body homepage: these students likely have greater financial strain in attaining higher education.

In a study conducted in California, the authors collected data about classrooms that had supplemental learning materials readily available to students versus classes that did not (Oakes & Saunders, 2002). The authors found that there was a relationship between students performing well in class and having the additional materials. These findings are significant to the purpose of this study; however, this research only examined high schools and it included supplemental materials rather than just required textbooks.

Other important factors to note for this research are the format of the textbook utilized by students. While research undertaken at Suffolk University found that there was not an impact on students' ability to learn from a digital textbook versus the traditional physical copy,

they did find that students preferred a physical textbook to a digital copy. The study cautioned, however, that as it was an ongoing study each academic year, student preferences did adjust more favorably in the direction of e-textbooks over time (Weisber, 2011).

Similarly, another study that looked at the impact on learning when using an interactive homework tool found that students seemed to have improved scores compared to other classes that did not use the interactive homework but were taught the same material (Qin Sun, Norman, & Abdourazakou, 2018).

Theory

This research utilizes the theory of utility maximization. This theory suggests that students choose between activities or purchases that result in the greatest received utility. Utility is maximized when the ratio of marginal utility divided by price is equalized for all goods. In the context of textbooks, if the marginal utility received from purchasing the required textbook divided by the cost of purchasing the textbook is less than the marginal utility of another good divided by the cost of that good, students will choose the other good over purchasing the textbook.

Another applicable economic theory is the law of demand. The law of demand states that as the price of a good increases, consumers will buy less of it. We would expect under this theory that as the prices of textbooks increased students would purchase fewer, leading to the theory of substitute goods. Students who are unable or unwilling to purchase textbooks at the new higher market prices would normally have created a market for substitutes. However, this unique market poses the problem that the consumers are not selecting the materials to purchase. There have been some professors who have taken note of the increased burden to students and have elected to use OER materials for their classes.

Economic Model

The model used in this research to measure the possible relationship between variables in a linear regression for the academic year 2017–2018 is modeled by the equation:

$$Y_{it} = \alpha_{it} + \delta T_{it} + \beta \chi_{it} + \omega_{it}$$

Where the dependent variable Y_{it} is the student's grade in the course for which they were surveyed, α_{it} is the y-intercept, δT_{it} indicates if the student has access to the textbook, $\beta \chi_{it}$ is a vector of control variables, and ω_{it} is the idiosyncratic error term. Control variables include: gender, students' overall GPA, if they felt having the textbook impacted their grade, class standing, students' method of access to the material, and the average textbook cost.

Data

The data for this project was collected first by surveying students that were enrolled in either ECON 2010, ENGL 2010, or PHYS 2210. Students were asked to answer questions about whether they purchased the textbook and if they felt having the textbook impacted their grade in the class, an estimate of the cost of the textbook if they did purchase it, and class standing. Additional data was provided by the university's Institutional Research Office (IRO): grades in the class, overall GPA, the semester that respondents took the class, and gender. A list of the variables along with a description can be found in Table 1, and statistics regarding the variables in Table 2.

Results

Of about 4,000 students surveyed, 569 replied to the survey (14.2% response rate). However, 77 of the responses had incomplete surveys and were not included in the regression, leaving a sample of 492 responses. Of the classes surveyed, 77% of respondents were enrolled in ENGL 2010, 15% were enrolled in ECON 2010, and 7% were enrolled in PHYS 2210. Of the respondents, 61% personally purchased the required textbook, 14% purchased the textbook using financial aid or scholarship money, 9% had acquired the textbook by other means, and 15% did not purchase the textbook. Of the respondents that did not purchase the textbook, 57% said that they did not feel that not having the textbook impacted their grade. Of the students that purchased the textbook, 12% felt the textbook definitely did not impact their grade and 24% felt it definitely impacted their grade, with the rest feeling somewhere in the middle.

An independent t-test was run to test for statistical significance within the model of differing classes that students were enrolled in. The results showed that the difference in mean price between the classes was significantly different using an unequal variances (P-value for two tail being .022 and right tail being .011). A chi squared test was done as well but found no significance on purchasing habits between the classes.

If GPA is not included as a control variable, not having access to the textbook is significant. Not having the textbook decreases grade points by .37 on a 4.0 scale (see Table 3). This could be problematic as not including it creates an omitted variable issue, because there are other factors that impact grade that are not being accounted for.

When including GPA, the only variable of significance was overall GPA. The argument for not including overall GPA in the model could be that it creates an issue of multicollinearity, since students would have been faced with the issue of purchasing or not purchasing the textbook for those classes, which could have impacted their grades in those classes as well.

Conclusion

The results depend on if you include overall GPA in the model. The argument could be made that including overall GPA accounts for student ability. So not including the variable is a challenge as you would expect that students who have a natural ability would perform better and it creates an issue of endogeneity when it is not accounted for. However, including overall GPA creates an issue of multicollinearity, where overall GPA is impacted by the focus variable. It does appear that higher-performing students do have access to the textbook, but this variable does not measure if students used the material to study. These results could be used to suggest that the availability of OER may be a good avenue for faculty to ensure all students have access to the material.

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Appendix

Table 1

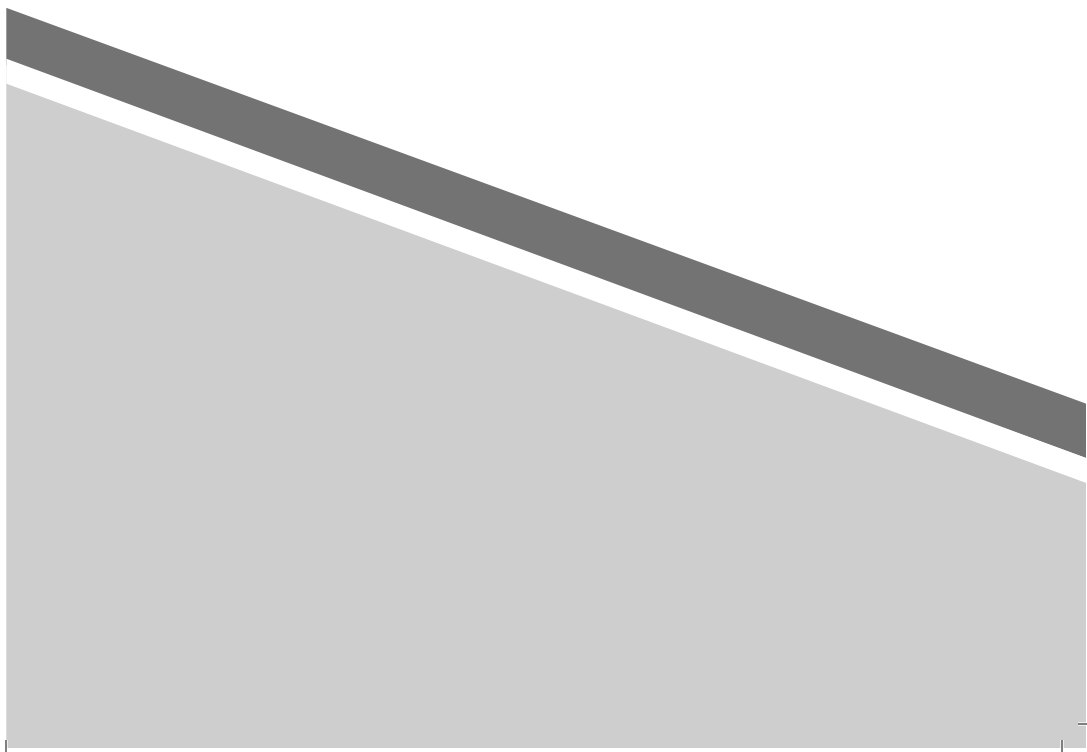
Variable	Description
Gender	Respondents self-identified
Class	Respondents selected what class they were enrolled in for the 2017–2018 school year. Classes surveyed were ENLG 2010, ECON 2010, and PHYS 2210.
Access to the Textbook	Respondents selected from four categories: 1- Purchased textbook out of pocket. 2- Used financial aid or scholarship money to purchase. 3- Had access through other means. 4- Did not have access.
Textbook Cost	Students who purchased responded.
Grade Impact	Respondents rated impact on grade.
Class Standing	Respondents selected year in school.
Class Grade	Grade students received in the class provided by IRO.
GPA	Students' overall GPA provided by IRO.

Table 2

Variable	Mean	Variable	Mean
Gender Female Male	63.1% 36.8%	Grade Impact with Textbook Definitely Yes Probably Yes Might or Might Not Probably Not Definitely Not	25.8% 22.6% 15.1% 21.5% 0%
Class ECON 2010 ENGL 2010 PHYS 2210	77.1% 15.9% 6.9%	Grade Impact without Textbook Definitely Yes Probably Yes Might or Might Not Probably Not Definitely Not	2.0% 0% 22.4% 22.4% 53.1%
Class Standing Freshman (yr. 1) Sophomore (yr. 2) Junior (yr. 3) Senior (yr. 4) Senior (yr. 5+)	37.9% 37.9% 17.3% 5.1% 1.7%	Grade Received in Class A: A- B+: B- C+: C- D+: D- F UW (unofficial withdraw) W (withdraw)	66.5% 20.2% 7.3% 2.0% 1.0% .6% 1.8%
Access to the Textbook Yes Purchased out of Pocket Used aid or scholarship Acquired Did not have access	84.2% 61.2% 13.9% 9.2% 15.7%	Cost by Class ECON 2010 ENGL 2010 PHYS 2210	\$107.07 \$67.59 N/A
Textbook Cost	\$72.28	GPA	3.41

Table 3

	Model without GPA		Model with GPA	
<i>Variables</i>	<i>Coefficient</i>	<i>P-Value</i>	<i>Coefficient</i>	<i>P-Value</i>
ECON 2010	-0.091	0.658	-0.013	0.083
ENGL 2010	0.395	0.046	0.002	0.800
PHYS 2210	Excluded	excluded	excluded	excluded
White	0.111	0.290	-0.005	0.200
Non-White	-0.132	0.229	0.005	0.217
Female	Excluded	excluded	excluded	excluded
Male	0.041	0.665	0.004	0.898
No Textbook	-0.370	**0.05	0.001	0.907
Financial aid/ Scholarship	0.018	0.927	0.004	0.571
Personally bought	Excluded	excluded	excluded	excluded
Senior	-0.575	0.097	-0.002	0.904
Junior	0.054	0.783	0.005	0.510
Sophomore	Excluded	excluded	excluded	excluded
Freshman	0.039	0.843	0.002	0.731
GPA	N/A	N/A	1.003	**0.00
Constant	3.217	**0.00	-0.023	0.028
R Squared	0.0503		0.9988	
Adjusted R	0.0283		0.9988	
**P-values significant at the 0.05 level				



Economic Growth In Singapore: What Factors Account For The Miracle?

Author

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Mentor

Nazneen Ahmad

Abstract

This paper investigates the sources of unprecedented economic growth in Singapore from 1960 to 2014. Our results indicate that the most important factor behind the growth in Singapore was increase of total factor productivity. While growth in capital stock and improvement of human capital contributed to the growth of Singapore, the effect of increase in the labor force on growth is found to be less significant.

Introduction

The four Asian Tigers (i.e., Hong Kong, Taiwan, Singapore, and South Korea) achieved global prominence as newly industrializing countries because of their exceptionally rapid industrial growth since the 1960s. All of these economies have since graduated into high-income economies. As one of the original Asian Tigers, Singapore's economic growth is outstanding. According to the Penn World data, the real GDP of Singapore has increased from about \$9,439 million in 1965 to about \$355,857 million in 2014. On average, Singapore had higher real GDP growth rate during 1960–2014 compared to the other Asian Tigers. Figure 1 shows that its real GDP per capita, which is widely accepted as a measure of standard of living, was above the other Asian Tigers during 1960–2014, and has been higher compared to the U.S. real GDP per capita since 2005.

The miracle of growth in Singapore interests us in the story behind the success of the economy. The Solow growth (Solow, 1956) model suggests that growth in capital stock, growth in labor force, and advances in technology all contribute to a country's economic growth. In the Solow model, *capital* is usually interpreted as physical capital. However, economic growth literature has emphasized that human capital is at least as important as physical capital in explaining differences in the standard of living across countries (Galor & Tsiddon, 1997). Human

capital refers to the knowledge and skills workers acquire through education; increase in human capital increases workers' ability to produce goods and services (Schultz, 1961).

This paper aims to investigate which of the above-mentioned factors contributed to economic growth in Singapore from 1960 to 2014. We rely on the growth accounting approach to shed light on the factors behind the economic growth of Singapore.

The remainder of the paper is organized as follows: section 2 discusses relevant studies, section 3 discusses the theoretical background of our study, section 4 discusses the methodology, section 5 discusses data and presents the summary statistics of our variables, section 6 presents our empirical results, and section 7 concludes the paper.

Literature Review

Empirical studies have investigated various factors behind economic growth in Asian Tigers. Young's 1995 findings suggest that it's not technological progress, but increase in labor force participation rates, educational attainment, and intersectoral transfer of labor from agriculture to manufacturing that have contributed to high economic growth in East Asian economies. According to Vu's 2011 findings, during 1965–2008, the average labor productivity's contribution to real GDP growth decreased in East Asia. The contribution of labor force to real GDP growth is found relatively stable at approximately 1.5 to 2 percentage points per year. Vu (2011) also finds that, since the mid-1980s, technology has significantly advanced in Singapore and has become a favorable factor of the country's GDP growth. Lu (2012) suggests that the increase of capital wedge was a primary factor behind the rapid growth in East Asia before the mid-1980s. However, after the mid-1980s, total factor productivity accounts for most of the economic growth in East Asia.

Our study contributes to the literature on the economic growth of one of the Asian Tigers, Singapore, by extending the sample period to 2014 and using a production function that incorporates human capital as a factor of production. Moreover, our paper contributes on measuring the human capital through the human capital index calculated by the Penn World Table 9.0, which measures the expected rate of return on average years of schooling (Feenstra, Inklaar & Timmer, 2015b). In addition to the impact of technology, capital stock, and labor force, our production function

allows us to look into the impact of growth in human capital index on the economic growth of Singapore.

Theory

According to the neoclassical growth model, output (Y) depends on the following: capital stock (K), work force (N), and level of technology (A). Hence, the production function is:

$$Y = A \times F(K, N) \quad (1)$$

Adding human capital (H), the production function is rewritten as: $Y = A \times F(K, H, N)$

Using the Cobb–Douglas production function, our production function takes the following form:

$$Y = AK^{\alpha} H^{\beta} N^{(1-\alpha-\beta)} \quad (2)$$

Where α is physical capital's share of income, β is human capital's share of income, and $(1-\alpha-\beta)$ is labor's share of income.

Methodology

In order to find out the strength and direction of a linear relationship between the real GDP growth and the growth of inputs of production, we calculate the correlation coefficients between the real GDP growth rate and the growth rate of factors of production included in our production function.

While the correlation coefficients between variables suggest the strength of relationship, it does not provide clear information about the impact of factors on growth. To get further insight, we estimate the contribution of factors to growth in Singapore using ordinary least squares method. Our static time-series model is given by:

$$\ln Y_t = \beta_0 + \beta_1 \ln K_t + \beta_2 \ln H_t + \beta_3 \ln L_t + \beta_4 \ln A_t + t + u_t \quad (3)$$

Where β_0 is the constant, and β_1 to β_4 are the estimated coefficients on each factor. Since time series data are used, to control for the possible effect of time trend, we include a time trend variable (t). u_t represents other factors that may affect Y_t . We run a regression using natural

logarithm on both sides which allows us to estimate the effect of rates of change in each factor on the rates of change in real GDP. In order to mitigate the heteroskedasticity problem, we use the robust standard error to calculate the t-statistics.

Data

Due to data availability, the sample period of our study spans from 1960 to 2014, resulting in 55 observations. The annual data of real GDP and capital stock are in millions of dollars at constant 2011 US\$. The annual data of the human capital index, average annual working hours and total factor productivity (hereafter TFP, a measure of technology) are at constant 2011 US\$. All data are collected from the Penn World Table 9.0. According to the Penn World website (Feenstra, Inklaar & Timmer, 2015b), human capital index is measured based on average years of schooling from Barro and Lee (2013) and an assumed rate of returns to education, based on Mincer equation estimates around the world (Psacharopoulos, 1994). Total factor productivity (TFP) is the portion of output not explained by the amount of inputs used in production. The data of TFP gathered from Penn World Table 9.0 reports the levels at constant prices against the reference year, 2011. Table 1 reports the summary statistics of our variables.

Figure 2 shows that both real GDP and capital stock in Singapore continuously increased, while the average annual working hours fluctuated from 1960 to 2014. Figure 3 shows that overall, the human capital index maintained an increasing trend, and total factor productivity increased with fluctuations.

Results

Table 2 reports the correlation coefficients between growth rate of real GDP and growth rate of factors of production. It indicates a weak positive relationship between the growth rate of real GDP and capital stock (0.31) and working hours (0.37). The correlation coefficient between the real GDP growth rate and TFP growth rate is 0.7939, indicating a strong positive relationship. Surprisingly, correlation with human capital index growth rate is -0.1149 indicating a negative relationship between growth rate of human capital index and real GDP.

Results of regression on equation (3) are reported in Table 3.

The predicted value of coefficient on total factor productivity is approximately 1.43. This implies that, on average, all factors held constant, 1% growth in total factor productivity is associated with 1.43% growth in real GDP in Singapore. This result is statistically significant at 1% level, which provides a strong evidence of positive impact of TFP growth on real GDP growth.

Compared to the TFP, estimated coefficient on capital stock and human capital index are smaller but positive. On average, real GDP of Singapore is predicted to grow by 0.77% if capital stock or human capital index grow by 1%, holding other factors constant. The results are significant at 1% level. Thus, the partial effects of growth in capital stock and human capital index on real GDP growth are also evident.

The estimated result on average annual working hours is negative and only significant at the 10% level. The estimated coefficient on time trend variable is insignificant. This implies there is no spurious correlation in this model which makes the estimators solid.

Conclusion

This paper investigates the factors that contributed to the extraordinary economic growth in Singapore from 1960 to 2014. Our results strongly suggest that growth in total factor productivity, which reflects the technology progress, had the greatest contribution to Singapore's economic growth. Our finding is consistent with Vu's 2011 findings on Singapore's economy. In addition to TFP, we find that growth in capital stock and improvement in human capital had significant contribution to the country's economic growth.

A limitation of this study is the use of relatively small samples due to unavailability of monthly or quarterly data. In future, if monthly or quarterly data are available, we would like to separate the sample into different periods to see the relative contribution of factors during different phases of economic growth in Singapore.

The implication of our study is particularly important for developing countries. As our results suggest, to achieve economic growth, a country should invest in technological advancement and human capital.

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Appendix

Figure 1. Real GDP Per Capita

From the Penn World Table 9.0, by R.C. Feenstra, R. Inklaar, and M. P. Timmer, 2015. Licensed by Creative Commons. doi: 10.15141/S5J01T

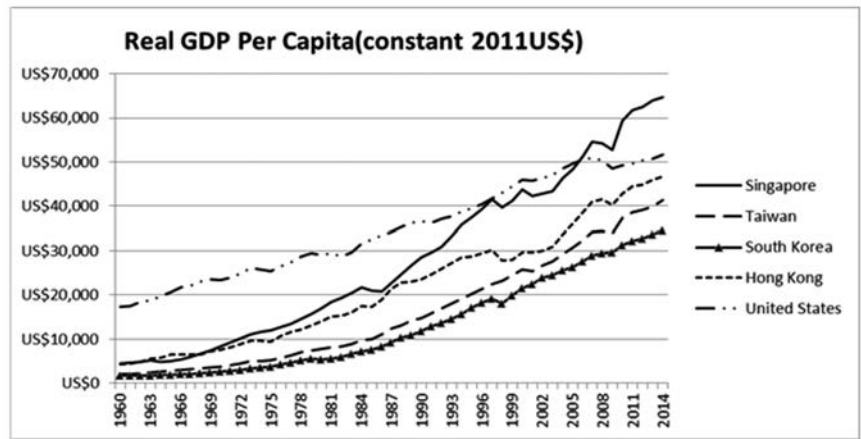


Figure 2. Trend of Real GDP, Capital Stock and Average Working Hours

From the Penn World Table 9.0, by R.C. Feenstra, R. Inklaar, and M. P. Timmer, 2015. Licensed by Creative Commons. doi: 10.15141/S5J01T

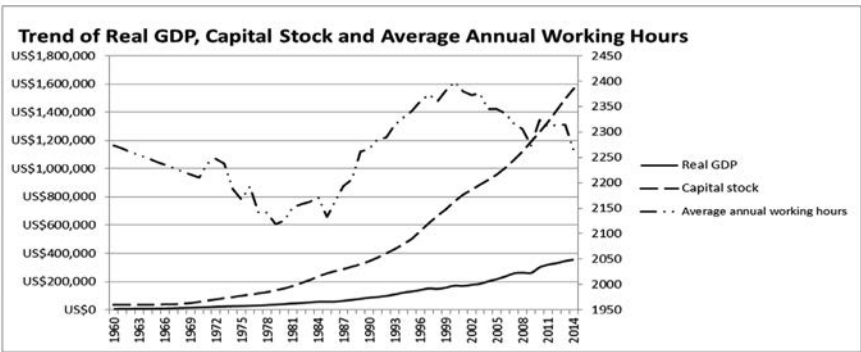
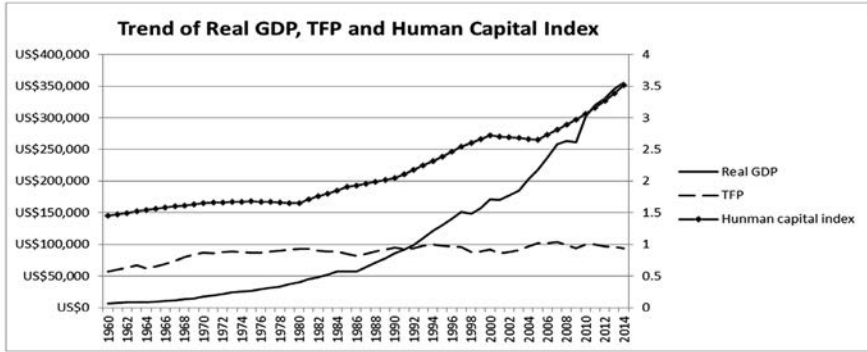


Figure 3. Trend of Real GDP, TFP and Human Capital Index

From the Penn World Table 9.0, by R.C. Feenstra, R. Inklaar, and M. P. Timmer, 2015. Licensed by Creative Commons. doi: 10.15141/S5J01T

**Table 1. Statistical Summary**

Variable	N	Mean	SD	Range
Real GDP (in millions)	55	108,647.01	103,116.13	7192.27 – 355857.5
Capital stock (in millions)	55	462,919.02	452,536.82	33550.02 – 1567K
Human capital index	55	2.15	0.58	1.46 - 3.52
Average annual working hours	55	2260.58	77.94	2118.08 - 2399.22
TFP	55	0.88	0.11	0.57 - 1.04

Table 2: Correlation Coefficient between Growth Rates of Real GDP and Factors of Production

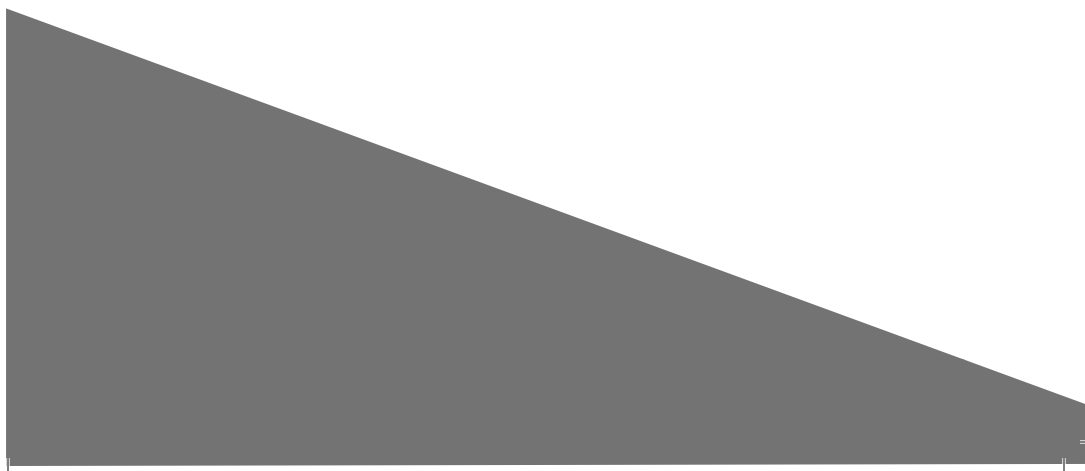
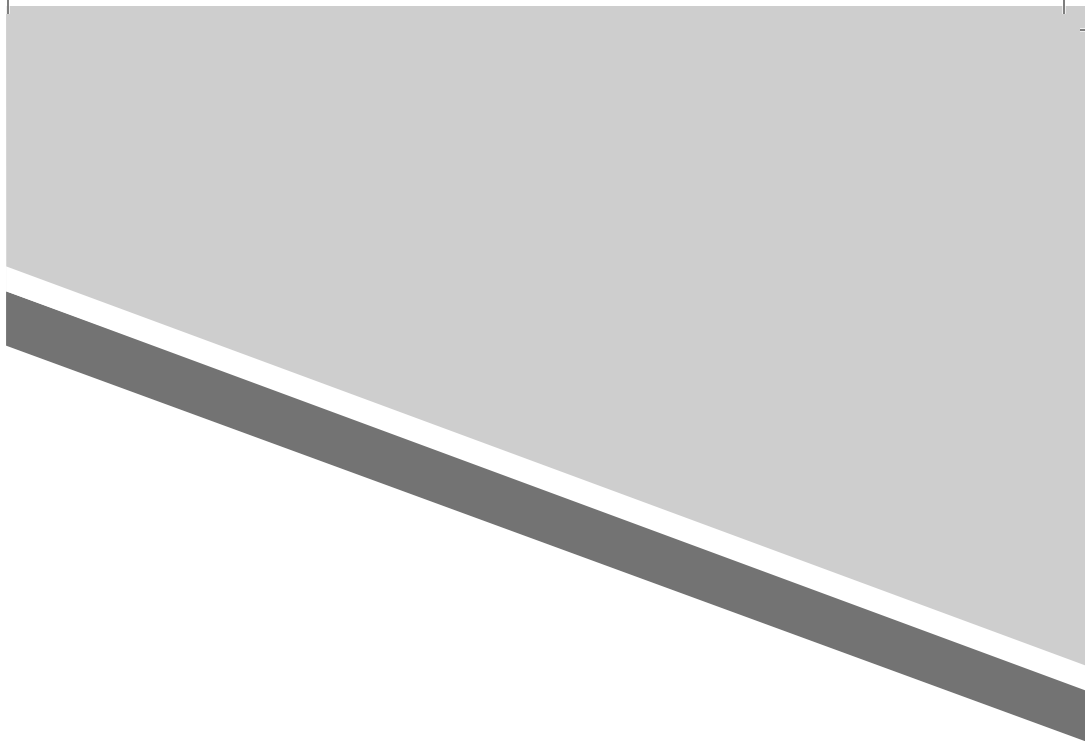
	Real GDP growth rate
Capital stock growth rate	.3137
Human capital index growth rate	-.1149
Average working hours growth rate	.3717
TFP growth rate	.7939
N	54

Table 3: Summary of Regression Results

Independent Variable	Y=ln(real GDP) (3)
ln(capital stock)	.7706*** (22.36)
ln (human capital index)	.7715*** (5.51)
ln(average annual working hours)	-.3826* (-1.91)
ln(TFP)	1.4322*** (31.63)
T	-.0056 (-1.19)
Constant	2.7416** (2.23)
N	55

Robust t-statistics are in parentheses.

***, ** and * indicate that the coefficient is statistically significant at the 0.01, 0.05 and 0.10 levels, respectively.



Marriage, Parenthood, and The Gender Wage Gap: A Utah Case Study

Author

Christopher Martinez

Mentor

Brandon Koford & Gavin Roberts

Abstract

Using the data obtained through the Integrated Public Use Microdata Series from the Current Population Survey (1995–2015), I investigate the effect marriage and parenthood have on the gender wage gap in Utah and compare it to the United States as a whole. The gender wage gap is growing at a faster rate in Utah than the national average. There is an equal percentage change in income with age in both genders and race does not show to be significant in Utah, which may be due to the lack of racial diversity. Education level has a stronger impact on women’s wages than it does for men. Culture may contribute to larger family sizes and could be a key indicator of gender wage inequality in Utah.

Introduction

The conversation surrounding gender wage inequality in the United States has been a topic of interest, and often controversy, for several decades. Overwhelming consensus and studies conclude that women are paid less than men in nearly every industry, irrespective of geography, nationality, education, or work experience. There are also social variables that may have an impact, such as marriage and parenthood. Utah is unique from the United States due to its dominant nationality, religion, large family size, and young age of first marriage. Utah also had the highest Gini Index in 2016, as recorded by the United States Census Bureau, establishing its place as holding the lowest income inequality in the country. However, what this coefficient does not measure is gender income inequality and how marriage and parenthood affect that inequality, the focal point of my study.

Literature Review

Research has shown that men receive a “marriage premium,” increasing men’s average annual income up to 40% more than a

single man of equal education and work experience (e.g., Hill, 1979; Korenman & Neumark, 1991; Antonovics & Town, 2004). Bang and Basu (2012) suggest in their findings that women receive a “marriage penalty,” but rather than a penalty for marriage, many have found that married women also have a wage premium over single women (e.g., Waldfogel, 1998; Killewald & Gough, 2013), just not to the extent that men experience (Petersen, Penner, & Høgsnes, 2014; Magnusson & Nermo, 2017). As they do for marriage, men also receive a “fatherhood premium” (Lundberg & Rose, 2000; Juhn & McCue, 2017), though in some cases it has been negligible (Wilde, Batchelder, & Ellwood, 2010). Mothers, however, have a widely-observed “motherhood penalty,” which increases with each additional child (e.g., Waldfogel, 1998; Budig & England, 2001; Wilde et al., 2010; Anderson, Binder, & Krause, 2002). The premium for fathers and a penalty for mothers accumulates to an observed “family gap.” I continue the studies on marriage and parenthood and its effect on the persisting wage gap.

Data

The data obtained for this study is from the Integrated Public Use Microdata Series using the Current Population Survey (IPUMS-CPS) between the years 1995 and 2015. Included in this data set is the Annual Social and Economic Supplement (ASEC), a supplemental survey prominently used by researchers both in social sciences and in policy. Using the Consumer Price Index Series (CPI-U), I have obtained data on 1,634,681 individuals from 1995–2014. Wages have been adjusted to 2015 dollars to account for inflation.

To best understand the gender wage gap, we need to look at wages as a result of other variables, or as being dependent on other variables. The key independent variables are related to gender, marital status, parental status, and location. I also control for age, work status, race, and highest level of education attained. For the dependent variable, I take the natural logarithm of each individual’s total pre-tax annual income, allowing estimates from the empirical model to be viewed as a percentage change.

I have excluded those who did not report net gains in the previous calendar year. This includes those who reported net losses and those

who worked but reported a zero-net gain. I have confined gender to be of the male or female sex only. I have defined marriage to be married with the spouse present. Being single is described as never having been married, divorced, separated, widowed, or married with the spouse absent. The male variable is assigned the 1 value if the individual is male and 0 if the individual is female. I have created several dummy variables to capture all the possible combinations of marital and parental status.

Methodology

To measure the effect of marriage and parenthood between men and women, I estimate using the Ordinary Least Squares method of linear regression:

1. $\ln(Y_{2015i}) = \beta_0 + G_i M_i P_i \beta_1 + G_i X_i \beta + \varepsilon_i$
2. $\ln(Y_{2015ui}) = \beta_0 + G_{ui} M_{ui} P_{ui} \beta_1 + G_{ui} X_{ui} \beta + v_{ui}$

where Y_{2015i} is the wages earned through income measured in 2015 U.S. dollars. β_0 is the estimated value of $\ln(Y_{2015i})$, when all dummy variables are equal to 0. β is the expected change in $\ln(Y_{2015i})$ for any positive dummy variables interactions. G , M , and P are dummy variables that are independently equal to 1 if the individual is a male, married, and/or a parent. X_i is a vector of individual characteristics, including age, work status, level of education, number of children, number of children under 5, and race, as defined by white or non-white. Subscript U denotes the variable to be Utah-specific.

I hypothesize that, consistent with literature, men will receive a “marriage premium” over single men. I hypothesize that women will also receive a “marriage premium,” but that the average premium will be less than that which a male receives. This smaller premium is often interpreted as a “marriage penalty.” I hypothesize there will be a similar effect with parenthood. Men and women will both benefit, regardless of marital status, but women will see less of a benefit than men:

$$1. \quad H_0: G_M M_1 P_1 = G_M M_0 P_0$$

$$H_A: G_M M_1 P_1 \neq G_M M_0 P_0$$

$$2. \quad H_0: G_F M_1 P_1 = G_F M_0 P_0$$

$$H_A: G_F M_1 P_1 \neq G_F M_0 P_0$$

$$3. \quad H_0: G_M M_1 P_1 = G_F M_1 P_1$$

$$H_A: G_M M_1 P_1 \neq G_F M_1 P_1$$

Where gender, G , is indicated by subscript M when the gender is male and F when female. Marriage, M , is designated with 1 when married and 0 when single. Parenthood, P , is also denoted by 1 and 0 when the individual is a parent or non-parent, respectively. Where P or M is omitted, the interaction hypothesis holds regardless of parental or marital status.

When relating the data to Utah, I hypothesize that all marital and parental interactions will see similar effects as that seen in the United States as whole, but at a smaller scale due to Utah's unique demographics.

Results

In line with my hypothesis, marriage has a positive and statistically significant impact on wages for both men and women, allowing me to reject the null hypothesis that marriage does not significantly affect wages. Where married men make an average of 24.65% more than single men, married women only make an additional 10.77%. Men also receive a larger premium with parenthood. On average, a single father receives 17.74% more than a single, non-parent male and a single mother makes 14.72% more than a single, non-parent female. This indicates parenthood may lessen the wage gap among single men and women. When incorporating both marriage and parenthood, the gender wage gap again becomes more prevalent. The gender wage gap among married individuals is 13.88%.

Also consistent with my hypothesis, marriage has a positive and statistically significant impact on wages for both men and women

in Utah with married men making on average 22.63% more than single men and women making an additional 7.71%. This indicates a 1.04% larger gender wage gap in Utah when accounting for marriage. Parenthood in Utah has a much different effect. Single mothers in Utah actually receive a 21.25% premium, compared to men's 19.12%. Similar to the observations in the rest of the United States, married fathers receive a far higher premium than married mothers.

Conclusion

The purpose of this study was to identify and test the magnitude of marriage and parental status in relation to the gender wage gap. I also aimed to relate those findings and their effects to compare Utah with the United States as a whole. I found that transitioning from a single individual to a married individual to a married parent provides the most consistent path of wage growth. Although both genders receive premiums with these increments, it does not always lessen the gender wage gap. In the United States, the gap increases with marriage and again with children. In Utah, however, the parenthood premium that women receive has a much stronger impact than it does for men and decreases the gender wage gap. Ultimately, the gender wage gap continues to grow at a quicker rate in Utah than the average state in the United States.

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Appendix

Table 1a. Premiums Over Single Non-Parents - United States

	Married Non-Parent	Single Parent	Married Parent
Male	0.2465***	0.1774***	0.3608***
Female	0.1077***	0.1472***	0.2169***
Difference	0.1388	0.0302	0.1439

Table 1b. Premiums Over Single Non-Parents – Utah

	Married Non-Parent	Single Parent	Married Parent
Male	0.2263***	0.1912***	0.3669***
Female	0.0771***	0.2125***	0.2407***
Difference	0.1492	-0.0213	0.1262

Table 2. Total Pre-Tax Annual Income (1995–2015) — United States

United States			
Variable	Observations	Mean ¹	Maximum ¹
Male	845,983	\$58,800.79	\$1,791,289
Female	790,698	36,820.65	\$1,301,559
All	1,634,681	\$48,168.96	\$1,791,289
Utah			
Variable	Observations	Mean ¹	Maximum ¹
Male	12,570	\$55,889.09	\$1,135,529
Female	9,948	\$30,394.02	\$ 619,070
All	22,518	\$44,625.88	\$1,135,529

¹Wage Income in U.S. Dollars

Table 3. Dummy Variable Observations (1995–2015)

United States				
	Single no children	Married no children	Single with children	Married with children
Male	255,083	150,651	50,685	387,564
Female	212,217	139,361	137,279	301,841

Utah				
	Single no children	Married no children	Single with children	Married with children
Male	3,050	1,898	564	7,058
Female	2,368	1,645	1,449	4,486

Table 4. Estimated Regression Coefficients

Variable	United States		Utah	
	Male	Female	Male	Female
Single Non-Parent	0.0645*** (0.0073)	- -	0.1266* (0.0672)	- -
Married Non-Parent	0.3110*** (0.0080)	0.1077*** (0.0028)	0.3529*** (0.0712)	0.0771*** (0.0276)
Single Parent	0.2419*** (0.0084)	0.1472*** (0.0037)	0.3178*** (0.0774)	0.2125*** (0.0340)
Married Non-Parent	0.4253*** (0.0081)	0.2169*** (0.0035)	0.4935*** (0.0716)	0.2407*** (0.0310)
Intercept		8.0590*** (0.0051)		8.0673*** (0.0521)
Sample Size	1,643,681		22,518	

Robust Standard Errors are given in parentheses.

Dependent variable: Log of wages.

***, **, and * Significant at $p < 0.01$, $p < 0.05$, and $p < 0.1$, respectively

Medical Marijuana Legalization and the Effect Upon Drug-Related Mortalities

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Abstract

This study estimates the effect that medical marijuana legalization (MML) has on state drug-related mortality rates using state panel data and difference-in-difference regression analysis. The results of the study indicate an increase of 3.548 state-wide drug overdoses per every 100,000 state citizens within states that adopt a comprehensive medical marijuana legalization policy. These results may allude to a complementary relationship between the use of medical marijuana and the use of illicit drugs.

Introduction

On October 24th, 2018, President Donald Trump signed bipartisan legislation to combat the growing opioid epidemic. However, opioids are not singularly responsible for the increased rate of drug overdoses; other drugs, such as cocaine and benzodiazepines (the chemical used in pharmaceuticals such as Valium), have contributed to the rise in mortalities. The drug-related mortality epidemic has reached such alarming levels that some experts believe it to be a major cause in the continuously sinking average life expectancy in the United States (Woolf, 2018). More alarming still, drug-related mortalities seem to coincide with the trend of state MML.

This study seeks to understand the relationship between MML and state drug overdose death rates in the United States. Largely influenced by the Cole Memo of 2012, which instructed federal prosecutors to not enforce federal marijuana laws in states with MML, this study analyzes state panel data for the periods 2010–2016, using a two-way fixed effects difference-in-difference model.

State MML programs vary greatly and range from policies that

allow for CBD oils exclusively for the most severe cases of epilepsy to policies that allow for the growth of cannabis plants within the private residences of citizens who have obtained a medical recommend (National Conference of State Legislatures, 2018). In this study, we define MML as state legalization policies that are comprehensive, as defined by the National Conference of State Legislatures (NCSL). According to the NCSL, a comprehensive MML program includes (1) protection from criminal penalties for using marijuana for a medical purpose, (2) access to marijuana through home cultivation, dispensaries or some other system of accessibility, (3) allowance of a variety of strains, including those of more than “low THC,” and (4) allowance of either smoking or vaporization of some kind of marijuana products. In defining MML in such a way, we have limited the scope of state legalization to those instances that provide for marijuana product offerings that can intoxicate users, and theoretically serve as either a substitute or complement to illicit drugs.

Literature Review

Using a difference-in-difference model, Bachhuber, Saloner, Cunningham, and Barry (2014) explored the relationship between MML and opioid analgesic mortality rates in the U.S., estimating a 25 percent reduction in the opioid-related mortality rate attributed to MML. Following the Bachhuber study, Powell, Pacula, and Jacobsen (2018) reaffirmed this relationship, while identifying the allowance of medical marijuana dispensaries as the key feature of an MML that affects opioid overdose death rates.

The relevant literature has identified a variety of additional benefits of MML in the realm of opioid use and abuse, yet we have not found any empirical papers analyzing the impact of MML on drug-related mortalities in general. This paper will contribute to the cumulative knowledge of the effects of MML by providing empirical evidence concerning the effects of such a policy on all illicit drug overdoses.

Model

As is common in policy analysis, a difference-in-difference model is employed to compare the difference in mortality rates among states who adopt MML against states who have made no such policy

adoption. Drug related mortalities are regressed as a function of MML with a general equation that can be expressed as so:

$$Y_{st} = \beta_0 + \beta_1 (MML_s \times After_t) + X\delta_{st} + \alpha_s + \tau_t + \mu_{st} \quad (1)$$

Y_{st} denotes the annual statewide drug-related mortalities per 100,000 state citizens. MML_s is a binary variable equal to 1 if the state has legalized medical marijuana, or equal to 0 otherwise. $After_t$ is a binary variable equal to 1 in years MML is present for the whole year, or 0 otherwise. The interaction term $(MML_s \times After_t)$ denotes the explanatory variable of interest, and will equal 1 when both MML_s and $After_t$ equal 1; so, the parameter β_1 represents the isolated effect that state MML has on Y_{st} after the initial year of the policy implementation, on average and with the control variables held constant. Simply stated, the study analyzes the effects that MML has on state drug overdoses only upon the first full year of implementation and onward using states that did not adopt MML as statistical controls.

The empirical model also includes state and year fixed effects. The parameter α_s captures the state-specific fixed effects, constant in a state across time. For instance, if the state of Alaska has a prevalent cultural factor that affects our metric of drug abuse in the years 2010 through 2016, but that same cultural factor is not prevalent in the state of Alabama, then this state unobserved factor can be controlled for. Likewise, the parameter τ_t , captures year-specific fixed effects across all states. This is especially useful in controlling for national and regional trends such as the ongoing opioid epidemic.

Following Powell et al. (2018) we include the annual average unemployment rate as a control to serve as a proxy for state economic conditions. Furthermore, we include the percentage of residents living below the state poverty threshold and the percentage of residents lacking medical insurance in an attempt to further explain the variance of state drug overdoses caused by macroeconomic conditions. Demographic information that has been identified by the National Institute on Drug Abuse (2015) as being statistically significant has been included as well. According to the National Institute on Drug Abuse, males and persons aged 15–29 are more likely to use drugs. Using a nationwide survey of self-reported illicit drug use, the institute estimated 22.6 percent of young adults aged 18–20 partook of illicit drugs, and every incremental year within the high-use age

range was within 4 percentage points of 15 percent illicit drug use. The proportion of users aged in the late 30s, the second highest proportion, in contrast reported only 9.3 percent illicit drug usage. The proportion of the population that is male, although having a greater variance at the local and county levels, has some variance at the state level, and is therefore included to better control for the variance of state drug overdoses that is unrelated to MML.

Data

The data has been gathered for the years 2010 to 2016. We believe the effects of MML will be more prominent within this timeframe than in years prior to the Cole Memo, when federal intervention was more common in state MML attempts. In these years, 11 states and the District of Columbia have periods both before and after initial implementation of MML; 13 states were legal through all periods of the study, and 26 states implemented no such policy throughout the said periods.

Drug-related mortality figures were obtained from the Center for Disease Control's Multiple Cause of Death, 1999–2017 database (MCOD). The database consists of U.S. residents' cause of death, as defined by death certificates, by county (Centers for Disease Control and Prevention, 2018). We then combine the MCOD data with the Census Bureau annual population data to derive our dependent variable, state drug-related mortalities per 100,000 state citizens.

A list of states that have currently adopted a comprehensive MML policy can be found at the National Conference of State Legislatures website (2018); however, in order to identify the year of adoption or the year the state medical marijuana legalization policy became comprehensive, every state MML policy had to be reviewed individually.

State unemployment rate information is sourced from the Bureau of Labor Statistics (2018) as monthly rates. We have derived the annual average of the monthly unemployment rates to serve as an annual economic performance proxy for each state. Hollingsworth, Ruhm, and Simon found that macroeconomic conditions have a significant effect upon opioid abuse (2017). In this study, the annual average unemployment rate proved significant in explaining total drug-related mortality as well.

Population statistics are sourced from the Census Bureau (2018). The figures for age, sex and total population are estimations for July 1 of each year. The figures are converted into percentages that serve as the model's demographic control variables. Summary statistics for the variables used in this study are contained in Table 1.

Empirical Results

The MML coefficient estimate of 3.5477 implies that states with MML adoption can expect drug-related mortality rates to increase by 3.548 persons per every 100,000 state citizens per year. This is more than a 22 percent increase from the national mean of 15.88 deaths per every 100,000. This estimate, as well as the proportion of the population lacking medical coverage estimate, is significant at 99 percent confidence. The unemployment rate and the proportion of the population of high-use age are significant at 95 percent confidence. All of the significant variables have a positive effect on drug overdoses. Estimation results and standard errors from the empirical model are contained in Table 2.

Using the Breusch-Pagan test for heteroskedasticity, problems of heteroskedasticity were discovered in the following variables: the proportion of the population living below the state poverty threshold, the proportion of the population that is of the high-use age, and the proportion of the population that is male. To better account for this presence of heteroskedasticity, heteroskedasticity robust standard errors are employed in the regression.

Discussion And Conclusions

Legalization of medical marijuana has a positive effect on drug-related mortalities. When considering why this might be, we consider the possibility of a complementary relationship shared by medical marijuana use and illicit drug use. In contrast, in the advent of MML, marijuana and opioids can both be professionally recommended by a doctor to remedy the same ailment (physical pain relief), placing the drugs in a more immediate competitive state within the medical arena. In order to draw conclusions concerning the *why* of this relationship, however, more research is ultimately needed.

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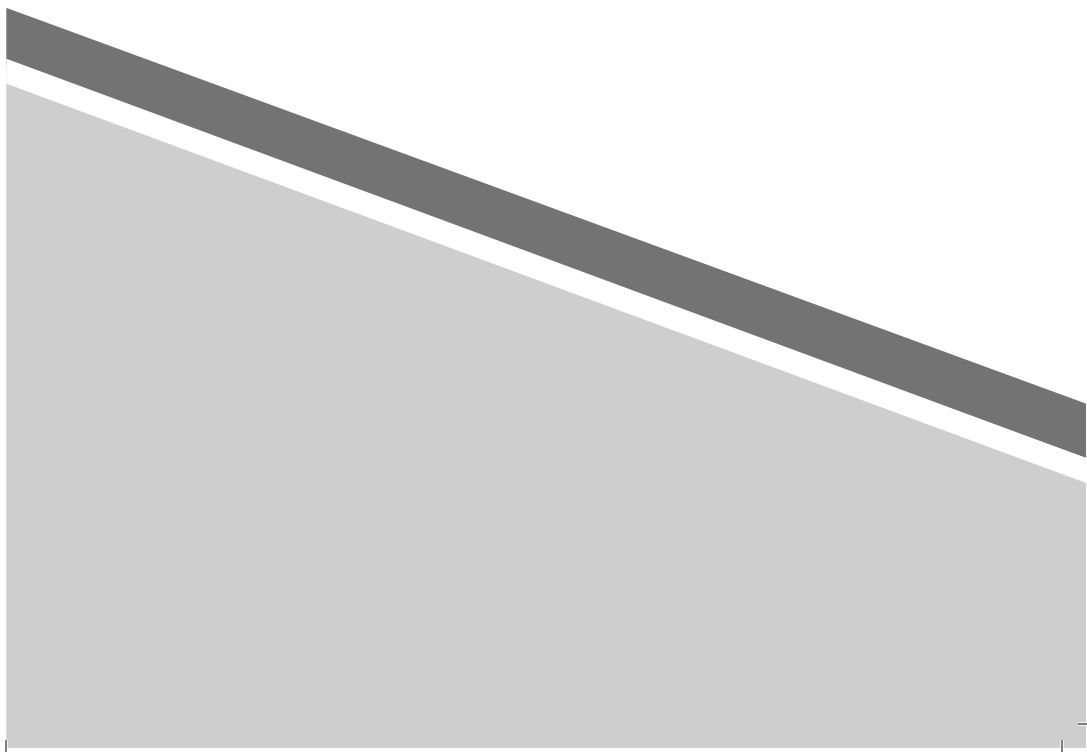
Appendix

Table 1. Summary Statistics

Dependent Variables	Mean	SD
Annual Drug-Related Mortalities	15.88	14.74
Independent Variable	Mean	SD
MML	0.36	0.48
Control Variables	Mean	SD
Annual Average Unemployment Rate (%)	6.66	2.13
Poverty Rate	13.76	3.43
Population Lacking Medical Coverage (%)	11.71	4.46
Male Population (%)	0.49	0.008
User-Age Population (%)	0.21	0.01

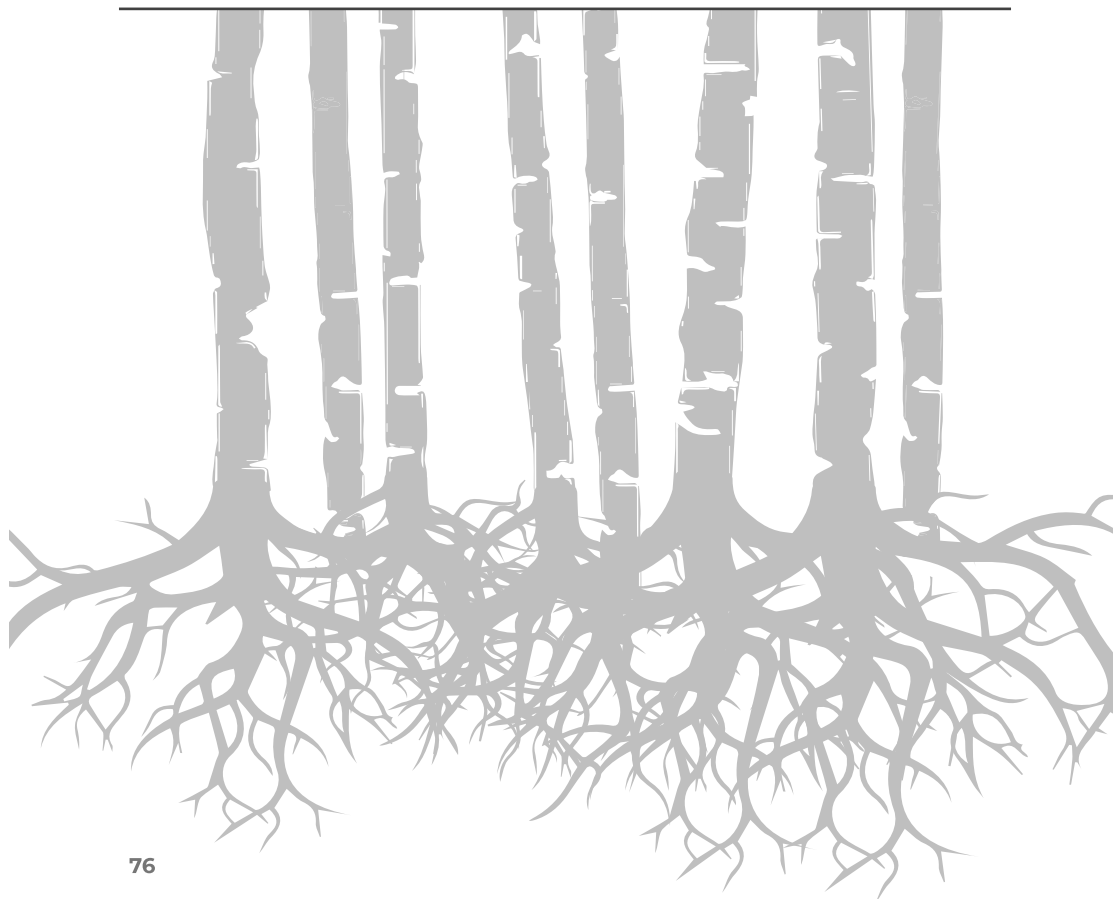
Table 2. Effect of MML on the State-Wide Drug-Related Mortality Rate

Variables	Mortalities
MML	3.548*** [0.000]
Annual Average Unemployment Rate (%)	-0.518** [0.042]
Poverty Rate	0.041 [0.763]
Population Lacking Medical Coverage (%)	0.844*** [0.000]
Male Population (%)	-67.743 [0.450]
User-Age Population (%)	156.183** [0.036]
P-Value in brackets. * indicates significance level (i.e. 90%=*, 95% =**, and 99%=***)	





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The Impact of a College-Level Stress Management Course on Self-Efficacy Among Undergraduate Students

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Abstract

This study examines the differences between self-efficacy levels for stress management among undergraduate students who have taken a stress management course compared to those who have not. The sample consisted of 224 current undergraduate students at Weber State University. A revised version of the general self-efficacy scale by Schwarzer, as well as techniques from Bandura on building a self-efficacy scale, were used to compile a ten-question survey for this study. Each participant took the survey to answer questions regarding personal levels of self-efficacy for stress management. The point scale ranged from 0 to 10 for each statement, with 10 indicating the highest degree of self-efficacy. A sum score between 0–100 was administered for each submitted survey, and these scores were not revealed to participants. An independent samples t-test demonstrated there was a significant difference in self-efficacy levels for stress management between the students in this study who have taken a stress management course and those who have not. This indicates that a college-level stress management course may increase the levels of self-efficacy for stress management among undergraduate students.

Introduction



The concept of *stress* takes on multiple definitions according to various fields and subjects. *Acute stress* can be defined as adaptive, instantaneous changes in the nervous, cardiovascular, endocrine, and immune systems in response to the perception of a sudden, threatening event (Schneiderman, Ironson, and Siegel, 2005). *Chronic stress* involves the acute stress response becoming maladaptive due to overactivation (Schneiderman et al., 2005). In biological systems, *homeostasis* is a fundamental component for the

proper maintenance of life and comprises the organism as a whole (Chovatiya and Medzhitov, 2014). When homeostasis is disrupted beyond its range of regulatory abilities, a stress response is activated to assist in the restoration of balance (Goldstein and Kopin, 2007).

This stress response produces numerous physiological adaptations to meet the demands of a perceived threat. The sympathetic nervous system (a component of the autonomic nervous system) is responsible for activating the stress response in moments of a perceived threat. This activation by the sympathetic nervous system will produce immediate physiological changes such as increased heart rate, increased blood pressure, increased respiration rate, increased muscular contraction, and more. Similarly, the nervous system will continue this response by suppressing the immune, reproductive, and digestive systems (Olpin and Hesson, 2014). When consistent, lasting episodes of stress occur in the human body, these physiological responses generate negative health effects. Studies suggest that up to 90% of visits to a primary care physician in the United States have a stress-related component (Boone and Anthony, 2003).

Penn State University released an annual report from its Center for Collegiate Mental Health (CCMH) that confirmed anxiety (a reaction to stress) as the number one reason for visits to the on-campus clinic (2018). Similarly, the American College Health Association found that students reported stress as the primary impediment to academic success and anxiety as the number two reason (2017). The report further revealed that the majority of students reported experiencing “more than average” stress within the last 12 months (ACHA, 2017). The reason for these high levels of stress among college students is ambiguous, but one study done among undergraduate students revealed that academic issues were the most significant stressors (Karagiannopoulou and Kamtsios, 2011).

Managing stress often requires a change of behavior. The desire and confidence to change can be convoluted, but there is a model that outlines various stages of intentional behavioral change among individuals: the transtheoretical model. This model was developed by Prochaska and DiClemente in the late 1970s and evolved through studies that investigated why some smokers were able to quit on their own while others needed further treatment. These studies revealed that people quit smoking when they were ready to do so,

implying that change is a continuous process (LaMorte, 2016). Subsequently, the transtheoretical model covers six different stages of change to explain this process: precontemplation, contemplation, preparation, action, maintenance, and termination. In precontemplation, the individual has no desire to change and does not likely see a problem in the specific behavior requiring change. Contemplation is the stage where an individual has recognized the need for change and plans on changing within 6 months. The preparation stage is where an individual is ready to make a change within the next 30 days. This model is effective in determining an individual's ability to recognize the need for change as well as the individual's confidence to do so.

Concerning the transtheoretical model of change, undergraduate students with low self-efficacy (the belief that one is capable of accomplishing specific tasks to achieve a desired outcome) were in the stages of either precontemplation, contemplation, or preparation for managing stress (Karagiannopoulou and Kamtsios, 2011). In other words, the students with lower levels of self-confidence were less likely to attempt to manage personal stress levels. There are various components involved with combating stress and its accompanying effects. Self-efficacy, for example, is associated with successful stress management. Albert Bandura, a pioneer in the research of self-efficacy, explains that personal beliefs in coping abilities directly correlates to stress levels (1994). Moreover, perceived self-efficacy in managing personal stressors plays a fundamental role in reducing anxiety (Bandura, 1994).

This was a quantitative study examining the differences between self-efficacy and stress management among undergraduate college students who have and those who have not taken a stress management course. The stress management course was taught by Dr. Michael Olpin and covered topics such as the science behind the stress response, mindfulness, meditation, deep breathing, and relaxation techniques. It was hypothesized that students who have participated in a college-level stress management course will report higher levels of self-efficacy for managing stress compared with students who have not taken such a course.

Methods

Participants

Undergraduate students (n=224) at Weber State University participated in this study. Students were recruited through professor emails, social media, and on-campus activity booths. Students were required to indicate whether they have (n = 71) or have not (n = 153) taken the stress management course (Health 1110) at Weber State University. Participation in the study was completely voluntary with no compensation of any kind. Each student was required to sign a consent form before participating in the study. The surveys were submitted anonymously through a secure, online source.

Procedure

The survey began by inquiring the class ranking of all participants to ensure each was an undergraduate student. The surveys completed by non-undergraduate students were not included in this study. The general self-efficacy scale by Schwarzer and a technique from Bandura were used to create a ten-question survey to most accurately fit the needs of this study (Schwarzer and Jerusalem, 1995; Bandura, 2006). The technique from Bandura involved using a larger scale to investigate the respondents' answers to survey questions. This increases the reliability of an individual's self-efficacy levels regarding the specific task (Bandura, 2006). Students indicated a personal aptitude level for managing stress in specific situations. This was done by participants selecting a number on a scale from 0 (cannot do at all) to 10 (highly certain can do) for every statement on the survey. Each student was given a single score anywhere from 0 (lowest score) to 100 (highest score) according to individual responses. Scores were determined by summing all the answers for individual participants. These scores were grouped by the students who had, and the students who had not, taken the stress management course. From here, the overall averages were calculated for the two groups. Scores were not disclosed to the students and were used for purposes of this study only.

Results

An independent samples t-test was run to determine the level of self-efficacy for stress management among undergraduate students who

have taken a stress management course compared those who have not. Results show that there was a significant effect among the participants of this study, $t(158)=4.78$, $p<.001$, and found that those students who have taken the stress management course have higher levels of self-efficacy ($M=73.39$, $SD=12.28$) as compared to those who have not taken a stress management course ($M=64.50$, $SD=14.35$). A Cohen's d effect size calculation indicates that the difference in self-efficacy for stress management between classes is moderate, $d=.67$.

Limitations

As a result of limited resources, a convenience sample was utilized for this study. Randomization of participant selection occurred via on-campus booths, social media, and emails. Acquiring a sufficient sample size was difficult using such methods. Participants were not specifically selected across major, gender, age, or any other demographical factors. Therefore, generalizability of this study is limited to only those who were surveyed.

Discussion

The hypothesis that undergraduate students who have taken a stress management course will report higher levels of self-efficacy for managing stress, compared with students who have not taken such a course, was supported for the participants of this study. The results from this study correlate with Bandura's beliefs on the existent relationship between self-efficacy and stress management. There are limited studies available for the relationship between these two concepts, so the results available from this research may act as a catalyst for future studies to be done on self-efficacy and stress management.

Furthermore, college life for undergraduate students can often bring changes and uncertainties that lead to high levels of stress and anxiety. These changes could include leaving home and forcing a new level of independence, educational and professional decisions, relationships, finances, balancing social interactions, healthy living, and studying. Chronic stress prevents the human mind from thinking clearly and logically, both of which are critical for optimal collegiate success. Students must recognize and feel capable that stress can be overcome. It is not uncommon for students to think that high,

constant levels of stress are a normal part of the college experience. Such thoughts can lead to the avoidance of trying to manage that stress. Stress management courses can offer students the chance to improve academically, socially, mentally, emotionally, and physically. This study demonstrates that helping students increase levels of self-efficacy is possible through a stress management course.

However, due to the small sample size, this study should not be considered representative of all college students. Additional studies should be done to test this hypothesis. Also, this study did not incorporate gender, major, or other demographical factors to the results. Such factors should be instituted to further investigate the relationship between self-efficacy and stress management among undergraduate students.

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The Effects Of Vitamin C, Hydrocortisone, and Thiamine in the Treatment of Sepsis

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Mentor

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Abstract

Bacterial sepsis kills around 250,000 Americans each year. Septic shock, resulting from bacterial sepsis, has a mortality rate of nearly 50%. Septic shock is the body's extreme response to an infection that causes inflammation throughout the body, resulting in severe tissue damage and organ failure. Tumor necrosis factor (TNF) has been shown to be the primary mediator of the inflammatory response in sepsis (Spooner, Markowitz, & Saravolatz, 1992). The combination of vitamin C, hydrocortisone, and thiamine given intravenously has recently been shown to improve the outcome for patients with sepsis and is still being researched. The goal of this research was to test the effects of these drugs on the TNF-alpha production in isolate peripheral blood mononuclear cells (PBMC). Minimal research has been done on how the three drugs in combination are treating sepsis. Human PBMCs were isolated from student donors and quantified. Then cell cultures containing 5×10^5 cells were plated and exposed to lipopolysaccharide, a component of Gram-negative bacteria outer membrane, to stimulate a response similar to *in vivo* septicemia. Cultures were then incubated for two, four, and six hours with hydrocortisone, vitamin C, thiamine, and a combination of all three. The three drugs were added in concentrations scaled to mimic intravenous doses both individually and in combination. After incubation, the supernatant was tested for TNF-alpha via enzyme-linked immunosorbent assay (ELISA) in duplicate to determine each combination effect on immune cell response to LPS. Statistical analysis yielded significant TNF-alpha suppression by hydrocortisone ($p < 0.001$). There was no significant decrease in TNF-alpha levels by vitamin C or thiamine, and the combination showed no synergistic effect among the three drugs.



Introduction

Sepsis is caused by an infection and leads to an immune response in the body. Widespread infection can lead to septic shock, severe inflammation, and organ failure. According to the Centers for Disease Control and Prevention, more than 1.5 million people in the United States get sepsis each year. Of these, 250,000 people die from complications due to sepsis (Centers for Disease Control, n.d.).

Traditional means of treatment by use of vasopressors and antibiotics have had a minimal effect in reducing the mortality rate in sepsis. In 2016, Dr. Paul Marik, a critical-care physician and head of the general intensive care unit (GICU) at Sentara Norfolk General Hospital in Virginia, administered intravenous vitamin C, thiamine, and hydrocortisone as a final life-saving attempt for his patients with sepsis. The treatment was effective; the mortality rate of sepsis patients in their ICU dropped from 40.4% to 8.5%. Those who died did not die directly from sepsis but from other comorbidities (Marik, Khangoora, Rivera, Hooper, & Catravas, 2017). The method of action of this treatment remains unknown.

To study how the treatment works during sepsis, cultures of PBMCs were stimulated with LPS, a molecule found in bacteria that the body recognizes as foreign, in order to simulate an *in vivo* septic system. TNF-alpha, a cytokine produced by immune cells to signal the body to respond to the bacteria, has been shown to be the prime mediator in the body's response to sepsis (Zee et al., 1992). Our research investigated if the combination therapy successfully treated sepsis through reducing inflammation, specifically, by reducing TNF-alpha production by stimulated PBMCs.

Methods

Isolation

In order to examine specific cytokine-producing cells, human PBMCs were isolated from the blood of the student researchers. First, blood was collected in eight sodium heparin tubes from each of two student researchers using standard venipuncture procedures. Aseptic technique was implemented by use of alcohol pads and povidone iodine swabs at venipuncture site and verified by negative blood cultures at 24, 48, and

72 hours of incubation. Then, blood was diluted in sterile phosphate buffered saline (PBS) in a 1:2 dilution. In 50 mL conical tubes, 36 mL of diluted blood was added on top of 10 mL of ficoll histopaque (density 1.077 g/mL). PBMCs were isolated by centrifuging the blood with ficoll histopaque at 400xg for 30 minutes with no decelerator brake (Doiron, 2014; Hougee et al., 2005). The buffy layer containing the PBMCs was extracted and placed into micro centrifuge tubes. The cells were washed three times with sterile PBS and were enumerated with a Beckman-Coulter CBC analyzer to determine the isolated PBMC concentration. PBMC solutions were diluted to equivalent concentrations of immune cells using 20% fetal bovine serum (FBS).

Stimulation

Cell cultures were stimulated with LPS, a component of Gram negative bacterial membranes, which causes cells to produce large amounts of TNF-alpha. Isolated PBMCs were added to 96 well cell culture plates in a concentration of 5.0×10^5 cells per well. Cells were suspended in 200 μ L of 20% FBS in sterile PBS. The culture plates were centrifuged at 200xg for 10 minutes. Cultures were then exposed to LPS of *E. coli* (serotype 055:B5) in a concentration of 0.1 μ g/mL. Vitamin C (0.1 mg), thiamine (0.01 mg), and hydrocortisone (0.003mg) were added to the cultures individually and in combination of all three drugs to determine their influence on TNF-alpha production. The cultures were incubated at 37° C with the drugs for two, four, and six hours with LPS exposure along with two levels of controls. Controls consisted of cultures exposed and unexposed to LPS, without the addition of drugs. The supernatant was extracted from the wells at the given time points. The supernatant was frozen at -80° C to prevent degradation before analysis.

TNF-alpha Analysis

To determine the cellular response after stimulation, TNF-alpha was measured by an ELISA. The ELISA was run according to the manufacturer's specifications (Thermo-Fisher). Concentrations of samples were calculated using an eight-point standard curve of known concentrations. Two levels of controls were also used to verify accuracy of the assay. All standards, controls, and samples were run in duplicate. The ELISA plate was read by the EPOCH 2 microplate reader at a wavelength of 450 nm.

Statistical Analysis

To analyze the data, R statistical software was used in all data analyses. Multiple ANOVA models were performed comparing different conditions on TNF-alpha levels, while controlling for time and donor effects. A Tukey Honest Significant Difference (HSD) test was used to perform all pairwise comparisons to determine significance of drug exposure on TNF-alpha production. Overall donor effects were tested by creating a two-way ANOVA model that controlled for variations in individual donors and possible donor interactions.

Results

To test the effects of each component of the new sepsis therapy, standard concentrations of PBMCs were co-incubated with LPS and various drug combinations for two, four, and six hours. The TNF-alpha levels were not significantly different between conditions during the two-hour time interval. TNF-alpha levels between conditions increased greatly during the four-hour and six-hour time intervals. During the four-hour and six-hour time intervals TNF-alpha levels were lower in the conditions that contained hydrocortisone and in the combination therapy (Figure 1). Mean TNF-alpha decreased from the positive control an average of 2652 pg/mL with hydrocortisone and 2668 pg/mL with the combination therapy ($p < 0.001$). The combination therapy and hydrocortisone did not have a significant difference between their reduced TNF-alpha levels ($p = 1.000$). There were no significant differences between the positive control and the conditions containing vitamin C or thiamine at any of the time intervals. The negative control had the same level of TNF-alpha as the positive control (see Table 1).

Discussion

Sepsis is a severe disease that kills hundreds of thousands of people a year, even in developed countries (Centers for Disease Control, n.d.). Hydrocortisone, vitamin C, and thiamine have recently been shown to be a successful form of treatment and has significantly decreased mortality in those treated with it (Marik et al., 2017). To determine the treatment's method of action, cell cultures of PBMCs were exposed to the three drugs during a septic simulation. TNF-

alpha was measured following treatment and compared to cells that did not receive the treatment. From the statistical analyses, it can be concluded that hydrocortisone was the only drug that significantly decreased TNF-alpha production. Although the combination therapy showed a significant decrease in TNF-alpha, it was from the hydrocortisone alone, not a synergistic effect between the drugs as was predicted (Figure 1). The reduction by hydrocortisone was to be expected, as hydrocortisone is a steroid and is meant to reduce inflammation. There appeared to be a significant donor effect on TNF-alpha production between the two donors; however, the effects of hydrocortisone on donor 1 and donor 2 cells were similar.

The negative and positive controls seemed to produce the same amount of TNF-alpha. No extra fluid was added to negative control wells to compensate for the absence of the drugs and LPS solution, which could have resulted in a dilution effect and falsely elevated TNF-alpha concentration. There could have also been carry-over contamination between wells during the ELISA wash stages which could have also falsely increased the measured concentration.

In conclusion, the vitamin C, hydrocortisone, and thiamine combination therapy treats sepsis by another means than reducing the TNF-alpha production directly. It could be that the drugs are helping with platelet function, and reducing clotting and disseminated intravascular coagulation (DIC). It could also be that the drugs are helping with vascular and organ function. Further research could utilize an animal model to more effectively study the septic response *in vivo* and further investigate the combination therapy's success.

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Appendix

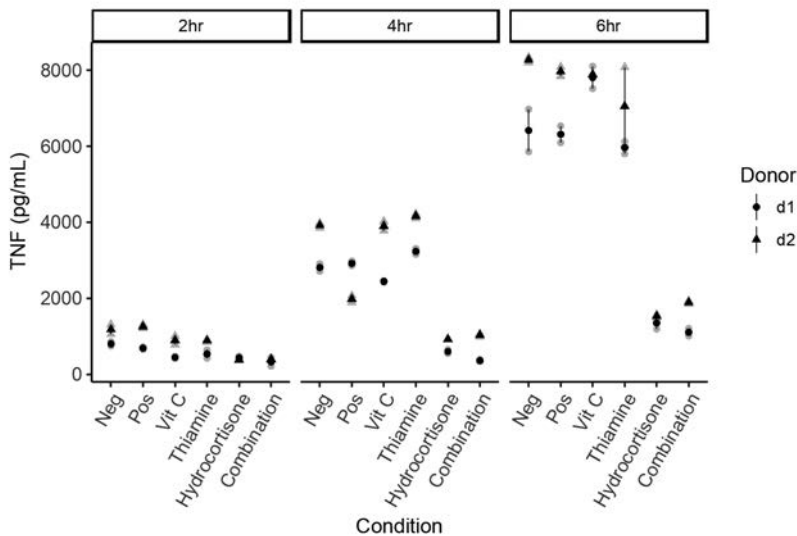
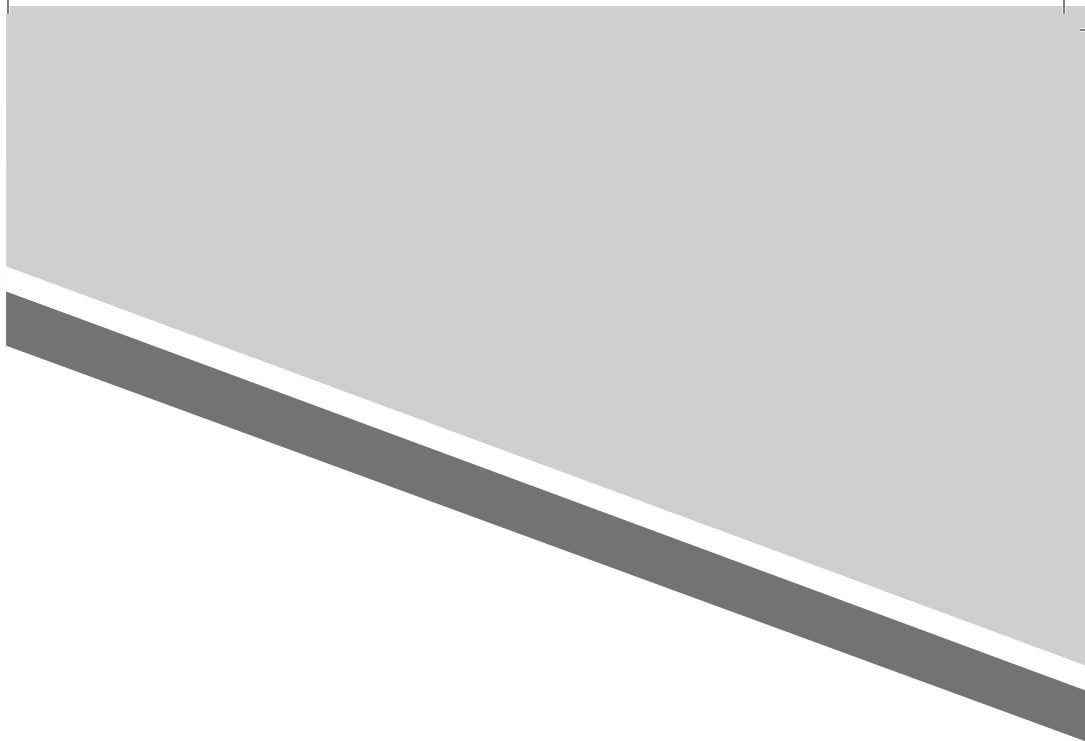


Figure 1. Tumor Necrosis Factor alpha (TNF-alpha) versus condition over time

Enzyme-linked immunosorbent assay (ELISA) results measuring TNF-alpha in pg/mL versus condition over the three separate time intervals. The circles correlate to donor 1 and the triangles to donor 2. The gray shapes represent individual data points. The black shapes indicate the mean TNF-alpha levels of corresponding donor and the black line corresponds to a 95% confidence interval. Conditions corresponding to Neg and Pos indicate the negative and positive control wells where lipopolysaccharide (LPS) was withheld and added respectively. The other conditions all had LPS added to stimulate TNF-alpha production. The other conditions were the addition of vitamin C, thiamine, hydrocortisone, and a combination of all three drugs.

Table 1. Pairwise Comparisons Between Donors of Tumor Necrosis Factor alpha (TNF-alpha) vs. Condition Over Time.

Comparison	Average Effect (pg/mL)	p-value
Positive vs Hydrocortisone	-2652	0.00002*
Positive vs Combination	-2668	0.00002*
Hydrocortisone vs Combination	-16	1.00000
Donor 1 vs Donor 2	+608	0.03799*
Donor x Condition	-	0.99790
Tukey Honest Significant Difference (HSD) comparisons of TNF-alpha production by peripheral blood mononuclear cells (PBMCs) between control and treatment groups, as well as between individual treatments and donors. Donor x Condition is an interaction term to determine if there were varying effects between donor and condition. Average effect of conditions is provided. Significant p-values were determined by exceeding the alpha threshold of 0.05 and indicated by an asterisk (*). All other comparisons not shown had a p-value exceeding 0.95 and were not significant.		



Electronic Cigarettes and the Risk of Rheumatoid Arthritis

Author

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Mentor

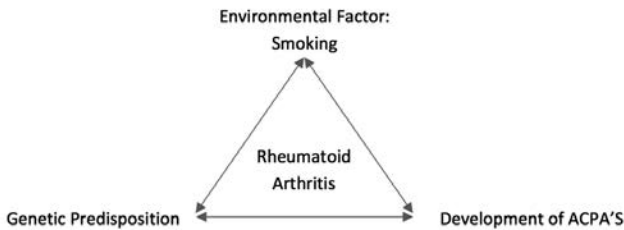
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Abstract

Electronic cigarettes have become increasingly popular in the past decade. Research has shown that smoking traditional cigarettes in conjunction with genetic polymorphisms results in significantly higher risk for developing seropositive rheumatoid arthritis (RA). RA is an autoimmune inflammatory rheumatic disease that affects approximately 0.5–1% of the population of the developed world with the seropositive form constituting two-thirds of all cases of RA (Kurkó et al., 2013). The purpose of this study was to investigate the potential health risks for developing seropositive rheumatoid arthritis in electronic cigarette users. EDTA blood samples were obtained from traditional smokers, electronic cigarette users, and nonsmokers totaling 44 participants. DNA was extracted from EDTA tubes and used to genotype for the presence of the risk alleles rs24766001 in *PTPN22* and rs6910071 in *HLA-DRB1* using TaqMan SNP Genotyping Assay. In addition, autoantibodies seen commonly in RA (i.e., antibodies to citrullinated proteins and rheumatoid factor) were measured in all samples using an Enzyme Linked Immunosorbent Assay (ELISA) and an agglutination test, respectively. Levels of the antibodies were compared across genotypes of each group using an analysis of variance (ANOVA) and then further compared to one another using a T-test. It was found that levels of antibodies among traditional smokers and electronic cigarette users were both significantly lower than Non-Smokers. These results could indicate a mechanism that is displayed in electronic cigarette users that causes them to produce less antibodies than other groups. The level of air quality in Utah could also explain why antibodies were elevated among the non-smokers, which was the control group.

Introduction

Smoking traditional cigarettes in conjunction with genetic polymorphisms results in significantly higher risk for developing seropositive rheumatoid arthritis (RA) (Kurkó et al., 2013; Malmström, Catrina, & Klareskog, 2016). Seropositive RA refers to the presence of antibodies to citrullinated protein antigens (ACPAs), whereas seronegative is the absence of these antibodies. A model showing how onset of RA is related to smoking, genetic polymorphisms, and development of ACPAs is shown below:



The purpose of this study was to investigate the potential health risks for developing seropositive RA in electronic cigarette users. It was expected that those who displayed genetic predispositions along with electronic cigarette use would develop ACPAs, which in turn would increase the risk of developing RA as proven with traditional cigarette users.

RA affects approximately 0.5–1 % of the population in the developed world and causes chronic synovial inflammation which eventually leads to joint destruction and disability (Kurkó et al., 2013). Cigarette smoking along with genetic risk factors in the PTPN22 and the HLA-DRB1 genes leads to inflammation and the production of the citrullinated proteins. Citrullination or deamination of a protein is the conversion of the amino acid arginine into the amino acid citrulline in that protein. These altered proteins cause the immune system to create ACPAs and rheumatoid factor, which mediate the pathogenesis of RA. (Malmström et al., 2016) (Kurkó et al., 2013; Malmström et al., 2016) (Van Der Woude & Catrina, 2015)

Previous diagnosis of RA was done by testing for rheumatoid factor (RF) in patients; however, that type of testing was often inaccurate. New research has found that rheumatoid factor can often be elevated in multiple other autoimmune diseases or infections. Emerging and successful research on the development of ACPAs in relation to risk factor of developing RA allows much more specificity in the diagnosis of RA.

In this study, each participant was genotyped for presence or absence of risk alleles which involved DNA extraction and a Taqman assay. Furthermore, each participant's serum was quantified for level of autoantibodies present in the blood along with a qualitative latex agglutination test for presence of RF factor in patient's serum. Our results showed that electronic cigarette users had significantly lower levels of antibodies than both smokers and non-smokers.

Materials & Methods

Study Population

The study population consisted of 44 participants overall: 16 non-smokers, 21 e-cigarettes users, and 7 traditional smokers. The non-smokers were used as healthy controls. Volunteer participants were found using flyers posted around the Weber State campus, local businesses, personal contacts, classroom announcements, and social media. This study focused on the relationship between electronic cigarettes and RA and was also part of a larger study focused on general effects of electronic cigarette use. A survey of basic demographics was completed, along with blood samples collected from each participant. A signed informed consent document was also obtained at time of collection. Samples were drawn in both an EDTA tube, a sterile vacutainer treated with Ethylenediaminetetraacetic acid to prevent whole blood from clotting, and a serum-separator tube (SST) which separates serum from the whole blood after centrifugation. The serum and whole blood was then aliquoted into cryotubes, and stored at -80 degrees Celsius until testing was performed. A random ID number was labeled to the participant's consent form, survey, and subsequent sample tubes, keeping samples anonymous. This project was reviewed by the WSU Institutional Review Board for Human Subjects.

DNA extraction

Using the EDTA tube aliquot, DNA was extracted via a Qiagen DNA Extraction DNeasy Blood and Tissue kit. Whole blood samples were transferred to a microfuge tube, included in the kit, along with AL buffer and Proteinase K. The microfuge tube was then incubated in a warming bath, per Qiagen protocol, thus lysing all cellular components found in the whole blood which released the DNA. Ethanol was added to precipitate the DNA out from the solution and the DNA was then isolated by centrifugation. The precipitated DNA was transferred to a solid silica matrix column that binds DNA. A series of buffers were used to purify the DNA and remove other debris left behind from the DNA removal. The DNA was extracted from the silica matrix into a cryotube and samples were tested using EPOCH spectrophotometer to determine their concentrations and purity, using a 260/280 nm ratio. Dilutions were made to standardize the concentrations of each sample. DNA samples were stored at -80 Celsius until genotyping was performed.

Genotyping

DNA samples were genotyped for each SNP (rs2476601 and rs6910071) using predesigned TaqMan assays from ThermoScientific. SNPs were selected using previous studies linking traditional smoking to RA (Malmström et al., 2016). Before assays were genotyped, all DNA samples were thawed and diluted to a concentration of 0.889 (ng/uL) per the ThermoFisher protocol. Genomic DNA was introduced into a reaction mixture consisting of TaqMan Genotyping forward and reverse primers. Each TaqMan MGB Probe anneals specifically to a complementary sequence, if present, between the forward and reverse primer sites. When the probe is intact, the proximity of the quencher dye to the reporter dye suppresses the reporter fluorescence. The increase in fluorescence occurs only if the amplified target sequence is complementary to the probe. Thus, the fluorescent signal generated by Polymerase Chain Reaction amplification indicates which alleles are present in the sample. This Quantitative Polymerase Chain Reaction assay was run on a Quantstudio 3 real-time PCR instrument in the MLS department located on the Weber State University campus.

ELISA testing/Latex Agglutination testing

Serum samples were used to give both qualitative and quantitative results of RF and the presence of ACPAs respectively. RF was tested using a Latex Agglutination Kit following standard procedure. The presence of ACPAs was quantified using an ELISA kit following standard procedure. Samples were tested with controls in duplicate using the EPOCH microplate spectrophotometer by BioTek. Standards and samples were read at 450nm. A 9-point standard curve was created using standards provided by BioEagle. The standard curve was then used to calculate the concentration of ACPA antibodies found in the participants' serum samples.

Statistical Analysis

An ANOVA was used to compare the mean values of all the ACPAs present in each of the patient sample groups. A T-test was then used to compare each group to one another. Each treatment group was stratified by genotypes. Analysis was performed using R software (R Foundation, 2008). The samples without the genetic polymorphism will be used as controls while the others will be grouped by presence of mutant allele. The treatment groups were divided into smokers, electronic cigarette users, and non-smokers by genotype.

Results

To assess the presence of ACPAs in each of the smoking groups, serum was tested using an ELISA. The mean ACPA levels for each group was as follows: non-smokers (control) (n=16) 8.056 (U/mL), traditional smokers (n=7) 6.236 (U/mL), electronic cigarette users (n=21) 3.783 (U/mL). An ANOVA test was used to compare variance between all groups which was found to be significant ($p < .05$). A T-test was performed between all groups to verify to what extent each group differed from the control. P-values are listed below (see Table 1). The electronic cigarette users had a significantly lower ($p = 6.67 \times 10^{-9}$) level of ACPA (3.783 U/mL) compared to the control (8.056 U/mL).

Each participant was genotyped for presence of mutation (rs2476601). One participant was heterozygous (A/G) for the mutation while just under half of the group was homozygous (G/G) for the mutation and just over half were homozygous for the wild-type (A/A) (see Figure 2). The graph

takes into account each group's ACPA concentration levels while also subdividing each group by genotype (mutation and non-mutation) (see Figure 1). Due to complications by ThermoFisher in producing a Taqman Assay for risk allele (rs6910071), results were skewed and were not able to be provided in this study.

All participant's samples were tested for presence of RF factor via a latex agglutination kit. One of the total samples measured was positive for agglutination. Each participant was then compared by smoking status and genotype to ACPA production (see Figure 1). Taken together we can see that there is an elevated level of ACPA found in those of non-smokers and a lower level in those using electronic cigarettes and traditional cigarettes.

Discussion

The data collected was used to assess a similar disease model between electronic cigarette users versus traditional cigarette users and the development of seropositive rheumatoid arthritis. Level of antibodies among all groups were tested and results showed significantly lower levels of ACPAs in electronic cigarette users and smokers compared to non-smokers. None of the participants were currently using or had recently used any form of immunosuppressant. A potential reason for this difference could be a mechanism for lowering levels of these antibodies that exists due to the presence of harmful toxins released from electronic cigarette vapor into the lungs. Although presence of ACPA concentrations varied between samples, all were below the reference value for rheumatoid arthritis (Negative < 30 U/ml). Due to all samples being negative, a big limitation to this study was not being able to measure antibodies for someone who is currently displaying rheumatoid arthritis.

Although our population contained diverse genotypes which allowed comparison of levels of ACPAs between mutations/non-mutations, we were not able to control for the amount of time each person had used traditional or electronic cigarettes. Because this study was done on a college campus, many of our participants were younger. The development of ACPAs may be lower in these groups due to lack of smoke exposure or time for ACPAs to develop.

Environmental factors could also be contributing to the ACPA levels in the participants. Northern Utah in the fall/winter has lowered air quality due to pollution and inversion. This air pollution could be

causing the activation of PAD's enzymes which start the production of antibodies. This would explain why the non-smokers had just as high of a level of antibodies as the traditional cigarette smokers. There was one sample positive for RF that showed low/normal levels for ACPAs. This further validates the argument that RF does not have high specificity for RA. This person could be positive for some other form of autoimmune disease.

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Appendix

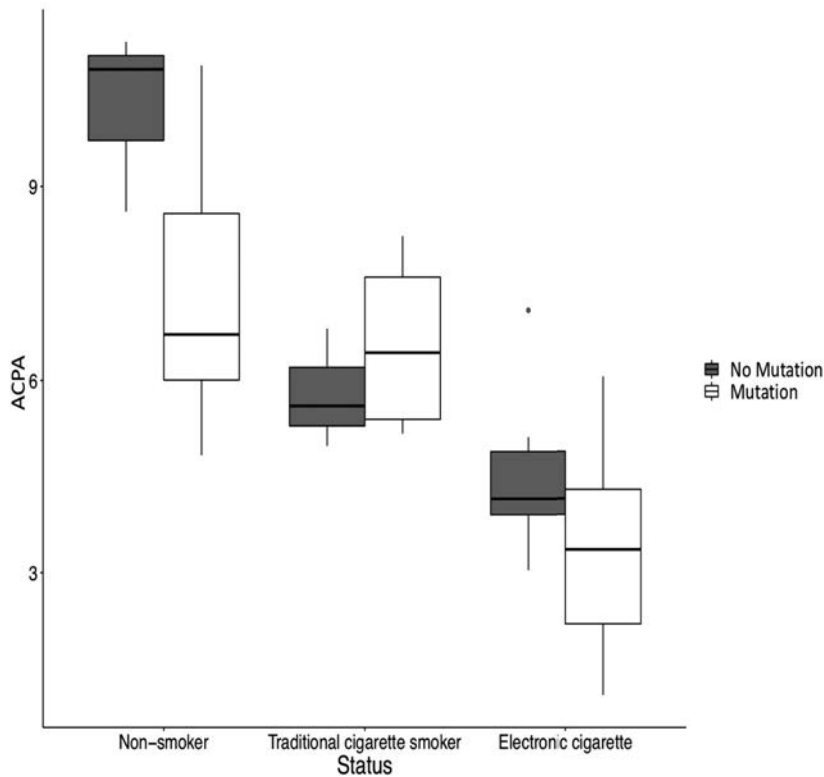


Figure 1. ACPA Concentration (U/mL) subdivided into genotypes across non-smokers, traditional cigarette users, and electronic cigarette users. Comparisons between smoking status, ACPA level, and genetic mutations are shown here. Both the traditional smoker and the electronic cigarette users had significantly lower levels of ACPAs.

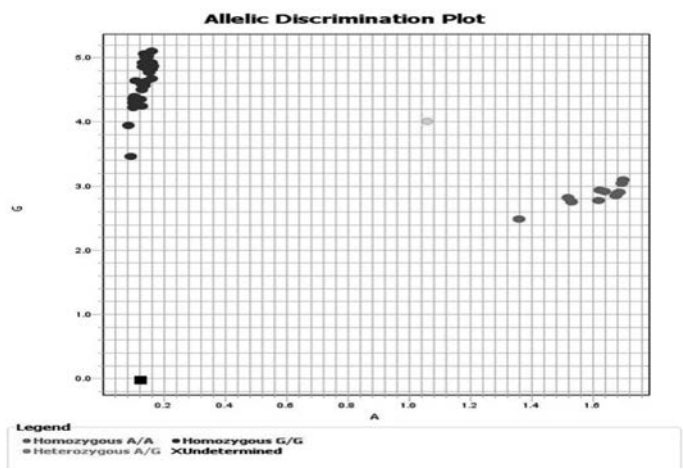


Figure 2. Allelic Discrimination Plot displaying genotype of participants with regards to rs24766001 in *PTPN22* plotted with Allele 1 A/A (wild type) vs Allele 2 G/G (mutation). Assay provided by ThermoFisher and all procedures completed as per ThermoFisher.

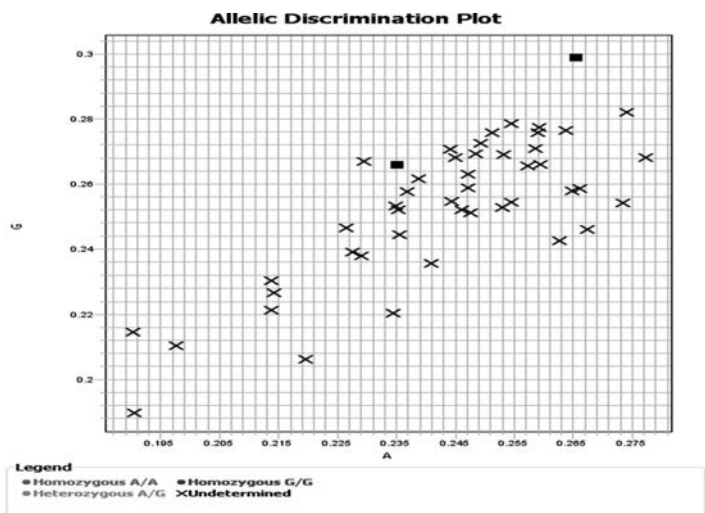


Figure 3. Allelic Discrimination Plot for rs6910071 in HLA-DRB1. Results invalid to due to poorly-designed Taqman Assay.

Systemic Inflammation in Electronic Cigarette Users Versus Traditional Smokers

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Abstract

The use of electronic cigarettes (e-cigs) has increased in popularity over the past several years; this can be attributed to multiple factors. Cigarettes cause inflammation both in the lung and systemically; this study tested differences in pro-inflammatory cytokines between those that use e-cigs, traditional-cigarette users, and non-smokers. Blood samples were collected from participants recruited from a suburban mountain west geographical area, mainly a university campus. Samples were centrifuged to isolate serum for cytokine testing using an enzyme-linked immunosorbent assays (ELISA). Pro-inflammatory cytokines, interleukin (IL)-1 beta, IL-6, and tumor necrosis factor-alpha (TNF-alpha) were evaluated. In conjunction, c-reactive protein (CRP) was quantified using an immunoturbidimetric assay on a benchtop clinical analyzer. An analysis of variance (ANOVA) yielded no significant difference between the groups.

Introduction

A study conducted in the U.S. found that 12.6% of adults have tried electronic cigarettes (e-cigs) and 3.7% of adults use e-cigs (National Center for Health Statistics, 2015). This can be attributed to multiple factors, (e.g., people thinking it is less harmful than traditional cigarette use, or people using it as a method to cut back on the amount of nicotine intake, or as a cessation to traditional smoking. These factors are specifically targeted by e-cig marketing efforts. E-cigs have been advertised as being healthier, cheaper, and more convenient (Grana & Ling, 2014). The possible effects of e-cigs on the health of an individual are still unknown. This study compared key pro-inflammatory cytokines found in the blood of traditional cigarette users, e-cig users, and non-smokers.

A recent study investigated the inflammation markers in keratinocytes and epithelial lung cells *in vitro* when exposed to e-cig vapor. Their results showed an increase in pro-inflammatory cytokines/chemokines IL-8, TNF-alpha, and IL-1 alpha in both the keratinocytes and epithelial cells. IL-6 was found to be decreased in both of the cell lines (Cervellati et al., 2014). The hyper-stimulation of the previously-stated cytokines and other pro-inflammatory cytokines can result in adverse health issues. Cigarette smoking has shown to cause lung cancer and respiratory disease via inflammation (van der Vaart et al., 2005). There have been chemical analyses done comparing e-cigs to traditional cigarettes and the disease burden that come from the vapor, smoke, and other substances contained in the e-cig juice or cigarette. They were able to find that e-cig vapor is less toxic than that of cigarette smoke (Oh & Kacker, 2014). Although the vapor may be less toxic, there is a need for better understanding how the user is affected.

This study investigated the *in vivo* pro-inflammatory cytokines levels in a controls group, traditional cigarette user group, and e-cig user group. Previous studies have looked at the inflammatory differences of e-cigs compared to traditional cigarettes *in vitro*. There has not been a significant amount of research looking at this difference *in vivo*. Aside from the pro-inflammatory cytokines that we measured, we also examined CRP levels. CRP has shown to be useful in monitoring disease progression and determining risk of future cardiovascular complications (Tonstad & Cowan, 2009).

Our study of inflammatory markers in e-cig users is part of an extensive investigation of these subjects, looking at behavioral and nutritional differences. Multiple physiological and biological factors were explored, which include: dependence, craving, withdrawal effects, heart rate, blood pressure, carbon monoxide levels, cortisol, high density lipoprotein cholesterol (HDL-C), and leptin. These measurements will be compared between the control group, traditional cigarette smokers, and e-cig users. The results from this study will clarify part of the assumption that e-cigs are healthier or better to use as an alternative to conventional cigarettes.

Methods

Population

43 participants were recruited from a suburban mountain west geographical area, mainly a university campus, with a mixed gender, and an age range of 18 to 56 years old during the months of September 2017 through January 2018. Blood samples were collected from participants that either currently smoke traditional cigarettes (n=7), use e-cigs (n=21), or non-smoker (control [n=15]). Participants were given a survey that asked about their smoking habits, medical history, and demographic information.

Sample Collection

Blood samples were collected in serum separator tubes via standard venipuncture, centrifuged to isolate serum for testing, and aliquoted into 500 uL microcontainer tubes. To preserve cytokine levels in the serum taken from the participants, samples were then stored in a -80° C freezer. The half-lives, ranging from 30 minutes to 2 days, were important to be able to evaluate the effects between cigarette smokers and vapers (someone who uses an e-cig). There were extenuating circumstance with some samples and we were not able to get them frozen within the time frame of their half-life.

Cytokine Testing

Serum pro-inflammatory cytokines were quantified using an ELISA, read on the Biotek Epoch 2 according to kit manufacturer's specifications. 96 microwell ELISA plates were used to test for specific pro-inflammatory cytokines: IL-1 beta, IL-6, and TNF-alpha. Controls, samples, and standards were all run in duplicate. A 9-point standard curve was formed and sample absorption values were measured at a wavelength of 450 nm and applied to the standard curve to calculate sample concentration. In conjunction, CRP was quantitated via immunoturbidimetric assay on the Mindray BS-200 chemistry analyzer according to manufacturer and analyzer specifications. Standards and controls were also prepared and tested per manufacturer specifications.

Statistical Analysis

The mean pro-inflammatory cytokine levels were compared between groups using an ANOVA, as well as two sample t-tests. Statistical analyses were performed using the R software package (R Foundation, 2008).

Results

To assess if e-cigs were associated with higher serum pro-inflammatory cytokine levels, CRP, IL-6, IL-1 beta, and TNF-alpha levels were evaluated from the 43 participants. Although no significant difference was present, there was a trend among the participants. The TNF-alpha levels were highest amongst the smokers with the vapers following (see Figure 1 and Table 1). The two-sample t-test further shows the trend of smokers' TNF-alpha levels being collectively higher than the other groups, with the vapers' levels being the next highest. This further confirms previous research findings of elevated TNF-alpha levels in vapers (Cervellati et al., 2014). The greatest finding among all of the pro-inflammatory cytokines measured was between the smokers and vapers, and their respective TNF-alpha levels (see Table 1).

Elevated CRP levels have been found in those individuals that smoke traditional cigarettes (Wu, Jiang, Minor, & Chu, 2014). The researchers were interested in the effects that vaping would have on CRP levels. Upon analysis, the smokers are producing the highest levels of CRP in their peripheral blood, followed by the vapers and control (see Figure 2 and Table 1). There is only a slight difference between the vapers and control group with respects to CRP levels (see Table 1). Data analysis of IL-6 revealed the vapers stimulated higher IL-6 values than the smokers and control group (Figure 3 and Table 1).

Discussion

Due to marketing as a healthier option, e-cigs have gained a lot of popularity among adolescents, young adults, and adults. Recent research conducted by the Utah Department of Health and Utah Department of Human Services suggest that up to 23.1% of students in grades 8, 10, and 12 have ever tried e-cigs and 11.1% have used within the last 30 days. Comparatively, 11.9% of those

same students have ever tried traditional cigarettes, and 2.9% had used in the last 30 days ("SHARP_Report_2017_(Final)," n.d.). This increased popularity raises the question of what biological processes are being altered by the inhalation of chemicals found in e-cig juice. Since e-cigs are a fairly new product on the market, not a lot of research has been performed to draw an insightful conclusion to this overarching question.

In an effort to find an answer, we collected peripheral blood from subjects. Upon data analysis no significant differences ($p < 0.05$) were found between the groups. Although, there was a trend which was anticipated: the mean smoker pro-inflammatory cytokine levels of TNF-alpha and CRP were higher than those of the control and vapor groups (see Table 1). Moreover, the mean vapor pro-inflammatory cytokine levels of IL-6 were higher than those of the control and smoker groups. Previous findings show that vapers express increased levels of TNF-alpha and IL-6 (Cervellati et al., 2014; Wu et al., 2014). Although there has not been a lot of research suggesting increased CRP levels in vapers, there has been research showing that IL-6 has a vital role in the progression of chronic obstructive pulmonary disease, in which disease process CRP is elevated (Wu et al., 2014). With these trends we can conclude that e-cig use is better than using traditional cigarettes, but still causing some systemic inflammation process *in vivo*.

Several limitations arose in the study. The group size and balance were not sufficient for the drawing of any significant statistical conclusions. We did not take into account the half-life of the cytokines *in vivo*. This was an issue because some of the samples remained unfrozen for 30–60 minutes. This may have skewed our results, making them appear lower than they actually were. Better control of the use of the e-cig or traditional cigarette prior to the collection of peripheral blood could have produce interesting insight to the differences in the pro-inflammatory cytokine levels. When data was evaluated for IL-1 beta, only some of the standards produced values. Upon evaluation of the step taken to make controls, it was found that the high control was higher than the largest standard. Due to cost, a second plate could not be prepared for analysis.

There is not a substantial amount of research on the half-life of these pro-inflammatory cytokines *in vivo*. Researching the optimal

time to collection prior to smoking or vaping would be beneficial. This would ensure the pro-inflammatory cytokines are present in the peripheral blood. Another avenue would be controlling between those individuals that have just smoked and never vaped, vaped and never smoked, and those that have done both.

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Appendix

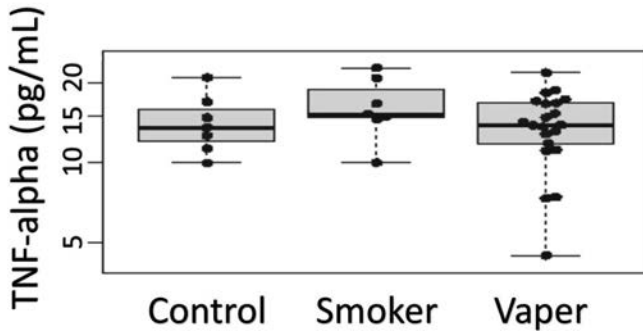


Figure 1. Tumor Necrosis Factor-alpha (TNF-alpha) levels from peripheral blood.

TNF-alpha pro-inflammatory cytokine levels were quantitated via ELISA on control (n=7), smoker (n=7), and vaper (n=21) groups. Serum was isolated from peripheral blood, an ELISA 96-well microplate was prepared and analyzed on the Biotek Epoch 2 at 450nm. Data are represented as mean; bars represent 1.5 IQR, $p=0.371$.

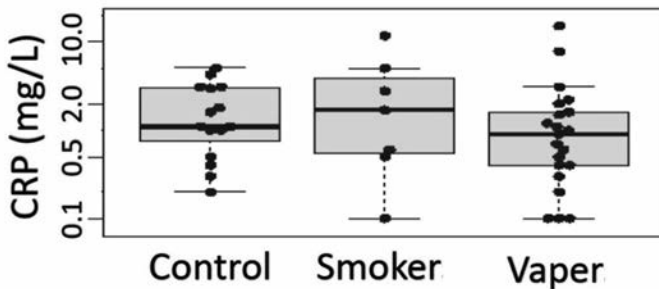


Figure 2. C-reactive Protein (CRP) levels from peripheral blood.

Quantification of CRP pro-inflammatory cytokine levels were performed via immunoturbidimetric assay for the control (n=15), smoker (n=7), and vaper (n=21) participants. Serum was isolated from peripheral blood and testing was performed on the Mindray BS-200. Data are represented as mean; bars represent 1.5 IQR, $p=0.59$.

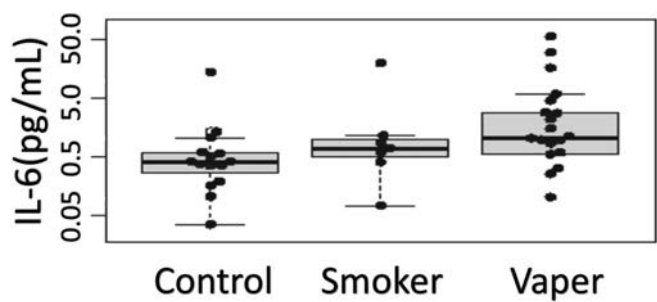


Figure 3. Interleukin-6 (IL-6) levels from peripheral blood.

Levels of IL-6 were measured, upon separation of serum from peripheral blood, via ELISA for control (n=15), smoker (n=7), and vaper (n=21) participants. ELISA 96-well microplate was prepared and analyzed on the Biotek Epoch 2 at 450nm. Data are represented as mean; bars represent 1.5 IQR, p=0.38.

Table 1. ANOVA and Two Sample t-test of data received from participants.

Groups	TNF-alpha (ANOVA p=0.37)	IL-6 (ANOVA p=0.38)	CRP (ANOVA p=0.59)
Control (C) vs. Smokers (S)	C=7, S=7 95% CI: -6.779 to 2.528 p=0.339	C=15, S=7 95% CI:-5.375 to 1.705 p=0.293	C=15, S=7 95% CI: -3.834 to <u>1.114</u> p=0.265
Control (C) vs. Vapers (V)	C=7, V=21 95% CI: -3.215 to 4.053 p=0.814	C=15, V=21 95% CI: -2.794 to 1.809 p=0.624	C=15, V=21 95% CI: -2.048 to 1.833 p=0.911
Smokers (S) vs. Vapers (V)	S=7, V=21 95% CI: -1.197 to 6.268 p=0.174	S=7, V=21 95% CI: -2.722 to 5.557 p=0.488	S=7, V=21 95% CI: -1.992 to <u>4.497</u> p=0.435

Two sample t-test comparing the different participants groups of the pro-inflammatory cytokine level analysis. The accompanying ANOVA values for the pro-inflammatory cytokine levels are in parentheses. |



Elimination of Antibiotic Resistant *Klebsiella Pneumoniae* Using a Crispr-Cas9 System

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Abstract

Antimicrobial resistance has become a growing threat worldwide. Using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated protein nine (Cas9) gene-editing technology, it may be possible to eliminate these resistant microorganisms with extreme precision. The goal of this study was to kill an ESBL (extended-spectrum beta-lactamase) quality control strain of *Klebsiella pneumoniae* (ATCC 700603) using CRISPR-Cas9 technology. Several plasmids were designed to target specific gene sequences to induce a double-stranded break in the DNA that could potentially kill the bacterial cell. These plasmids targeted the housekeeping *DeaD* gene and the *bla*(SHV-1) gene coding for beta-lactam resistance. A non-specific GFP plasmid was designed to show that a plasmid could successfully be introduced into the bacterial model without altering the genetic makeup in a detrimental or beneficial way. *Klebsiella pneumoniae* was transformed with these CRISPR-Cas9 plasmids by electroporation to observe the targeted gene's effect on colony count. Successful transfection of the CRISPR-Cas9 plasmids led to a significantly decreased colony count of *K. pneumoniae* with the *DeaD* gene when plated on Trimethoprim antibiotic selective media.

Introduction

As of 2017 it is estimated that a minimum of 2 million illnesses and 23,000 deaths occur each year as a direct result of antibiotic resistance in the United States (CDC, 2017). Misuse of antibiotics and the continued evolution that occurs in bacterial genomes both contribute to antibiotic resistance. In an outbreak that occurred in the Second Affiliated Hospital of Zhejiang University, 21 strains of carbapenem-resistant *K. pneumoniae* were collected from five patients; all five later died due to severe lung infection, multi-organ failure, or shock. A representative strain from each

patient was analyzed and identified as being hypervirulent, highly drug-resistant, and highly transmissible even to healthy individuals. (Gu et al., 2018). We speculated that introduction of a CRISPR-Cas9 plasmid, specifically targeting a *DeaD* and a *bla*(SHV-1), would result in the death of *K. pneumoniae*. By using a system of bacterial enzymes and proteins to detect and destroy DNA from invaders by specifically cleaving or degrading the target nucleic acid, CRISPR acts as the adaptive immune system of bacteria (Beisel, Gomaa, & Barrangou, 2014). The protospacer adjacent motif (PAM) component of the CRISPR system is a sequence of 2-6 DNA bases that must immediately follow the target sequence for recognition to occur. The target section of the genome should be unique compared to the rest of the genome for site specificity. Another necessary component of the CRISPR system is the guide RNA (gRNA), which is about 20 bases long and is complementary to the target sequence. In theory, gRNA only binds to the target sequence (Anders, Niewoehner, Duerst, & Jinek, 2015) and the Cas9 enzyme is directed by the gRNA to the target location in the genome and then cuts the DNA once in the appropriate location (Your Genome, 2016).

The use of modern CRISPR technology has been suggested as a solution to the threat of antibiotic resistance due to the extreme precision of targeting specific sequences in the bacterial genome. The accidental or intentional altering of a bacterial genome utilizing CRISPR leads to cell death. Extreme precision in targeting specific sequences eliminates the target strain, which means that non-target strains will be untouched (Gomaa et al., 2014). This has beneficial implications for human natural flora to be maintained while killing specific strains of harmful bacteria.

We found that successful transfection using these plasmids resulted in cell death. By reducing colony counts on antibiotic media we showed that our CRISPR-Cas9 system targeting was successful.

Methods

Plasmid Design

CRISPR plasmids specific to *K. pneumoniae* (ATCC 700603) were created by the University of Utah CORE lab and were designed to target specific sequences for the housekeeping *DeaD* gene, beta-lactam resistance *bla*(SHV-1) gene, and a non-specific control plasmid targeting GFP (green fluorescent protein). Target sites were selected

based on low GC (guanine cytosine) content and proximity to PAM sequence. Each plasmid also contained a Trimethoprim-resistant gene which helped to select for positive transformants when grown on 30 µg/mL Trimethoprim trypticase media (Fig. 1). Sterile water was added instead of plasmid which served as a negative control when plated on 30 µg/mL Trimethoprim trypticase media.

Colony Types

Two separate distinct colonies grew from the ATCC 700603 *K. pneumoniae* lyophilized strain. One colony was named "gray" and the other "yellow" due to appearance on SBA (sheep blood agar) plates. A Beckman Coulter MIC (minimal inhibitory concentration) Neg Urine Combo 51 panel was performed on each colony, confirming that the colonies were both EBSL *K. pneumoniae*. The gray colony was found to be resistant to the 2/38 µg/mL MIC panel well containing Trimethoprim/Sulfamethoxazole and the yellow one was found to be susceptible. The concentration of Trimethoprim for our plating purposes was increased to 30 µg/mL to maintain selective pressure so both colony types would uptake and keep the plasmids.

Electrocompetency

Klebsiella pneumoniae was made electrocompetent by taking a colony less than 48 hours old and growing it up in 15 mL of SOB (super optimal broth) for at least 18 hours on a rotating air incubator at 37 degrees Celsius with speed set to 250 rpm. With an OD600 (optical density) between 0.50–0.70, cells were spun down at room temperature on a Thermo Scientific STI 6 Benchtop Centrifuge at 3,800 rpm for 10 minutes. After decanting, cells were re-suspended in 8 mL ice cold 10% glycerol; 2 mL were then placed into four 2 mL aliquots. Aliquots were spun on a Sorvall Legend Micro 21 R refrigerated microcentrifuge (4,700 rpm, 10 min., 4 degrees Celsius). Cells were decanted and resuspended in 1 mL ice cold 10% glycerol and aliquots were combined in pairs. Aliquots were spun down at the same speed, time, and temperature conditions; after each centrifugation step, aliquots were combined in pairs after decanting and re-suspending in 1 mL ice cold 10% glycerol. On the final resuspension of the last aliquot, 100 µL of bacterial suspension was pipetted into 10 separate fresh aliquots that were stored at -80 degrees Celsius until further testing.

Electroporation & Plating

For transformation using electroporation, 50 μL of competent cells and 1 μL of the specific CRISPR-Cas9 plasmid were combined, placed in a 0.1 cm Gene Pulser/MicroPulser cuvette, and electrically shocked at 1,700 V, 25 μF , 200 Ω , with a time interval 4.0–4.4 seconds, using a BioRad MicroPulser electroporation machine. The suspension was immediately added to 500 μL of SOC (super optimal broth with catabolite) recovery broth and placed in an air incubator at 37 degrees Celsius for 1 hour before being plated. Electrocompetent gray and yellow *K. pneumoniae* each had a blank, GFP, DeaD, and bla(SHV-1) cuvette electroporated; each cuvette was plated on 30 $\mu\text{g/mL}$ Trimethoprim trypticase media plates (warmed for 1 hour in air incubator at 37 degrees Celsius) in 10 μL , 30 μL , and 50 μL amounts. Plates were placed in an air incubator at 37 degrees Celsius to grow for 24 hours, at which time the plates were read for colony counts.

Statistical Analysis

CFU (colony forming units) were calculated by taking averaged colony counts from two runs multiplied by the dilution factor and dividing by the volume plated in mL.

Transformation efficiencies were calculated by taking the average colony count, dividing by the plasmid concentration (ng/mL), multiplying by the total volume of the sample divided by volume plated, and then multiplied by the dilution factor.

An ANOVA test was performed to compare the average CFU/mL from two runs between the colony types. A two-tailed Two Sample t-test was used to compare the plasmid CFU/mL and were calculated assuming normal distribution, independence of variables, and equal variances.

Results

The University of Utah CORE department was able to create the CRISPR-Cas9 plasmids targeting each specific sequence. We did not have the “best” sequence (lowest GC content) for target sites; by conferring with the CORE lab, two other sites were selected.

Confirmation of specificity was performed by the CORE department.

The two distinct colony types from ATCC 700603 *K. pneumoniae* were made electrocompetent then electroporated separately with bla(SHV-1), DeaD, and GFP plasmids and plated on 30 µg/mL Trimethoprim trypticase agar at 1 µL, 30 µL, and 50 µL amounts. After growing in an air incubator at 37 degrees Celsius for 24 hours, colonies were counted on each plate. A blank with sterile water served as the negative control for the electrocompetent bacteria during electroporation. This demonstrated that the bacteria without the plasmid's antibiotic resistant gene would not grow on the antibiotic media.

Transformation efficiencies for the gray colony for run 1 and 2: 1.07×10^5 and 2.79×10^4 . The yellow colony transformation efficiencies for run 1 and 2: 2.50×10^4 and 1.06×10^4 . The runs were considered to have sufficient transformation efficiency.

The average colony counts were compared across the two colony morphologies and four plasmid conditions (neg, GFP(pos), DeaD and bla(SHV-1). Analysis showed a statistically significant pvalue ($p = 3.262 \times 10^{-6}$) for the gray colony and a p-value ($p = 0.04729$) for the yellow colony, meaning the groups were different.

The gray *K. pneumoniae* after growth resulted in an average of 20.748 colonies per µL for the GFP, 0.768 colonies per µL for the DeaD, and 0.504 colonies per µL for the bla(SHV-1). Analysis of this for bla(SHV-1) vs. GFP ($p = 0.000963$) and DeaD vs. GFP ($p = 0.00100$) were both statistically significant, meaning the bla(SHV-1) and DeaD were needed for survival of the bacteria; the bla(SHV-1) vs. DeaD ($p = 0.496$) was not statistically significant, meaning that reduction in colony count was not improved by targeting one sequence or the other (see Figure 2).

The yellow *K. pneumoniae* after growth resulted in an average of 5.496 colonies per µL for the GFP, 0.036 colonies per µL for the DeaD, and 3.012 colonies per µL for the bla(SHV-1). Analysis of this for DeaD vs. GFP ($p = 0.0142$) and bla(SHV-1) vs. DeaD ($p = 0.000162$) were both statistically significant; however, bla(SHV-1) vs. GFP ($p = 0.135$) did not have a statistically significant difference, meaning the DeaD was needed for survival as shown by reduction in colony count

but the bla(SHV-1) targeting in this bacterial strain was not effective in reduction of colony count and therefore is not needed for survival.

Transformation using the DeaD housekeeping gene successfully reduced growth in both colonies, showing that is crucial for the survival of both organisms (see Figure 2). The yellow colony transformed with the bla(SHV-1) plasmid did not show significantly reduced growth compared to the positive control GFP, which may indicate the bla(SHV-1) is not necessary for the survival of the yellow organism (Fig. 2B).

Overall this proves that the DeaD gene is the optimal target for future CRISPR experiments in this bacteria model. A future experiment may involve the development of a phagemid. A phagemid is a bacteriophage that could be utilized to carry a specific CRISPR plasmid targeting the DeaD gene.

Discussion

Bacteria are becoming an even deadlier threat on a global scale due to antibiotic resistance. By looking at other methods to avoid using antibiotics the CRISPR-Cas9 system showed promising potential. We hypothesized that by targeting the DeaD or bla(SHV-1) genes colony counts would be significantly reduced in ESBL *K. pneumoniae*. To test this hypothesis, we made this bacteria electrocompetent before electroporating with the target plasmids, then counted the resulting colonies. This research demonstrated that the CRISPR-Cas9 system was able to successfully reduce growth in this bacterial model.

The bla(SHV-1) for the yellow strain, which was susceptible to Trimethoprim MIC panel result, did not reduce growth to a statistically significant amount. The yellow strain could have reverted back to a wild-type strain, rendering the ATCC 700603 type specific CRISPR-Cas9 system ineffective. Another possible explanation may be that the bla(SHV-1) did not incorporate into the genome as a crucial gene for survival like the gray strain did. This could mean the yellow strain was harboring this gene on a pathogenicity island, a genomic sequence acquired by certain bacteria through gene transfer.

The Neg Urine Combo 51 MIC panels used for the verification of the

ATCC 700603 *K. pneumoniae* colonies were expired, which may have caused the variability in Trimethoprim-resistant interpretation. When the distinct colonies were grown on the Trimethoprim trypticase media originally the yellow colony appeared to thrive better; however, this was the colony confirmed to be susceptible to Trimethoprim and was verified by a second MIC panel.

A concern with any antimicrobial therapy is the long-term effect of usage, mainly how evolution of the microbe leads to resistance. Previous studies have concluded that bacterial survivors of the CRISPR-Cas systems either did not receive the system or a defective system was received. These findings would lead to the conclusion that bacteria are not developing resistance to the CRISPR-Cas system, an advantage the CRISPR-Cas system has over antimicrobials (Beisel, Gomaa, & Barrangou, 2014).

For future studies, crucial CRISPR-Cas9 plasmids could be fused with a bacteriophage to create a phagemid which would then act as the vector to deliver this novel treatment against antimicrobial resistant organisms. For the next phase of research, an appropriate bacteriophage would need to be found before further testing could be conducted. The *DeaD* target could be a good target to start with for this phagemid model.

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Appendix

Table 1. Plasmid sequence name with genetic sequence targeted

<u>Plasmid sequence name</u>	<u>Genetic sequence targeted</u>
DeaD	GGATCGATGTTGTTTCAGCAG
Bla(SHV-1)	ACTAAGCGAAAGCCAGCTGT
GFP	GGCCACAAGTTCAGCGTGTC CGG

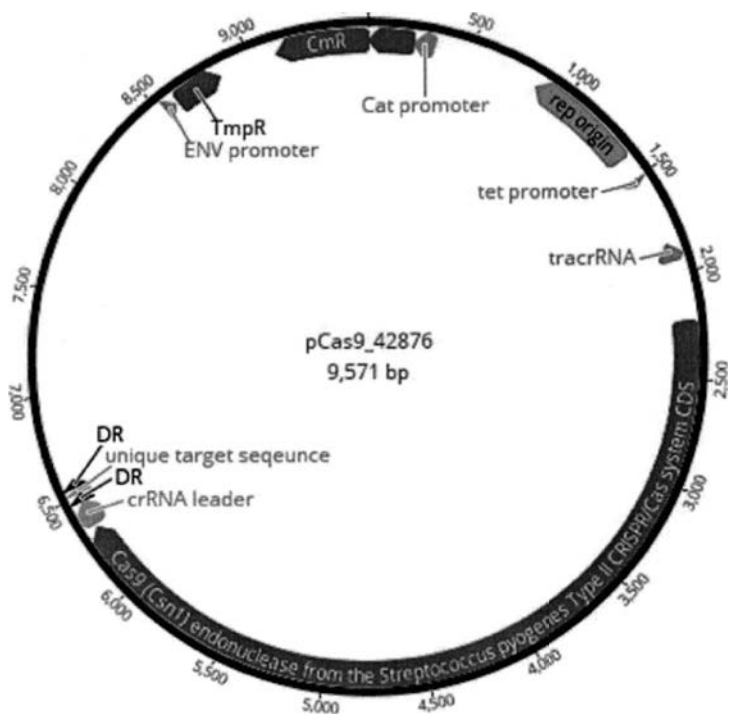


Figure 1. pCas9 Plasmid Design

Sequence specific to the targeting done in this research.
CmR: chloramphenicol resistance gene
TmpR: trimethoprim resistance gene
ENV & Cat: bacterial promoters driving expression of resistance genes
tracrRNA: trans-activating CRISPR RNA
crRNA: CRISPR RNA
DR: direct repeats
Unique target sequence: gRNA sequence specific for each gene

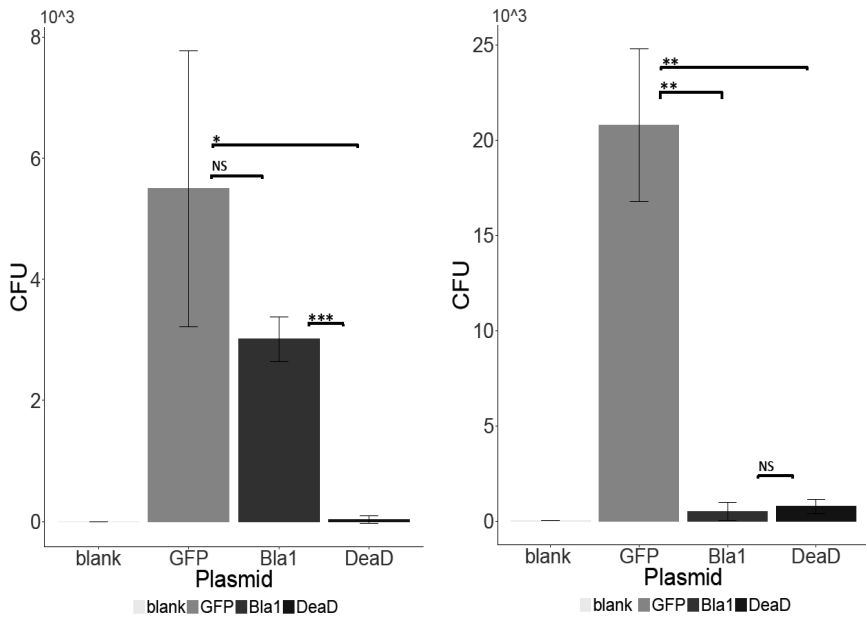


Figure 2. Mean CFU (colony forming units) per mL in two distinct colony types from *K. pneumoniae* ATCC 700603

- (A) Gray strain *K. pneumoniae* with different target plasmids
 (B) Yellow strain *K. pneumoniae* with different target plasmids

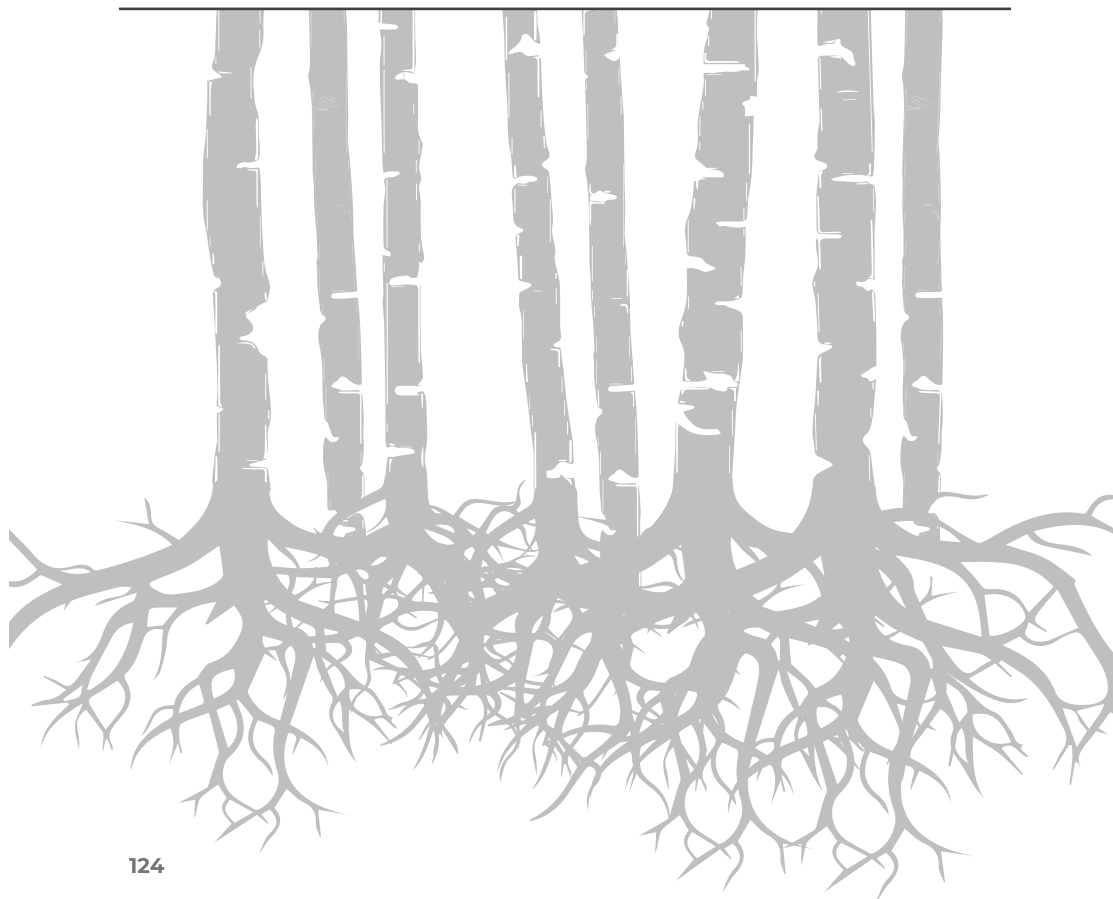
Error bars indicate standard deviation from the mean. The “blank” was the negative control for both colony types; instead of a plasmid sterile water was used in the electroporation. The GFP non-specific target served as a positive control for each experiment.

NS= no significant difference

P-values are indicated by: * <0.05, ** <0.005, *** <0.0005



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Structure-Activity Relationship of Chalcone and *In Vitro* Cancer Cell Inhibition

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Abstract

The molecule (E)-1,3-diphenylprop-2-en-1-one, commonly called chalcone, has two aromatic rings and an α , β -unsaturated carbonyl system. It is a versatile molecule which (through addition or substitution of functional groups) has many derivatives, referred to as chalconoids, that can serve as pharmaceutical agents. This study is primarily focused on the structure-activity relationship of chalcone to its anti-tumor properties. Chalcone has a half-maximal inhibitory concentration (IC_{50}) of 33.9 μ M in the HeLa and A549 cell lines. Cinnamaldehyde, which has a hydrogen in place of the 1-phenyl ring of chalcone and is a component of cinnamon, was found to have an IC_{50} of 8.8 μ M. Other substitutions have proven effective in determining which portions of the chalcone molecular structure are utilized in cell growth inhibition. Key aspects of chalconoid activity include alkene conjugation to an electron-withdrawing group (EWG), and aromaticity at the β carbon.

Introduction

Chalconoids are a class of compounds derived from the molecular structure of chalcone (see Table 1, Compound 7) and have great value in medicinal chemistry. In addition to their anti-cancer properties, their potential as pharmaceutical agents includes antimicrobial, anti-inflammatory, analgesic, antimalarial, antiviral, antioxidant, antitubercular, and antihyperglycemic properties. (Patil, Mahajan, & Katti, 2009; Prashar, Chawla, Kumar Sharma, & Kharb, 2012; Wang et al., 2015). The potential of chalconoids lies in the adaptability of chalcone's structure. There are 15 carbons at which substitutions can be made. Most research until now has focused on addition of functional groups to enhance activity of chalconoids. This study has relied on the removal and replacement of existing functional groups of chalcone to identify regions of importance.

Some work has been done to identify how chalconoids interfere with the rampant mitotic division seen in tumor cells (Das & Manna, 2016), yet little work has been done on the structure-activity relationship (SAR) of chalcone. The goals of this study include: 1. synthesize chalconoids with the purpose of making more effective (lower IC₅₀) cancer drugs, and 2. carry out a SAR of chalcone to its chemotherapeutic activity levels. We suspected that the α , β -unsaturated carbonyl system is a key structure based upon prior research of a unsaturated carbonyl compounds' inhibition of DNA replication (Erwin, Hoffman, Bastian, Deininger, & Scheckenbach, 1990).

IC₅₀ (a concentration that inhibits cell proliferation by 50%) is used as an effective measure of cell growth inhibition in *in vitro* trials. Our SAR focused on removal of functional groups to better understand their necessity in treatment efficacy.

Methods

Compounds 4, 5, 7, and 11 (see Table 1) were synthesized by aldol condensation. A modified form of the aldol condensation known as a Knoevenagel condensation was used to form compounds 2 and 8 due to their unique structures. Esterification of carboxylic acids using dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) produced compounds 6, 10, 12, 18, 22, and 32. We used mostly cinnamic acid and acrylic acid as starting material. Compounds 1, 3, 9, 13–17, 19–21, 23–31, and 33–38 were purchased from Sigma-Aldrich Corporation, St. Louis, Mo., and tested to develop the SAR.

In vitro Testing on Cancer Cell Lines

Each compound was dissolved in dimethyl sulfoxide (DMSO) to form a 0.1 M solution. The solutions were tested *in vitro* on HeLa, A549, and HTC116, which are respectively cervical, lung, and colon cancer-cell lines. One mL of cell suspension in Dulbecco's Modified Eagle Medium (DMEM) media was placed in each 12-well plate and incubated at 37° C, 5% CO₂ for 24 hours. One μ L of the 0.1 M solutions was added to each plate giving a 100 μ M dose. Two wells were set aside as control for each cell line and treated with only DMSO. Plates were incubated for 72 hours. after treatment.

Upon removal from incubation, cells were placed on ice. The medium was gently aspirated from the adherent cells. The cells were fixed with cold methanol. Cells were stained by a 4% crystal violet dye in ice-cold methanol solution, which was removed after 1 minute of agitation. After being left to dry overnight, the dye was extracted from the stained cells in 500 μL of 10% acetic acid. 100 μL of these solutions were placed in microwell 96 plates. The color absorbance of crystal violet (590 nm) was measured with a Tecan Infinite Microplate reader. When compared to a control set of cells, the effectivity of each trial was measurable across time.

Three categories were established based upon initial trials. If the compound had an absorbance of 0–45% of the control, they were considered *active*; if 46–80%, *semi-active*, and if 85%+, *non-active* (see Table 1). All active compounds were diluted through 1:3 serial dilutions up to 100-fold, providing results on a logarithmic scale from 0 (1 μM) to 2 (100 μM). The estimation of remaining cell numbers provided an indirect means of measuring cell growth up to a given point in time, as well as data to calculate the IC_{50} .

Results

Thirty-seven total compounds were tested. Nine (compounds 1–9) were initially classified as active. Though compound 9 was estimated to be active upon initial testing, a dose curve showed an IC_{50} of 51.3 μM . Thus, only compounds 1–8 were confirmed to have growth inhibitory properties.

The lowest IC_{50} (8.8 μM) was attributed to compound 1 (cinnamaldehyde) which is approximately four times more potent than compound 7, which represents the original chalcone structure (33.9 μM). Compound 1 contains a simple substitution of the 1-phenyl ring of chalcone with a hydrogen atom). The logarithmic dose curves for active compounds are shown in Figure 1.

Absorbance levels varied in controls by 8%. Though the active compounds are listed in order of decreasing IC_{50} activity, the semi-active and non-active compounds are grouped primarily by similarities in structure (see Figure 2).

Discussion

The results of the SAR seem to indicate two main properties leading to compound activity: aromaticity at the β carbon and alkene conjugation to an electron-withdrawing group (EWG).

A phenyl group at the β carbon to an EWG is seen in almost all active and semi-active compounds, excluding 4, 13, and 14. Compound 4 was a unique active compound because it utilized the aromaticity of furan groups rather than phenyl groups. Addition of an EWG was seen to increase activity (see compounds 3/28 and 5/27).

Nearly all active and semi-active compounds contain a carbonyl group in some form, other than 5 and 9. The nitro group on compound 5 is the best indicator of the EWG increasing reactivity.

The 1-phenyl group of chalcone likely does not play a direct part in the reaction that inhibits cell growth, since its substitution with a hydrogen, a benzyl ester, or a nitro group increased activity (see 1, 5, and 6) while substitution with a methyl group, phenyl or ethyl ester, or alcohol group decreased activity (see 10, 11, 12, and 15).

The 3-phenyl group of chalcone conjugated with a π bond is a key part of the inhibition process (see 1/37), but it must be coupled with additional substituents to be active (see 5/27). This suggests that a Michael reaction is involved in cell growth inhibition. It is possible that chalconoids utilize the same pathway that Curcumin utilizes (due to similarities in structure including the carbonyl conjugation, α , β π bonds, and aromaticity at the β carbons) in inhibition of histone hyperacetylation in both PC3-M prostate cancer cells (where curcumin has already been used for treatment in clinical trials) and peripheral blood lymphocytes, specifically by binding to p300. This is thought to occur through a Michael reaction, and is therefore suggestive of a similar mechanism for chalconoids (Neckers et al., 2006).

Another potential anti-tumorigenic effect of chalconoids is disruption of the cytoskeleton. Chalconoids have been identified as tubulin assembly inhibitors (Ducki, 2007). Microtubules are composed of the protein tubulin. Deficiency of microtubules frustrates cell division.

Recent research has proposed a potential mechanism of action of the most active compound of this SAR, compound 1. Compound 1—

cinnamaldehyde—and berberine (a naturally-abundant quaternary ammonium salt) have been used together to induce A549 cell apoptosis and inhibit cell proliferation. An *in-vivo* trial of mice with A549 indicated no berberine or cinnamaldehyde toxicity in mice (Meng et al., 2017).

It is not surprising, therefore, that cinnamaldehyde was found to have such a high activity level in this SAR. Though there is more research currently being done on its mechanism of inhibition of cell growth, it has been demonstrated to inhibit the inhibit Wnt/ β -catenin pathway proteins (Wu et al., 2017). This pathway is involved in cell proliferation and stem cell maintenance (Rao & Kuhl, 2010). Whether or not inhibition of this pathway is the mechanism by which all chalconoids operate is unknown.

Further research should focus on reproduction of the experiments carried out to uncover the mechanism of cinnamaldehyde-induced growth inhibition and its relationship to other active compounds in this study. This, in combination with a crossed analysis of curcumin and chalconoids, could identify a specific protein binding site for the Michael reaction to take place.

Conclusion

IC₅₀ estimation was possible though standard dose curves of thirty-seven compounds with structural similarity to chalcone. The necessity of an α , β -unsaturated carbonyl system of chalconoids was demonstrated in this SAR as well as the increased benefits of heightened aromaticity at the β carbon and alkene conjugation to an EWG. Though the mechanism of action is unknown, research on compounds such as curcumin and cinnamaldehyde suggest that chalconoids, through the process of a Michael reaction, can induce apoptosis and inhibit proliferative cell growth.

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Appendix

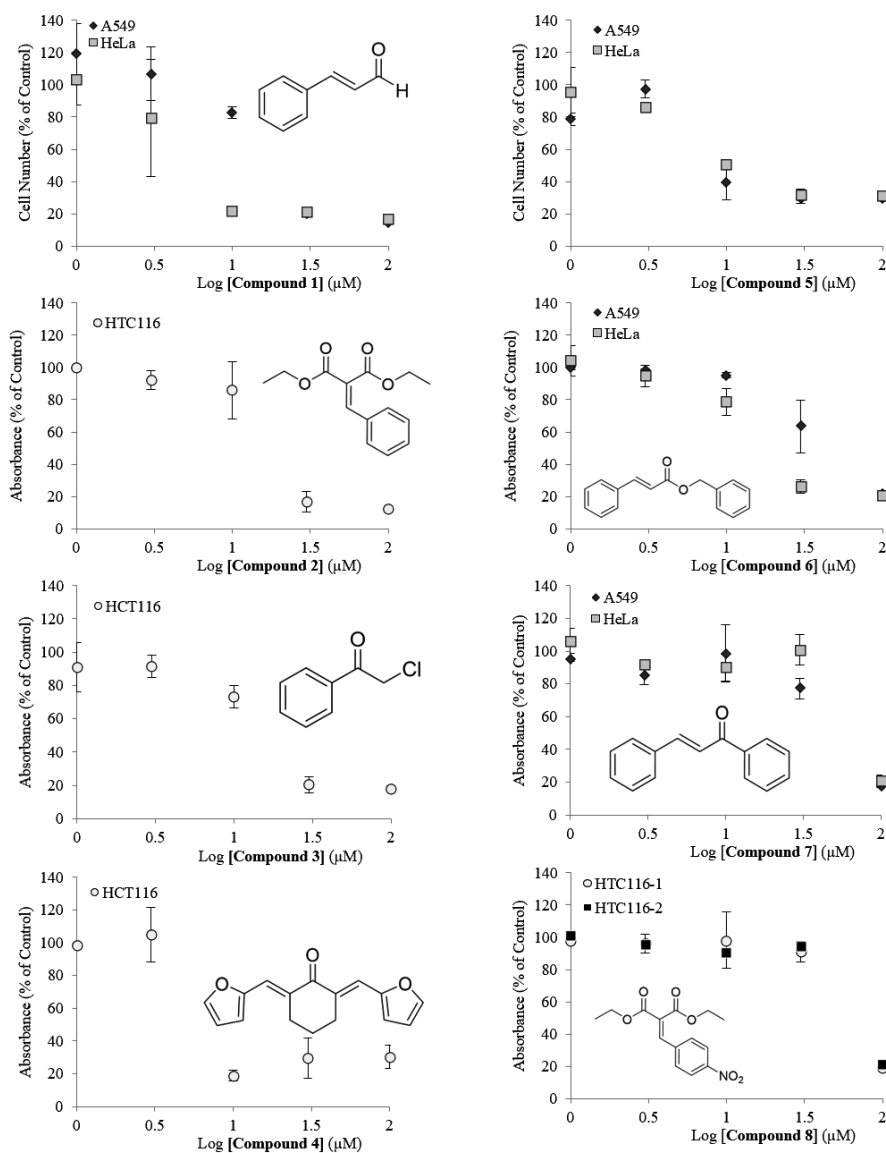


Figure 1. Logarithmic dose curves of active compounds.
 Error bars indicate standard deviation.

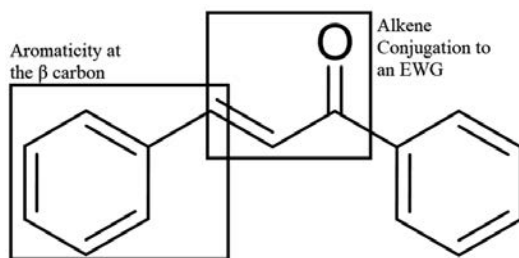


Figure 2. Key chalcone structures promoting antitumor activity.

Table 1. Compound Number Assignments, IUPAC nomenclature, structures, and IC₅₀ when applicable.

#	Active Structure	IC ₅₀ (μM)	#	Non-Active Structures
1		8.8	15	
2		10.0	16	
3		14.1	17	
4		15.1	18	
5		18.2	19	
6		21.4	20	
7		33.9	21	
8		34.7	22	
#	Semi Active Structure		23	
9			24	
10			25	
11			26	
12			27	
13			28	
14			29	
			30	
			31	
			32	
			33	
			34	
			35	
			36	
			37	

Applying Computer Aided Drug Design Resources To Increase Ivacaftor Solubility While Maintaining Target Interactions

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Abstract

Ivacaftor is the first Food and Drug Administration–approved drug that treats cystic fibrosis by treating the underlying cause rather than the symptoms. Ivacaftor is an orally-delivered drug that is absorbed in the gastrointestinal tract and travels to its target cells bound to plasma proteins. Ivacaftor has low water solubility, which causes a large percent of it to remain bound to the plasma proteins without reaching its intended target. Using free computer-aided drug design resources, structural modifications of Ivacaftor are proposed that would increase the water solubility of Ivacaftor, decrease its binding affinity to alpha-1-acid glycoprotein, and maintain favorable bind contacts with the target cystic fibrosis transmembrane conductance regulator protein. Docking experiments performed *in silico* show that selected structural variants of Ivacaftor have increased water solubility, decreased binding interactions with alpha-1-acid glycoprotein, and maintain favorable free energies of binding with the target protein. These results illustrate how online resources can be used to investigate relevant medicinal chemistry problems.

Introduction

Cystic fibrosis is an autosomal recessive genetic disorder characterized by improper production and function of the cystic fibrosis transmembrane conductance regulator protein (CFTR). The mutated gene that causes cystic fibrosis is carried by 1 in 25 people of European descent, which makes it the most common lethal recessive genetic disease (Davidson & Porteous, n.d.). The CFTR protein is a chloride ion channel found on the cell membrane, which maintains ion and water balances inside and outside of the cell. Improper CFTR function leads to lung problems such as mucus buildup, infection, inflammation, and continually decreasing lung function. Cystic

fibrosis also causes problems in other organs such as the pancreas, *vas deferens*, and other exocrine glands (Strausbaugh & Davis, 2007). Currently, the expected lifespan of individuals with cystic fibrosis is 37 years (Cystic Fibrosis Foundation, 2015).

There are over 1,700 mutations of the CFTR gene that are classified into six classes depending on the stage of CFTR production and function that is interrupted. Ivacaftor [VX-770; KALYDECO™] is an orally-administered drug that has been approved to treat 33 mutations in classes 2–4 of cystic fibrosis (Ong & Ramsey, 2016). It is the first drug to treat the cause of the disease rather than the downstream effects of cystic fibrosis. Ivacaftor binds the mutated CFTR protein, causing a conformational change that allows for chloride ions to pass through the protein channel (Rowe, 2010; Hwang & Kirk, 2013).

While Ivacaftor is the first to directly target the cause of cystic fibrosis, it is not an ideal drug for several reasons. Ivacaftor is absorbed in the digestive system and is >97% bound to plasma proteins in the blood due to its low solubility (< 0.05 µg/mL) and favorable interactions with serum proteins alpha-1 acid glycoprotein (AGP) and albumin (Fohner et al., 2017; Van Goor et al., 2009). To reach a therapeutic dose, excess Ivacaftor is given to account for the plasma protein binding. This is costly in terms of drug use and patient's financial cost. Ivacaftor can be displaced by other plasma protein binding drugs, such as ibuprofen and omeprazole (Schneider et al., 2015); this increases the free drug concentration in the plasma and hence the *in vivo* efficacy of ivacaftor. However, this is not always ideal since other effects such as drug-drug interactions, dosage, half-life, and off-target effects need to be well-understood when displacing a drug *in vivo*. A more ideal drug would have a better solubility and less plasma protein interaction, while maintaining target interactions.

Computer-aided drug design (CADD) has improved rapidly over the last 20 years and offers scientists tools to streamline rational drug design by using structural information of the drug and/or the target. Computer-based tools such as pharmacophore modeling, molecular docking, and target-protein interactions are readily available and can be applied to medicinal chemistry problems (Liao, Sitzmann, Pugliese, & Nicklaus, 2011; Rodrigues et al., 2015). CADD online websites and software allow predictions to be tested *in silico*, saving both time and money. Herein, we report the use of free CADD tools to predict

and test *in silico* molecular modifications of Ivacaftor that increase its solubility, decrease *in silico* interactions with AGP, and maintain interactions with its target CFTR.

Materials And Methods

Chemical Properties

The chemical properties of Ivacaftor were found in the Guide to Pharmacology (Harding et al., 2018).

3D-Binding Site Exploration

Schrodinger's molecular visualization program, Maestro (Schrödinger, 2018), was used to explore the binding sites using structures from the Protein Data Bank (PDB) (Berman et al., 2000) of AGP (PDB: 3KQ0 [Schönfeld, Ravelli, Mueller, & Skerra, 2008]) and CFTR (PDB: 1Q3H [Lewis, Buchanan, & Burley, 2004]). PDB structures were imported and pre-processed using the protein preparation wizard in Maestro. Ligand binding sites were explored for size and polarity in the 2D and 3D viewer using Maestro software.

Pharmacophore Modeling

PDB structures 3KQ0 and 1Q3H were loaded into ZINCpharmer (Koes & Camacho, 2012). Important pharmacophore features between proteins and ligands were selected and highlighted in ZINCpharmer online software.

3D Drawings

A 3D SDF structure file of Ivacaftor was downloaded from Pubchem (Kim et al.,

2016) and imported into Maestro software. 3D structures of the variants were created in Maestro 3D Builder. The lowest energy conformations were made using the minimization tool in Maestro. Structures were exported as MOL2 files.

Determination of Molecular Properties

Lipinski's rule of five (Lipinski, Lombardo, Dominy, & Feeney, 1997) was used to evaluate the chemical properties of Ivacaftor

and the proposed variants. Each modification was evaluated using Molinspiration's property calculation service (www.molinspiration.com) to find molecular weight and calculated LogP values.

Docking of Ligands onto Protein Targets

Ivacaftor and the variants MOL2 files were then uploaded to SwissDock (Grosdidier, Zoete, & Michielin, 2011) along with the accompanying target protein PDB files (3KQ0 and 1Q3H). Best fit docking was calculated by the SwissDock online software, with results displaying multiple possible docking conformations and their corresponding ΔG values. These docking conformations were evaluated to find conformations that mirrored Ivacaftor's interaction with the two target proteins, AGP and CFTR. The docking location and binding affinities of the modified drug structures were then compared with the original drug.

Results

Pharmacophore modeling is a tool that allows one to identify important steric and electronic features important for a drug binding a target. To begin, the important interactions of Ivacaftor and AGP were investigated. Zincpharmer (Koes & Camacho, 2012) was used to explore the AGP binding site. As there is no crystal structure of AGP bound to Ivacaftor, AGP bound to another ligand (2R)-2,3-dihydroxypropyl acetate (PDB: 3KQO) was used to model important binding site interactions in Zincpharmer Dihydroxypropyl acetate interacts with AGP through two hydrogen bond acceptor sites (yellow balls), one hydrogen bond donor site (white ball), and hydrophobic interaction (green ball). These electronic features are found on Ivacaftor as well, which presumably potentiates its binding to AGP. Using Maestro, the binding site of AGP was further investigated. Other than key H-bond interactors, the AGP binding site is relatively large and hydrophobic which explains why Ivacaftor binds well as it is larger and more hydrophobic than didydroxypropyl acetate.

In order to propose structural modifications of Ivacaftor that disrupt AGP binding but not CFTR binding, the binding site of CFTR was also examined using Zincpharmer and Maestro. Phosphoaminophosphonic Acid-Adenylate Ester was used as the ligand because there are no crystal structures of Ivacaftor and CFTR available in the PDB

yet. Key interactions of CFTR and its ligand in 1Q3H show several H-bonding sites and an aromatic interaction. It was noted that Phosphoaminophosphonic Acid-Adenylate Ester is much more polar than Ivacaftor, indicating that CFTR binding site can accommodate more polar ligands. Investigating the binding site with Maestro confirms a polar side to the binding site. This is important since the proposed modifications of Ivacaftor should improve drug solubility while maintaining binding interactions of the target.

Lipinski's rule of five is a general set of guidelines in drug design to improve the probability of creating an orally-available drug. Lipinski's rules state that most successful oral drugs meet the following guidelines: a molecular weight less than 500 kDa, less than 5 hydrogen bond donors (HD), less than ten hydrogen bond acceptors (HA), and a logP of less than 5 (Lipinski, 2000). Ivacaftor currently has a molecular weight of 392.21 kDa, 4 hydrogen bond acceptors, 3 hydrogen bond donors, and a logP value of 6.48. A logP value is an octanol-water partition coefficient; the lower the value the more it partitions in water. Ivacaftor obeys all of Lipinski's rules except for the logP. The high logP value of Ivacaftor shows that the drug partitions more into octanol than water.

Three structural modifications of Ivacaftor were proposed at the site of the tert-butyl groups. The goal of proposed modifications was to increase Ivacaftor's solubility, decrease binding to AGP, and maintain binding affinity in the binding site of the CFTR protein. The tert-butyl groups were selected for modification due to their high lipophilicity and size, which would fit into the AGP binding site. Adding H-bond acceptors, such as the nitro group, is proposed to increase solubility and interact favorably with CFTR. The first proposed structure replaces one of the tert-butyl groups with a nitro group, abbreviated Iv1. The second structure proposed replaces both of the tert-butyl groups with nitro groups, abbreviated Iv2. The third structure completely removes one of the tert-butyl groups on the aromatic ring, abbreviated Iv3. Calculated logP values were determined for each of the proposed Ivacaftor variants and, as predicted, all had a lower logP value indicating greater water partitioning.

Maestro software was used to make the 3D structural files for Ivacaftor and its variants. Docking with SwissDock was used to virtually test the binding affinity of Ivacaftor and its variants to the alpha-1-acid

glycoprotein (PDB: 3KQ0) and to CFTR (PDB: 13QH). Results of docking of Ivacaftor and the variants showed similar binding locations with AGP and CFTR, indicating that the variants bind in the correct location. The results of the binding energies from *in silico* docking showed that Ivacaftor bound to alpha-1 acid glycoprotein most favorably, while the variants had a less negative free energy of binding. *In silico* docking showed that both Ivacaftor and the variants maintain favorable binding energy to CFTR. The best proposed variant of Ivacaftor is Iv2, which reduces its binding energy to AGP by 12% while maintaining 100% of the binding energy to CFTR.

Discussion

Ivacaftor is a first-in-class drug that directly targets the CFTR ion channel to treat cystic fibrosis (Rowe, 2010). However, Ivacaftor is poorly soluble in water and primarily bound to plasma proteins such as AGP which greatly reduces the *in vivo* efficacy (Van Goor et al., 2009). To address this problem using freely available CADD resources, the binding sites of AGP and CFTR were investigated. Modifications of Ivacaftor were proposed to increase the water solubility, decrease the AGP binding, and maintain CFTR interactions. Using calculated log P values and *in silico* docking, we have shown that structural variants of Ivacaftor can be designed that accomplish each of the three desired outcomes.

Iv2 was the best proposed Ivacaftor variant in terms of water solubility, decreasing AGP binding interactions, and maintaining CFTR interactions. Iv2 replaces both t-butyl groups of Ivacaftor with nitro groups. This greatly reduces the calculated logP value from 6.48 down to 2.26, which indicates a much lower octanol to water partitioning than Ivacaftor. Using binding energies from Swissdock, it was determined that Iv2 reduces the free energy of binding to AGP by 12%. Although it is hard to predict what this would mean *in vivo*, even a small reduction of the binding energy of a drug that is largely bound to plasma protein may result in a large difference in the free drug concentration (Scheife, 1989). Finally, Iv2 maintains 100% of the predicted free energy of binding to its target CFTR. This is likely due to the H-bond acceptor sites added by the proposed nitro groups, which were found to be important pharmacophore features of Phosphoaminophosphonic Acid-Adenylate Ester and CFTR. Compared to Ivacaftor and the other proposed variants, Iv2 maintains a relatively high molecular weight and a low calculated LogP value. This

provides insight about optimal steric and electronic interactions that are important for both AGP and CFTR interactions with drug molecules.

There are, of course, limitations to using CADD. Ideally, the structure of the target and the ligand must be available. For example, investigating the ligand-binding interactions of Ivacaftor with AGP and CFTR would streamline the prediction process. Using an alternate ligand and extrapolating out to Ivacaftor may not reflect true target-ligand interactions. Additionally, the results of *in silico* docking may not directly reflect the results performed from *in vitro* docking and *in vivo* testing. Despite limitations, CADD has aided in the field's understanding of drug-target interactions and has helped narrow the focus for lead candidates that should be taken through the costly and time-consuming process of *in vitro* and *in vivo* testing (Macalino, Gosu, Hong, & Choi, 2015). Although Iv2 was shown to meet the desired goals of the project *in silico*, *in vitro* docking should be done next to confirm reduced interaction with AGP and maintained interactions with CFTR. Even if Iv2 were successful *in vitro*, it is unlikely to be a good candidate for an oral drug. This is primarily because aromatic nitro groups are often reduced *in vivo* to highly reactive hydroxylamines and nitrosamines, which cause unwanted secondary effects (Tatsumi, Kitamura, & Narai, 1986). However, selected isosteric replacement of the nitro groups which maintain H-bond acceptors, relative sterics/molecular weight, and water solubility would be a good place to start testing further variants of Ivacaftor.

We have shown that freely available CADD resources can be used to investigate pharmaceutically relevant problems by building on medicinal chemistry foundations. Use of these resources allows young investigators to develop and test hypothesis relating to drug design, medicinal chemistry, and pharmacokinetics. In addition, these resources can be used to develop and refine scientific hypotheses while saving time and money.

Conflicts of Interest/Disclosures: The authors have no conflicts of interest nor disclosures applicable to this research.

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Evaluation of Chemiluminescence Reactions using Factorial Experimental Design

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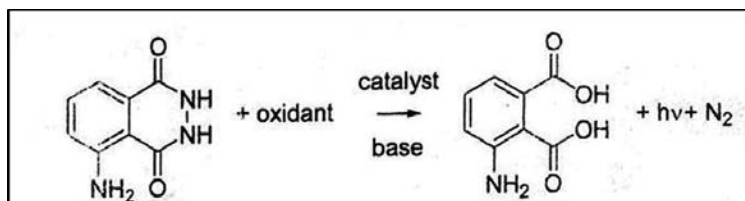
Abstract

The objective of this study was to evaluate chemiluminescence reactions using factorial design of experiments (DOE). The duration of a luminol-based chemiluminescence reaction was tested using factorial experimental design. The effect on reaction duration of three variables: a base (NaOH), an oxidant (UHP), and a catalyst (Hemin Porcine), were measured using DOE and analyzed using statistical analysis. The screening tests showed that UHP, NaOH, and hemin porcine were statistically important variables in the chemiluminescence reaction. A full factorial design confirmed these findings and showed an additional interaction effect between UHP and NaOH. In conclusion, this study showed that factorial design was effective in identifying important variables in chemiluminescence reactions.

Introduction

A common experimental design taught in many undergraduate classrooms changes one variable at time while holding the remaining variables constant. While this method has been proven effective in simple studies, statistical design of experiments (DOE) has proved more effective because DOE varies more than one variable (factor) at a time. Benefits of DOE include greater precision, estimates of variable interactions, decreased time and material, and fewer experimental runs (Czitrom, 1999). DOE applications are not only used in scientific research, but have also been applied in medical, dental, orthodontic clinical trials, and in business and economic studies (Pandis, Walsh, Polychronopoulou, Katsaros, and Eliades, 2013). This paper focuses on the fundamental mechanics of DOE and analysis of two-level factorial experiments. The application of DOE was tested by studying the duration of luminol reactions. A two-level factorial design evaluated the amount of catalyst, oxidant, NaOH, stirring, and temperature on the duration of reaction time. Luminol

(C₈H₇N₃O₂, CAS No. 512-31-3) when reacted with an oxidant, a base, and a catalyst, exhibits chemiluminescence and emits a blue glow. The reaction proceeds as follows:



Materials And Methods

Urea hydrogen peroxide (UHP, CAS No. 124-43-6), Sodium Hydroxide (NaOH, CAS No. 1310-73-2), and Hemin Porcine (C₃₄H₃₂ClFeN₄O₄, CAS No. 1316-91-2) were utilized as the oxidant, base, and catalyst respectively. Standard solutions of Luminol, UHP, NaOH, and Hemin Porcine were made at concentrations of 0.44 g/L, 0.22 g/L, 100.00 g/L and 0.02 g/L respectively. Each test was run by adding UHP to various mixtures of high and low concentrations of NaOH and Hemin containing 10 mL of Luminol in a 100 mL beaker. Each test was conducted using a total of 50 mL of the reaction mixture in a dark-opaque box with a 5"x5" hole in the bottom to accommodate the beaker. Two slits were used so two observers could view the reaction duration as shown in Figure 1. Immediately upon adding the UHP to the reaction mixture stopwatches were started and the box was placed over the beaker. An average of the two observed times was taken and recorded. The high (+) and low (-) values used for the different test mixtures are shown in Table 1.

Screening Test Results

A 12-run Plackett-Burman partial factorial screening design was used to identify the most significant variables which affect the duration of the chemiluminescence reaction. The five variables tested are given in Table 1 and include temperature, stirring, and concentration of UHP, NaOH, and Hemin. Table 2 shows the results of these twelve tests. The top row gives the variable tested. The left-most column gives the experimental run number 1 through 12. The right-most column gives the results for duration of the chemiluminescent reaction in seconds.

The unassigned factors (x6) through (x11) provide an estimate of error for the overall experimental study. The specific values tested for each variable as designated in the screening design are given by either a (+) or (–) sign. The experimental runs 1 through 12 were conducted in a randomized order to mitigate any systematic error.

The observed reaction durations ranged from 13.8 to 76.9 seconds. The data was analyzed using the computations shown in Table 2. The sum (+) values in the table are calculated by adding the duration times of each experimental run which have a high (+) value for the given variable tested. For example, UHP was tested at a high value with experimental runs 1, 2, 4, 5, 6, and 10. These runs had reaction durations of 54.0, 14.6, 25.6, 42.6, 25.4, and 13.8 seconds respectively. The sum of these times is 176 seconds as shown in Table 2. The sum (–) value is calculated by the same method. For example, UHP was tested at a low value in experiments 3, 7, 8, 9, 11 and 12. These runs had durations of 54.8, 45.6, 54.4, 73.6, 76.9, 72.1 seconds respectively. These times when added give the sum (–) value of 377 seconds shown in Table 2. The average effect of a variable is determined by taking the difference between the sum (+) and the sum (–) and dividing by the number of high values in the column, which is 6 for a 12-run Plackett-Burman design. For example, the difference for UHP between the high and low levels is -201. This value divided by 6 gives the average effect of -34. This indicates that increasing the UHP concentration will decrease the duration of the chemiluminescence reaction.

The minimum value for which an effect is determined to be significant is found using the equation:

$$[\text{Min}] = \text{Error}(t) \quad (1)$$

where Error is the estimated error in the design and t is the student- t value for the given degrees of freedom at the desired confidence level. The degrees of freedom for the design is equal to the number of unassigned factors. The error is estimated by taking the root mean square of the unassigned factor effects in the Plackett-Burman design using the equation:

$$\text{Error} = (\text{UFE}_1^2 + \text{UFE}_2^2 + \text{UFE}_3^2 + \dots/n)^{1/2} \quad (2)$$

where UFE is the unassigned factor effect and n is the total number of UFEs. The estimated error in the chemiluminescence is

$$\text{Error} = [(4^2 + (-5)^2 + (-4)^2 + (-7)^2 + (-6)^2 + (-6)^2)/6]^{1/2} = 6$$

Using the student-t table, gives a t value at a 90% confidence level for six degrees of freedom as 2.01, and the minimum difference is:

$$[\text{Min}] = \text{Error}(t) = 6(2.01) = 12.06$$

A variable will have a significant effect when the absolute value of its effect is greater than 12. The screening test results show that UHP, NaOH, and Hemin all have significant effects on the duration of chemiluminescence. Sodium hydroxide has a positive effect, so it increases the reaction time, while UHP and Hemin have a negative effect, so they decrease the reaction time. The significant effects are indicated by an asterisk (*) in Table 2. The variables, temperature and stirring did not have any significant effect on the chemiluminescent reaction.

Full Two-Level Factorial Test Results

A 2^3 factorial design was used to further evaluate the main and interaction effects of the significant variables on the chemiluminescence reaction. A 2^3 factorial requires 8 tests to evaluate all possible combinations of the variables. The test conditions and results for the full factorial are shown in Table 3. The concentrations for the main factors, UHP, NaOH, and Hemin, used for these tests are given in Table 1. Each of the eight tests were run in duplicate in a random order. Also, four midpoint tests were done at mean concentrations between the low and high point concentrations; UHP (0.12 g/L), NaOH (15.0 g/L), Hemin (0.04 g/L). The main effect of the variables is evaluated by comparing the average of four tests done at the high level (+) to the average of four tests done at the low level (-).

As seen in Table 3, the sum (+) value is calculated by adding the results of each experimental run which have a high (+) condition for the given variable tested. For example, NaOH was tested at a high level in experimental runs 3, 4, 7, and 8. These runs had reaction durations of 162.5, 47.8, 150.7, and 28.6 seconds respectively. The sum of these times gives the sum (+) value 389.5 as shown in the

table. The NaOH was tested at its low level in experimental runs 1, 2, 5, and 6. The sum of these times gives the sum (-) value 184.6. The difference is calculated by subtracting the sum (-) value from the sum (+) value. The average effect for each variable is determined by dividing the difference by number of (+) values or (-) values in the column, which is 4 in a 2^3 factorial. For example, the difference for NaOH is 205. This value divided by 4 gives the average effect of 51.2 for NaOH as seen in Table 3. These same calculations are done for all the other columns in the table to determine their average effects. In a manner similar to that in the Plackett-Burman screening, a minimum value is calculated which is used to determine if the effect of a factor is statistically significant or not. The equation is:

$$[\text{Min}] = ts(2/mk)^{1/2} \quad (3)$$

where t is a value from the t -table, s is the pooled standard deviation, m is the number of plus signs, and k is the number of replicates. In this experiment, the degrees of freedom was found by subtracting the number of averages used (9) from the total number of observations (20) as given in the factorial results in Table 3. The t -value for 11 degrees of freedom (20-9) at 95% confidence is 2.2. The pooled standard deviation calculated in Table 3 is 10.5, the number of plus signs was 4, and the number of replicates was 2. Plugging these numbers into equation 3 gives:

$$[\text{Min}] = 2.2(10.5) [2/(4 \times 2)]^{1/2} = 13.1$$

This means that in order for a factor to be considered significant, the absolute value of its average effect must be greater than 13.

As shown in Table 3, there are four factors that have effects on duration of the chemiluminescence greater than 13. These four factors are concentrations of UHP, NaOH, Hemin, and the interaction between UHP and NaOH. A full factorial analysis allowed for the determination of interactions between variables affecting the chemiluminescence reaction as shown in Table 3.

Mathematical Model

The factor effects, whether statistically significant or not, represent the difference between the responses of the reaction at the high and low levels. Therefore, if the factor effect is divided by 2, (because the levels of +1 and -1 differ by two units), the result is equal to a coded-unit change in the factor. Herein lies the predictive power of the two-level factorial design as it allows for the prediction of future outcomes based on the results of the initial experiment. A mathematical model of the two-level factorial takes the form of:

$$Y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{23} x_2 x_3 + b_{123} x_1 x_2 x_3 \quad (4)$$

where Y is the predicted response, b_j $\frac{1}{2}$ (factor effect for x_j), and b_0 is the average Y value. Using the data from Table 3 gives the coded equation:

$$Y = 71.7 - 41.6x_1 + 25.6x_2 - 8.2x_3 - 17.7x_1x_2 + 0.8x_1x_3 + 0.5x_2x_3 - 2.7x_1x_2x_3 \quad (5)$$

The coded values and the original design factor variables can be related by the following equation:

$$x_j = \frac{(\text{Factor Level}) - (H_i + L_o)/2}{(H_i - L_o)/2} \quad (6)$$

Using equation 5 for each factor gives:

$$x_1(\text{UHP}) = \frac{(\text{UHP} - 0.12)}{0.06}, x_2(\text{NaOH}) = \frac{(\text{NaOH} - 15)}{12}, x_3(\text{Hemin}) = \frac{(\text{Hemin} - 0.04)}{0.02}$$

Substitution of these values into equation 5 gives the following uncoded equation:

$$Y = 95 - 326(\text{UHP}) + 5(\text{NaOH}) - 410(\text{Hemin}) - 24.5(\text{UHP})(\text{NaOH}) \quad (7)$$

The uncoded equation provides a mathematical model of the chemiluminescence results.

Conclusion

Both the screening and DOE studies showed that different concentrations of UHP, NaOH, and Hemin have significant effects on the duration of chemiluminescence. In addition, factorial studies showed that there was an important interaction between UHP and NaOH on chemiluminescence. Increasing UHP and Hemin concentrations decrease the duration of chemiluminescence, while NaOH increases the duration. A mathematical model was developed from the DOE study results that successfully predicts experimental results.

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Appendix

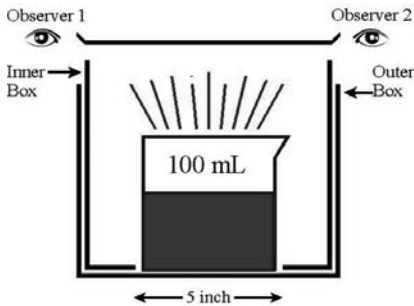


Figure 1.
Box reactor for creating dark conditions for chemiluminescent reaction.

Table 1.
High and low values used in factorial designed chemiluminescence tests.

Variable Number	Variable Identity	High (+)	Low (–)
x1	UHP	0.18 g/L	0.06 g/L
x2	NaOH	27 g/L	3.0 g/L
x3	Hemin	0.06 g/L	0.020 g/L
x4	Temperature	50 C	22 C
x5	Stirring	On	Off

Table 2.

Twelve-run Plackett-Burman screening design and computations used to determine the most significant factors in the chemiluminescence reaction duration in seconds.

Exp #	UHP	NaOH	Hemin	Temp	Stir	x6	x7	x8	x9	x10	x11	Result (sec)
1	+	+	—	+	+	+	—	—	—	+	—	54
2	+	—	+	+	+	—	—	—	+	—	+	14.6
3	—	+	+	+	—	—	—	+	—	+	+	54.8
4	+	+	+	—	—	—	+	—	+	+	—	25.6
5	+	+	—	—	—	+	—	+	+	—	+	42.6
6	+	—	—	—	+	—	+	+	—	+	+	25.4
7	—	—	—	+	—	+	+	—	+	+	+	45.6
8	—	—	+	—	+	+	—	+	+	+	—	54.4
9	—	+	—	+	+	—	+	+	+	—	—	73.6
10	+	—	+	+	—	+	+	+	—	—	—	13.8
11	—	+	+	—	+	+	+	—	—	—	+	76.9
12	—	—	—	—	—	—	—	—	—	—	—	72.1
Sum +	176	328	240	256	299	287	261	265	256	260	260	Error: 6 Min: 11 at 90% Confidence
Sum —	377	226	313	297	255	266	293	289	297	294	294	
Diff	-201	102	-73	-41	44	21	-32	-24	-41	-34	-34	
Effect	-34*	17*	-12*	-7	7	4	-5	-4	-7	-6	-6	

Table 3.

Data and computations for 2-level factorial of chemiluminescent reaction showing significant factor effects, where x1=UHP, x2=NaOH, x3=Hemin.

Data Summary					Computations									
Trial	Result		Avg	S.D.	Trial	Mean	UHP	NaOH	Hemin	x1x2	x1x3	x2x3	x1x2x3	Result
1	72.1	92.1	82.1	14.1	1	+	—	—	—	+	+	+	—	82.1
2	25.2	29.7	27.5	3.2	2	+	+	—	—	—	—	+	+	27.5
3	153.6	171.3	162.5	12.5	3	+	—	+	—	—	+	—	+	162.5
4	50.9	44.7	47.8	4.4	4	+	+	+	—	+	—	—	—	47.8
5	54.9	60.9	57.9	4.2	5	+	—	—	+	+	—	—	+	57.9
6	17.3	16.8	17.1	0.4	6	+	+	—	+	—	+	—	—	17.1
7	170.4	130.9	150.7	27.9	7	+	—	+	+	—	—	+	—	150.7
8	27.6	29.5	28.6	1.3	8	+	+	+	+	+	+	+	+	28.6
9	35.9	34.5	33.9	3.1	Sum+	573.9	120.9	389.5	254.2	261.4	290.2	288.8	276.4	
	29.3	35.8			Sum—	0	453.1	184.5	319.8	357.6	283.8	285.2	297.6	
Pooled S.D. = 10.5					Diff	573.9	-332.3	205.0	-65.6	-141.3	3.4	3.5	-21.3	
[Min] = $ts(2/mk)^{1/2} = 11.5$					Effect Sig	71.7	-83.1	51.2	-16.4	-35.3	1.6	0.9	-5.3	
t = from table (11 DF @ 95%) = 2.2														
m = number + signs = 4														
k = number of replicates = 2														
s = pooled standard deviation														

Changes in Xylem Anatomy During Fire Contribute to Post-Fire Mortality

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Abstract

Wildfire increases are putting forest populations at risk, both with initial deaths and post-fire mortality. Populations at the periphery of wildfires are at risk for post-fire mortality, and two hypotheses are attempting to explain this phenomenon. The hypothesis attributing post-fire mortality to xylem conduit failure due to high temperatures has had an increase in support from various studies. The size and shape of the xylem conduit impacts its hydraulic vulnerability, which could be increased with changes to xylem conduit shape. This study examined anatomical changes to xylem conduits due to heating. Results showed a significant difference in conduit area in control and heating groups. This supports the hypothesis that high temperatures from wildfire cause changes in xylem anatomy, which contributes to post-fire mortality.

Introduction

Over the past four decades, the wildfire season in the western United States has increased by more than seventy days (Westerling, Hidalgo, Cayan, & Swetnam, 2006). Global climate change is predicted to drastically increase the rate and intensity of forest fires (Westerling et al., 2006), and may have dramatic, irreversible effects on forest ecosystems. Wildfires increase carbon dioxide in the atmosphere, impact ecosystem stability, reduce biodiversity, and harm nearby homes and communities (Pastro, Dickman, & Letnic, 2011).

Forest fires reduce forest stability and biodiversity by killing trees in one of two ways: by burning and killing trees immediately, or by damaging tissue which results in post-fire mortality (Bär, Nardini,

& Mayr, 2018). The latter scenario is more likely to occur on the periphery of forest fires or in areas of low fire intensity (Bär et al., 2018). Although the exact physiological mechanisms that lead to post-fire mortality are not clearly known, heat-induced changes in the xylem may be a major factor in post-fire tree mortality (Bär et al., 2018). Understanding the mechanisms behind post-fire mortality of trees will broaden the understanding of the ecological impact that ever-increasing wildfires have in forested areas.

Cambium necrosis resulting from exposure to high temperatures is the most cited hypothesis for post-fire mortality of trees (Dickinson, Jolliff, & Bova, 2004). However, evidence of alteration to water-conducting tissues (xylem) as a cause of post-fire mortality is building (Michaletz, Johnson, & Tyree, 2012). Excessive heat associated with wildfires has been shown to cause anatomical changes to xylem conduits, which has the potential to increase cell-wall plasticity, causing permanent anatomical deformations upon cooling (Bär et al., 2018). Permanent changes in shape, volume, and wall thickness negatively impact the conductive ability of xylem (Bär et al., 2018). These changes increase the risk of air entering the xylem (cavitation), and rendering the cell nonfunctional (embolism), thus severely inhibiting the plant's overall hydraulic conductance.

Variations in anatomical features could impact a tree's post-fire mortality rate. Gymnosperms are less vulnerable to embolisms than angiosperms (flowering plants), due to the absence of vessel elements in their xylem. The large diameters of vessel elements along with the presence of a perforation plate allow for increased cavitation events and subsequent embolisms. Thus, vessel elements have a greater potential for hydraulic vulnerability and failure.

This is a relatively new field of study and consequently, the evidence supporting post-fire mortality resulting from heat-induced anatomical changes to xylem lacks quantitative data, and is coupled with an ongoing debate about the mechanism of post-fire mortality. The objective of this study was to quantify the effects of heat from fire on hydraulic conduits. Two dominant tree species found in the Uinta Mountains of Utah were selected, the first a gymnosperm, *Pinus contorta*, and the second an angiosperm, *Populus tremuloides*. By studying the effects on both tracheary elements and vessel elements this study was able to determine which species are more prone to hydraulic failure due to permanent changes to xylem anatomy.

Methods & Materials

Field Methods

P. contorta and *P. tremuloides* tissue samples were collected from burned and unburned sites in the Uinta Mountain range of Utah, along the perimeter of the Cobblerest and Murdock Fires (40°36'23"N 110°58'56"W, 2850m). At each site, twelve branches ~2.5 cm in diameter were cut at the node, at breast height, for each species (n=48). Each specimen was labeled and GPS location recorded (see Figure 1).

Laboratory Methods

In the laboratory, branches were cut twice under water ~5 cm to the proximal ends to remove any vascular tissue that was exposed to air during transportation. Branches were left in room temperature water overnight then stored at 4°. Using a modified version of the methods laid out by Bär et al. (2018), control samples were taken from the proximal end of each branch. Following the collection of control samples, the effects of fire were simulated in a laboratory setting by submerging the proximal ends of every branch in 90° water for one hour. After an hour, heating samples were collected by taking another cut from the proximal end. All tissue samples were chemically fixed in formaldehyde-acetic acid alcohol (FAA) for 14 days.

Samples were then placed in a Sakura Tissue Tek processor for dehydration and paraffin infiltration of the tissue. Samples embedded in paraffin were then sliced into ~4.5 µm thick sections with a rotary microtome. Sections were cleaned of excess paraffin with a series of xylene and 100% ethanol solutions then stained with Calcofluor white. Then stained sections were observed and photographed using an Olympus FluoView Confocal Fluorescence Microscope.

Data Analysis

We used a random number generator to select six conduits per specimen in the microscopy images. The cross-sectional area of each conduit was measured using ImageJ software. Conduit area was analyzed using separate two-way analysis of variance for each species, with treatment and collection site as fixed factors. Conduit area was then compared using Tukey's HSD post hoc analysis. All data were checked for homogeneity of variances using Levene's test, and for

normality using the Shapiro-Wilk test. All statistical analyses were performed using RStudio version 1.1.419.

Results

On average, *P. tremuloides* conduits had a smaller area in heated treatments compared to control treatments ($p < 0.001$; see Figure 2). There was no significant effect of collection site (Burned, Unburned) on conduit area. There was significant within treatment variance in *P. tremuloides* due to the fact that conduits included tracheids, vessel elements and vessel elements with cell walls blown out (see Figure 3). Damaged vessel elements measured over $2,000 \mu\text{m}^2$. Similarly, *P. contorta* tracheids had a smaller area in heating treatments in both sites ($p < 0.001$), but there was no significant effect of site alone on conduit area ($p > 0.05$; see Figure 2).

Discussion

We found that heat altered conduits in all heat treatment groups. Smaller, deformed conduit sizes (see Figure 2) and clear damage to the cell walls of vessel elements (see Figure 3) in heating groups support our hypothesis that changes in xylem anatomy may contribute to post-fire mortality. Additionally, our research also builds on the findings of Bär et al. (2018). Despite differences in site location, elevation, and species sampled, similar results were achieved, suggesting that xylem responses to wildfire are not site or species specific.

Anatomy is an effective tool for studying xylem conduction. Conduit shape in xylem anatomy is reflective of xylem function, and alterations to original xylem shape decreases conductivity. It is apparent that angiosperm anatomy trades cell wall stability for faster, more efficient hydraulic conduction. This leaves angiosperms at higher risk for post-fire mortality than gymnosperms. The anatomy of the xylem contributes to gymnosperm longevity and height and could play a role in resistance to conduit damage from wildfire.

Post-fire mortality due to xylem failure occurs much faster than death from cambium necrosis (Midgley, Kruger, & Skelton, 2011). This is concerning when we consider that the duration and frequency of wildfires and droughts are increasing with global climate change. Fires followed by drought would result in rapid and widespread

death of populations at the edges of wildfires. Increases in wildfire occurrence could cause shifts in forest ecology, as we demonstrated that angiosperm populations are potentially more vulnerable than gymnosperms to post-fire mortality. This could contribute to community composition shifts, which have long-lasting effects across ecosystems.

Future research on this topic is still necessary. Our findings support the hypothesis that changes in plant cell walls of xylem tissue contributes to post-fire mortality, and bring up many more questions, including questions regarding the long-lasting effects that post-fire mortality could have on forest ecology.

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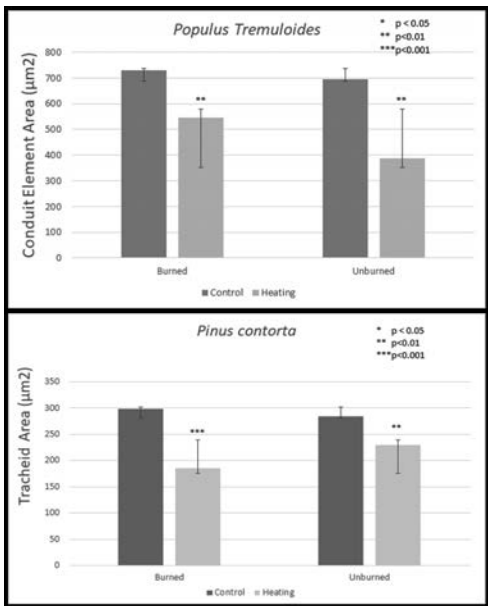


Figure 2. Statistical analysis of results of heating experiments with burned and unburned samples of *P. tremuloides* and *P. contorta*. Significant differences were found between control and heating treatments for both species. There were no significant differences found when the control groups of burned and unburned samples were compared within a species. However, inclusion of more data points might reveal significant differences between control groups.

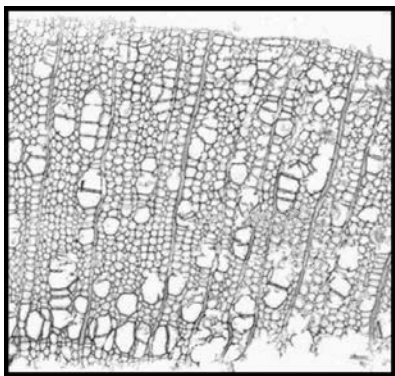


Figure 3. Cross section of *P. tremuloides* from burned area. Damage to vessel element cell wall is visible across sample.



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Obscenity, Censorship and Playwright Frank Wedekind in Wilhelmine Germany

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Modern Germany was founded in 1871 through the unification of central European German-speaking states. Many new challenges accompanied the new nation's founding, including the establishment of law and order. One of the law-and-order challenges that quickly emerged in the young nation was the proliferation and purveyance of obscene and pornographic material. German leadership attacked this societal ill and criminal activity via the passage of a new legal statute and subsequent aggressive enforcement efforts. In January 1872 the German Reichstag (Parliament) passed Paragraph 184 of Germany's Criminal Code, thereby codifying the elements of Germany's obscenity law and commencing the government's censorship of material deemed obscene or pornographic (Stark, 1981, p. 213). By the turn of the century, a revised Paragraph 184 formed the foundation of a censorship attack on Germany's artistic community.

Paragraph 184 as passed in 1872 stated "Whoever sells, distributes, or otherwise disseminates obscene publications, or representations, or who exhibits or displays these shall be fined up to 300 marks or imprisoned up to six months," obscene material being "anything that offends the modesty and morality in a sexual sense" (Stark, 1981, p. 213). Enforcement officials subjectively determined whether material "offended," hence whether the material warranted enforcement action (Stark, 1981, p. 213). In the two decades following the law's passage, enforcement was narrowly applied and only targeted persons selling and distributing obscene material. Conviction required proof that both elements of the crime had been violated; i.e., that the accused knew the material qualified as obscene, and that they intended to distribute the material (Stark, 1981, p. 213). An average of 299 persons per year were prosecuted under Paragraph 184 by the 1890s, and the conviction rate was 70% (Stark, 1981, p. 214).

The 1872 version of Paragraph 184 was valid until 1900, when a revised law was enacted. Legislative efforts to modify the law began in 1892, inspired by an event seemingly unrelated to obscenity, pornography, and Paragraph 184, but which ultimately broadened the law's scope far beyond its original intent. In September 1887, in Berlin's Latin Quarter, the "seedy side and home to its criminal elements," a night watchman, Friedrich Braun, was patrolling the neighborhood. He was murdered during his shift and found the next morning hanging from a tree with stab wounds to his neck. In late 1890, Hermann and Anna Heinze, a pimp and prostitute, married to disguise their business relationship, were arrested and charged with premeditated murder. They were tried in a two-part trial in October 1891 and June–July 1892. The jury convicted both of them on the lesser charge of "doing bodily harm with outcome." Both were sentenced to hard labor (Hett, 2004, pp. 55–99).

Sensational news coverage during the trial broadcast to all of Germany that Germany was not immune to the salacious criminality of prostitutes, pimps, and murder. Germany's king, Kaiser Wilhelm II, was agitated by the trial's sensational media coverage and how it publicized to all of Germany that the new nation—the Kaiser's Germany—had elements of prostitution, pimping, and murder. The Kaiser's Germany did not reflect a pure and pristine morality. In response, Kaiser Wilhelm II issued a decree addressing the immoralities and dangers the Heinze trial exposed (Hett, 2004, p. 86). The decree stated in part:

The Heinze trial has shown in an alarming way that pimpdom, along with extensive prostitution . . . in Berlin, has developed into a common danger for state and society. The call for an energetic struggle against this problem will in the first place bring into question how far emphatic steps against pimps can be taken on the basis of the already existing laws. . . . In connection with this, it will be discussed whether and how there may be an amendment or extension of the existing laws. (Hett, 2004, p. 86)

Braun's murder, the Heinze trial, and the Kaiser's resultant decree calling for legislative review of existing laws launched a nearly decade-long legislative debate in the Reichstag that resulted in a revised Paragraph 184 called the "Lex Heinze," or the Heinze law (Ritzheimer,

2016, pp. 54–55). Enacted in 1900, Lex Heinze reached far beyond the Kaiser's original concerns about prostitutes, pimps, and murder. It redefined the obscenity law by criminalizing not just the distribution of obscene material, but also the manufacture, possession, advertising, or public extolling thereof (Stark, 1981, pp. 216–217).

In addition to the originally criminalized material that visually depicted sexual activity, Lex Heinze re-defined obscenity to include material that depicted ideas implying sexuality (Stark, 1981, pp. 216–217). For example, “a series of drawings that began with a man and woman meeting in the park and ended with a room in which male and female clothing was scattered about was ruled obscene, even though the drawing did not actually show . . . any immoral act” (Stark, 1981, pp. 217–218). Lex Heinze was applied across all facets of German society, including the artistic community, where its application would prove oppressive to German artists engaged in any pursuit involving human sexuality.

As often happens with the enactment of a new law, enforcement of Lex Heinze yielded unintended consequences. German artists were caught up in an aggressive anti-obscenity enforcement campaign (Stark, 1981, pp. 219, 224–226, 1989, pp. 447–468). German officials subsequently recognized that not all depictions of nor inferences to sexuality had obscene intent, and were, in fact, justified. Recognizing that the “narrow prescriptions” of the law would limit artistic freedom, officials sought to “suppress products that endanger public morality without . . . inhibiting the legitimate, free development of art” (Stark, 1981, p. 222). The quandary German officials faced was how to allow for artistic freedom but still protect the public morality. They were not naïve to the notion that any clause legalizing artistic freedoms would also be abused. Manufacturers, purveyors and possessors of obscene material would defend their actions with claims that their material had artistic value (Stark, 1981, pp. 222–224).

Anticipating such abuse, officials implemented the “relative obscenity clause” in order to eliminate abuse but still provide for the artistic community's needs. The clause stated that an object may be deemed illegally obscene in one setting but legal in another (Stark, 1981, p. 224). For example, a sculpture containing human genitals was legal if it served a legitimate artistic purpose within an

appropriate artistic setting and was viewed by a “qualified audience” for relevant purposes; however, outside of those defined contexts the same item was illegally obscene because it may “arouse . . . sexual lechery” (Stark, 1981, p. 224).

Despite the relative obscenity clause, Lex Heinze continued to cast a shadow over the artistic community. The artist to whom a potentially obscene work of art was attributed was also liable for how the art was displayed, even if they played no part in its display (Stark, 1981, p. 224). Any art form properly displayed and available only to audiences “capable of appreciating it for its higher artistic . . . value as distinct from purely sexual content” was deemed legal and the artist was not legally liable. Conversely, art displayed to a public audience, i.e., persons not “capable of appreciating it for its higher . . . artistic value,” and in a public venue, even without the artist’s knowledge or approval, was illegal, and the artist was legally liable and subject to criminal penalties (Stark, 1981, p. 224).

The literary works of German author and playwright Benjamin Franklin “Frank” Wedekind (1864–1918) provide an example of the application of Lex Heinze and the relative obscenity clause. Wedekind’s career was underway by the time Lex Heinze was enacted in 1900 (Jelavich, 1979, p. 212). A number of his plays, including *Frühlings Erwachen* [*Spring Awakening*], *Die Buchse der Pandora* [*Pandora’s Box*], and *Totendanz* [*The Dance of Death*], contained overt sexual content (Borowitz, 1974, pp. 219–220; Stark, 1989, pp. 455, 466). *Spring Awakening* contained depictions of hetero- and homosexual activity, masturbation, sexual dialogue, implied rape, incest, abortion, sexual sadism, sado-masochism, and child abuse (Borowitz, 1974, pp. 219–220; Journey, 2015, p. 21; Wedekind, 1891/2012).

As an author and playwright, Wedekind condemned what he believed were incompetent parenting and puritanical sexual attitudes that resulted in the stunted sexual development of Germany’s youth (Journey, 2015, p. 21). His works emphasized conflicts between normal human sexual development and the absurdity of bourgeois Germany’s societal norms (Borowitz, 1974, p. 219); exposed sexual hypocrisies by focusing on taboo sexual behaviors (Libbon, 2010, p. 166); emphasized sexuality-based generational conflicts (Borowitz, 1974, p. 220); revealed the

audacities of “[a] bumbling education system, adult bureaucratic ineptitude, and criminally ignorant parenting” (Journey, 2015, p. 21); and “illustrate[d] how the rationalizing structures of family, school, and religion all conspire to suppress the nascent sexuality” of German youth (Jelavich, 1979, p. 213). Through his *Spring Awakening*, Wedekind condemned German society’s “refusal to provide humane guidelines to aid children in coping with their inevitable sexual awakening,” and how that failure “leads variously to sadism, masochism, autoeroticism, homosexuality, suicide, unwanted pregnancy . . . [and] abortion—everything, that is, except a ‘normal’ heterosexual relationship” (Jelavich, 1979, p. 213).

Per the relative obscenity clause, Wedekind’s sexually-charged works qualified as obscenity when presented outside of the proper context, as they were often presented. With the enforcement of Lex Heinze, he became very familiar with Germany’s censorship laws. He was twice arrested and stood trial on obscenity charges—for *Pandora’s Box* and *The Dance of Death*—but was acquitted both times (Stark, 1989, pp. 455, 466).

Though not convicted, Wedekind nevertheless suffered consequences for challenging the censorship boundaries. *Pandora’s Box* was ruled obscene, the court ordered all printed copies destroyed, and many of his other plays were banned from public performance on grounds they violated Lex Heinze (Stark, 1989, pp. 455, 466). Theatrical production of his works was achieved only after they were modified to pass the censor’s inspection (Libbon, 2010, p. 166).

Wedekind battled Germany’s censors and endured continual challenges to his works for the remainder of his career, fighting to receive public performances and to prevent their destruction. (Stark, 1989, pp. 455, 466). He once commented, “what a singularly thankless honor it is to be a German [writer] in Germany” (Stark, 2009, p. 249). That Germany twice prosecuted Wedekind for Lex Heinze violations demonstrated how German officials believed he was a purveyor of obscenity. However, his acquittal by jury on obscenity violations suggests that German society was not in agreement with German officials and that society was not morally offended or endangered by Wedekind’s works.

Wedekind was not alone in his plight against German censorship.

Between 1885 and 1914, all facets of Germany's artistic community were prosecuted for violating Lex Heinze. Twenty of Wedekind's peer authors were prosecuted and received fines or prison sentences of up to a year confinement. The works of "scores of authors" were confiscated, publication prohibited, and performances banned. German censorship sent artists to prison, estranged them from their society, and had a chilling effect on an era of Germany's artists (Stark, 1989, pp. 449–450).

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Proposal for Climate Change Policy in Face of Impacts on Federal Lands

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Introduction

America's federal public lands, which are owned equally by all American citizens, cover 640 million acres, with the majority in the western United States. Debates over management and use of these lands have been simmering even before the 1872 creation of what we know as public lands. Policies have become increasingly polarized in fast-growing western states, making political standoffs over endangered species, oil and gas extraction, grazing, and recreational access, to name just a few, common and contentious. Population growth in the western United States will place a burden on the available resources located in public lands as the need for water and energy grows. Census data on regional growth shows that "[t]he West had the highest share of its population living within an incorporated place (76.7 percent)" (U.S. Census Bureau, 2018.) Because the West is home to much of these needed resources it is vital that everyone benefit from their management, properly addressed through policy. As climate change has become a factor increasing the variability of these resources, it must be considered when making these important policy decisions regarding public lands. We need to come to a consensus that climate-related issues are real, that they will impact our public lands and affect the cost and management of resources, and that we must address the fact that we lack a climate-change policy coordinated with the federal agencies that manage our public lands.

Mapping Federal Lands

Historically, the western United States was annexed as territories before being divided up and organized into states. Hence, the federal government disproportionately owns large portions of public land in the West; these lands fall under the jurisdiction of some of the major federal agencies including: Department of the Interior, Bureau of Land Management, Fish and Wildlife Service, National Parks Service,

and Department of Agriculture, which houses the Forest Service and is responsible for the management of national forests. A look into proportions show that 46.4% of public lands reside in 11 of the contiguous western states, compared with just 4.2% in the eastern states. Policy makers are tasked with funding the management of these lands. Because climate change will make an impact on their reliability for economic growth there is good reason to investigate sustainability and pursue smart policy that is beneficial to the future of these lands.

Climate Change

Climate sensitivity, a key term best described as the response of the climate to concentrations of greenhouse gases, means that “Earth’s surface temperature would likely increase by somewhere between 1.5 degrees Celsius and 4.5 degrees Celsius once the climate came to equilibrium after a doubling of CO₂ from preindustrial 280 ppm.” (Wolfson, 2017, p. 347) Warming above 2 degrees Celsius would be catastrophic. NASA indicates that global temperature has risen 0.9 degrees Celsius, which correlates with the rise of CO₂ emissions in the atmosphere, around 409 ppm (National Aeronautics and Space Administration, 2018). As energy trends continue, we can expect CO₂ concentrations to increase, ensuring warming over 1.5 degrees Celsius by the end of the century (see Figure 1).

The International Panel on Climate Change (IPCC) found it necessary to release a special report on 1.5 degree Celsius warming earlier than planned. Policy makers should read and try to understand the report due to its urgency and implications. “Global warming is likely to reach 1.5 degrees Celsius between 2030 and 2052” (Masson-Delmotte et al., 2018, p. 6) The timing is clearly marked. “Climate-related risks to health, livelihoods, food security, water supply, human security, and economic growth are projected to increase with global warming of 1.5 degrees Celsius” (Masson-Delmotte et al., 2018, p. 11). In the Fifth Assessment Report’s summary for policy makers the IPCC specifies: “The design of climate policy is influenced by how individuals and organizations perceive risks and uncertainties and take them into account. Methods of valuation from economic, social and ethical analysis are available to assist decision-making” (Pachauri & Meyer, 2014, p.17). In the United States the lack of a national climate policy coordinated with the management of public lands is unjustified as resources are available to formulate it effectively.

The congressionally-mandated *Climate Science Special Report (CSSR)* “is designed to be an authoritative assessment of the science of climate change, with a focus on the United States, to serve as the foundation for efforts to assess climate-related risks and inform decision-making about responses” (Wuebbles, Fahey, Dokken, Stewart, & Maycock, 2017). This two-volume report is a step in the right direction for understanding the risk imposed on federal lands and evidence for the much-needed policy. Topics including how rising temperature, precipitation, droughts, floods, and wildfires will affect the 46.4% of federal lands in the West and how damages will heavily burden the U.S. taxpayers. The West will be at high risk of extreme temperatures. “Substantial reductions in western U.S. winter and spring snowpack are projected as the climate warms. Earlier spring melt and reduced snow water equivalent have been formally attributed to human-induced warming (high confidence)” (U.S. Global Change Research Program, 2018, p.159) Drier conditions hurt wildlife and plants. “Under higher scenarios, and assuming no change to current water resources management, chronic, long-duration hydrological drought is increasingly possible by the end of this century (very high confidence).” (U.S. Global Change Research Program, 2018, p.159). This would ensure that land management complications will be exacerbated for all federal agencies associated, giving more push for action.

Increased Cost Of Management

Congress appropriates the budget for the agencies that oversee management of federal lands. Overall there is a trend in declining budgets while the costs of management increase due to a multitude of factors. Adequate funding is vital to adapt to the challenges associated with climate change. The cost of fighting fires has already skyrocketed, with \$3.5 billion for wildfire appropriations between the Department of Interior and Forest Service in the fiscal year of 2013, and the cost is rising. “Since 1960, the eight largest fire years by acres burned have all occurred since 2000. In the past decade, annual appropriation for federal wildfire suppression and protection have more than tripled the funding levels since the 1990s” (Center for Western Priorities, 2014, p.3). Fires are proving to be deadlier at current global temperatures. Costs, including loss of forests, property, and lives, will mount as warming goes on.

Energy Profile Of The West

Each state has their own market for energy development plans which are continually variable, illustrated by the states with natural gas–fired electricity and a trend in some states prioritizing the cleanest option, renewable energy (see Table 1). Even with a push for electricity generation from renewable energy sources, western states have not made it past the 50% mark for energy consumption from renewable sources, so the potential for clean energy will expand.

Fossil fuel reliance has worsened the effects of climate change. Federal lands have been a source of fossil fuel exploration and mineral extraction. Leasing public lands under the Mineral Leasing Act of 1920 has allowed for even more oil and gas exploration, a major contributor to each state’s energy profile. “Royalty rates, bid minimums, and lease development terms have largely remained stagnant, resulting in a system heavily favored to the interest of oil and gas companies rather than to the American taxpayer” (DeSantis, 2018). Although amended, the act is outdated in terms of energy trends. Because of “the federal government’s failure to modernize its oil and gas program, U.S. taxpayers are now losing out on more than \$730 million in revenue every year” (DeSantis, 2018.) Incentives that provide leases for renewable energy would cut out this major fossil fuel subsidy and should be made a priority.

One bright spot regarding public land–leasing programs is through the Bureau of Land Management (BLM). “In order to create jobs, cut carbon pollution and develop clean domestic energy, the BLM is updating its policies to formalize key aspects of the BLM’s existing ‘Smart from the Start’ approach to renewable energy development” (U.S. Bureau of Land Management, 2016). This would be done with implementation of the new Solar and Wind Energy Rule, which “will facilitate responsible solar and wind energy development on public lands and ensure that American taxpayers receive a fair return from use of those resources” (U.S. Bureau of Land Management, 2016). This would yield a higher return on investment over the long term than would leases given to the fossil fuel industry. Clean energy policies could be promulgated using existing technologies that provide energy once installed, lead to cleaner air, shutting off excessive greenhouse gas emissions, and mitigation efforts that can help lessen the burden of land management.

Responsible land use is a moral argument for future residents. “We can be fairly certain that they will need food, clean air and water, shelter, open space for recreation, and natural beauty for sustenance and meaning in their lives” (Beatley, 1994, p.152). The current generation inherited this planet with resources which were overused, and as a result, ecosystems were disrupted. Fortunately, there has also been an increase in scientific knowledge regarding climate, ecosystems, and sustainable resource management. Applying knowledge with an eye to the future is a responsible way to pursue land policy.

Protecting resources is a core part of the mission statements of the federal agencies who manage federal lands. They may have different challenges, but overall, they are concerned with future use of the lands that they are preserving for future generations of Americans. Coordinating climate-change policy at the national level will facilitate responsible management. Currently, outdated national policies must consider the reality and severity of climate change, including: increasing pressure on public lands management, warmer and drier summers, decreased snowpack, and increasing populations. However, the potential for clean energy, economic development based on renewable energy, public opinion strongly in favor of ethical use of public lands, and the preservation of these resources provide opportunities and highlight the necessity to unite our efforts with federal, state, and local policy making, to protect America’s public lands.

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Appendix

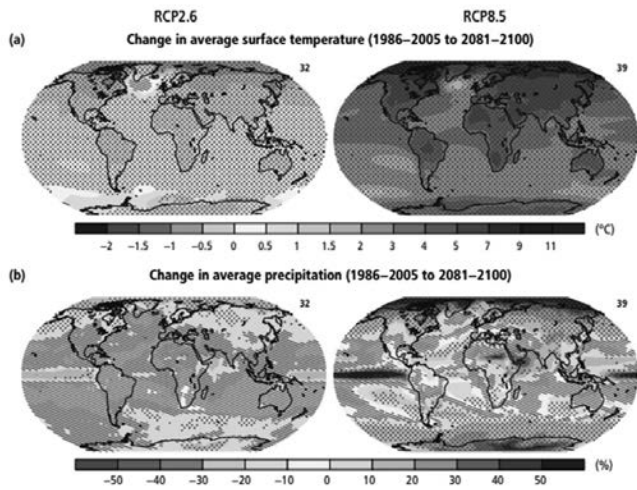


Figure 1. Projected temperature and precipitation changes by the end of the century. Reprinted from the *Climate Change 2014 Synthesis Report*, by R. K. Pachauri & L. A. Meyer (eds.), 2014, p. 12, retrieved from IPCC website: <https://archive.ipcc.ch/report/ar5/syr/>

Table 2. Energy Profile of the Western United States

Energy Profile of the Western United States						
Source:	Utility-Scale Net Electricity Generation (Share of Total)					Energy Consumption from Renewable Sources
	Petroleum-Fired	Natural Gas-Fired	Coal-Fired	Nuclear	Renewables	
Alaska	10.6%	60.50%	10.30%	0%	18.70%	3.20%
Arizona	0%	41.10%	25%	23.70%	10%	10.80%
California	0%	51.50%	0.1%	7.80%	39.70%	13.40%
Colorado	0%	31.60%	49.90%	0%	18.80%	10.30%
Idaho	0%	30.10%	NM	0%	69.40%	27.00%
Montana	0.1%	3.20%	49.80%	0%	43.40%	31%
Nevada	0%	69.50%	9%	0%	21.40%	14.40%
New Mexico	0.1%	39.70%	43%	0%	16.80%	8.70%
Oregon	NM	41.40%	3.70%	0%	54.70%	47.70%
Utah	0.1%	25.80%	63.80%	0%	10%	5.40%
Washington	NM	17.40%	8.70%	8.90%	64.40%	45.30%
Wyoming	0%	2.10%	89.40%	0%	7.60%	10.80%

Note. Data collected from “Energy Consumption from Renewable Energy,” by U.S. Energy Information Administration. Last updated Aug. 2018, *Selected States Comparison*. Retrieved from <https://www.eia.gov/state/compare/?sid=AK#?selected=US-AK-AZ-CA-CO-ID-MT-NV-NM-OR-UT-WA-WY>

Common Ground Community Garden: Its Impact On Surrounding Residential Property Values Over Time

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Abstract

This paper examines the Common Ground Community Garden, in North Ogden City, Utah, and its impact on the surrounding residential properties. Community gardens are a means of social engagement and networking for the communities that use them. Gardens can have an economic impact on the surrounding residential properties by either increasing or decreasing the property values over time. This study looks at the property values through an eleven-year timespan and how the presence of the garden has impacted those prices. The study area is comprised of just under 200 single-family residential properties. Matched pair analysis was used to analyze data for the five-year timeline before and after the garden was established in 2012. Matched pair analysis results are consistent with previous studies that identify the greatest impact is found within 1,000 feet of the open space. A more detailed analysis would include home characteristics to help explain prices.

Introduction

Community gardens can help a community grow through meaningful social work that provides a means of civic engagement and social cooperation. Community gardens can reconnect people, food, and nature to create a more sustainable future (Schemelzkopf, 1995; Macias, 2008). Community gardens do this by upholding three sociological components of local agriculture: food equity, social integration, and natural human capital (Macias 2008). The Common Ground Community Garden is located in North Ogden City, Utah, and has been a popular place for people to grow their own produce. However, no records pertaining to the impact of the garden have been kept. The impact of the community garden on the surrounding properties would be a vital piece of

information to have when planning a future garden development project. The overall nature of this research is to gather empirical data on the community garden's impact on the surrounding property market values. Based on the literature reviewed, it is hypothesized that the presence of the Common Ground has increased the market values of the surrounding residential properties since its creation in 2012.

Background

Numerous researchers have found that community gardens empower the communities that utilize them. Bailey, Hendrick, and Palmer (2018) highlight the implications that community gardens have for social work and how they become staging points for action, observations also made by numerous others (Schmelzkopf, 1995; Macias, 2008; Voicu and Been, 2008; Wakefield, Yeudall, Taron, Reynolds, and Skinner, 2007; Rosol, 2012; Teig et al., 2009; Hanna and Oh, 2000; and Cumbers, Shaw, Crossan, and McMaster, 2018). Community gardens allow for the sharing of food, plant knowledge, and gardening skills, as well as the building and construction of ideas. Members can network with each other to share the latest news and information and build upon skills that can be translated into life outside the garden. Community gardens provide a place for children and adults to work, play, and learn. Community gardens act as sanctuaries that protect users from the dangers, stresses, and temptations of the streets (Schmelzkopf, 1995; Teig et al., 2009; Hanna and Oh, 2000; Cumbers et al., 2018; Wakefield et al., 2007; Bailey et al., 2018).

Furthermore, community gardens have also been at the center of political and commercial development disputes as they are often erected on lots that are most desired for commercial development (Schmelzkopf, 1995; Cumbers et al., 2018). These contested spaces often involve squatter gardens that utilize the land without any agreements made with the city or the landowners (Schmelzkopf, 1995). In some cases, often seen in New York City, cities will offer one-year lease agreements, sometimes longer, that the community gardeners pay for. The presence of the garden is tolerated until the lot gains any plans for development in the future (Schmelzkopf, 1995). The gardeners can sometimes be unsure of when that time will come. Disputes in the community can arise between members of the garden and organizations that support the development plans (Schmelzkopf, 1995; Cumbers et al., 2018; Rosal,

2012). These land-use disputes can be a challenge to overcome and endure. Such disputes can create difficulty in establishing gardens.

Community gardens, in addition to these social implications, may also have an economic impact. When green spaces are created, they can impact the surrounding community. However, not all green spaces will necessarily have the same effect on home prices. Members of community gardens feel that their gardens increase the value of the surrounding residents' properties. Property values have been seen to be impacted after the installation of a community garden (Voicu and Been, 2008). Market values of homes are increased up to \$2,100 USD within 1,500 feet of green spaces (Bolitzer and Netusil, 2000), by 7% in value for every 1% increase in the open space size (acres) (Conway, Li, Wolch, Kahle, and Jerret, 2008), and \$28 USD for every acre increase in green space (Bolitzer and Netusil, 2000). The impact of a community garden is strongest in the immediate area surrounding the garden and will decrease as the distance from the garden is increased (Voicu and Been, 2008; Bolitzer and Netusil, 2000).

Despite there being few studies that look at the impact of community gardens on home values, there is a link between the garden's proximity to a property and the market value of that property (Morancho, 2003). Voicu and Been (2008) indicate residential property values are most impacted within a 1,000-foot radius of a public green space. Properties located within 100 feet of a green space do not show a statistically significant relationship between property value and the presence of a community garden (Conway et al., 2010; Boltizer and Netusil, 2000). This lack of significance is expected, as the properties experience both positive and negative effects, such as an increase in traffic or noise. Conway et al. (2010) determine that proximity has the most significance when properties are 200 to 300 feet from a green space. Increasing the amount of green space by 1% can increase the sale price of a home by 0.07% which is equivalent to \$171 on average. Analysis indicates that the distance from a home to an open green space of any type can have statistically significant effects on a home's sale price (Boltizer and Netusil, 2000; Morancho, 2003). Depending on what method is used to analyze the relationship between open space and home value, proximity has been found, in some research, to be the most important in the literature (Boltizer and Netusil, 2000; Morancho, 2003).

Using linear and semi-log functional forms, green space significance can be determined using hedonic valuation and assuming a constant variable for housing characteristics (Owusu-Ansah, 2011; Moranchó, 2003; Bolitzer and Netusil, 2000; Conway et al., 2010; Voicu and Been, 2008). Using hedonic valuation to explain housing prices, three important environmental variables are highlighted: proximity of the household property to green space, the existence of view from property to green space, and the size of the green space (Moranchó, 2003).

Most of the literature defines open and green space as parks, cemeteries, and golf courses, each with the potential to generate additional property value. Hanna and Oh (2000) and Rosol (2012) are some of the few known studies that recognize green space as not just parks, cemeteries, and golf courses, but also community gardens.

There does exist a gap in the literature. There is little that focuses on how the presence of a community garden may impact the property values of the surrounding residential properties. Community gardens have become popular, and cities have acted blindly in creating them. These open space findings (Bolitzer and Netusil, 2000; Moranchó, 2003; Conway et al., 2010; Fan, Hansz, and Yang, 2016; Owusu-Ansah, 2011) could be used to analyze the impact of a community garden installation. Without understanding its impacts on the community, it would be unwise to install a community garden until looking deeper, as Macias (2008), for example, concludes.

There are few studies that specifically focus on the impacts of community gardens on residential property values. Voicu and Been (2008) are among the very few studies to look specifically at the effect of community gardens on the surrounding property values. Fan et al. (2016) are among the few that consider both open space proximity and home value while also factoring in the current housing market status to prevent biased results when looking at the resulting impact. Previous research (Macias, 2008; Teig et al., 2009; Bailey et al., 2018; Schmelzkopf, 1995; Cumbers et al., 2018; Wakefield, 2007; Hanna and Oh, 2000) on community gardens tend to focus on the social implications within the community and the benefits of producing food. Studies that take an economic approach (Fan et al., 2016; Bolitzer and Netusil, 2000; Moranchó, 2003; Conway et al., 2010) typically focus on open space and housing prices without incorporating community gardens. The focus of the current research is to address this gap in the literature and understand how the installation of a community garden can impact the surrounding

residential community when the property values are compared to each other through time before and after a garden's installation.

The Common Ground

The research project described in this paper looks at the Common Ground Community Garden, located at 2557 North 550 East, North Ogden, Utah (see Figure 1), and how it has impacted the surrounding residential properties' market values over an eleven-year timeline.

In 2012, the North Ogden Parks and Recreation Department created this garden on city-owned land to accomplish its mission of "providing open space and wholesome activities for individuals and families." The garden has been a very popular place for people to learn about and grow their own produce. Since the garden was established in 2012, according to the parks and recreation director, all available garden beds have been rented out each season except the 2017 season (two garden beds went untouched). The 2018 growing season had 14 of the 39 growing boxes used. The garden does not limit how many garden beds a person may use each season so long as they are paid for. Therefore, multiple boxes may be used by a couple people. The decline in utilization for the 2018 growing season was caused by the loss of a few gardeners.

In the 2017 and 2018 growing seasons, the garden experienced problems related to gardeners not paying for the boxes they used. This can be a problem since, according again to the director of North Ogden Parks & Recreation, the registration fees that are paid per box are used to help partially fund and maintain the garden. The registration funds gathered from the garden are used to install and repair the garden boxes (4'x12') as they age over time and provide topsoil each growing season. The rest of the garden's costs are supplied by the city and cost approximately \$2,000 USD each year. It is up to gardeners to maintain their boxes, and volunteers to maintain the landscape.

Methodology

By sending a Weber County Government Request for Records (GRAMA) form to John Ulibarri, the Weber County Assessor, market value information was obtained for every parcel in Weber County for the years 2007–2017. The number of parcels ranged from 90,900–100,000 depending

on the year. Parcels that exist outside the census tract and the final study area were eliminated. 1,981 parcels were identified within census tract 2102.04. The study area (see Figure 2) contains 1,588 properties. Geographic Information Systems (GIS) software was used to create the study area and identify the parcels for each zone within the study area. These zones can be seen in Figure 2. The established zones are as follows from the garden: zone 1 (0–100 feet), zone 2 (200–300 feet), zone 3 (300–500 feet), zone 4 (500–1,000 feet), zone 5 (1,000–1,500 feet), and zone 6 (parcels that are more than 1,500 feet from the garden). The zones do not entirely encircle the garden plot. To allow for equal comparison between parcels, the zones are restricted to census tract 2102.04. The location of the garden in census tract 2102.04 creates a unique circumstance where the zones increase in sample size the farther they are from the garden (see Figure 2). There were some parcels that did fall within the established zones; however, they were removed from the study because the market value data did not extend back as far as 2007, they did not have addresses, they did not have residential structures built on them, or they were not classified as a single-family residential type (e.g., duplexes, commercial (grocery), and city-owned buildings/facilities).

With the study area identified and each zone established, the needed parcels were filtered out of the information received from the Weber County Assessor's Office. Each parcel had an assessed property market value for each year between 2007 and 2017. The parcels were grouped by zone and listed in chronological order. Five years of market values existed on each side of 2012. This allowed for an equal before and after comparison between the two time periods.

The averages for each zone were calculated for each year. The averages were used to calculate an anomaly value to see how far they existed from the average. The anomaly aids in understanding the impact of the garden before and after 2012, when it was installed. It was calculated by subtracting the overall average for each year from the average of each zone for each year. Two t-tests (paired two sample for means and two-sample assuming unequal variances) was conducted on the before (2007–2011) and after (2013–2017) time periods for zones 1–6.

The same tests were conducted again on zones 1–6. Each zone in this series of tests had a sample size of $n=6$. It was thought that since zone 1 has such few samples, it might be skewing the results. The second series of t-tests was conducted with an equal sample size, $n=6$, for each zone.

Three tests were conducted on this sample size. The samples for zones 2–6 (since zone 1 already had 6 samples) were randomly selected for each test using a random number generator. This action produced new average and anomaly values for each test.

To further investigate zone 1, and if its small sample size was impacting the results, it was removed entirely from analysis. Zones 2–6 each held their original sample sizes from the first t-tests. A t-test was conducted on the before and after anomaly values, as for all other tests to this point.

The fourth series of t-tests was conducted with an equal sample size, $n=14$, for each zone (excluding zone 1 which was removed once again). Three tests were conducted on this sample size. The sample sizes for zones 2–6 were randomly selected for each test. The removal of zone 1 created different averages and anomaly values for each test.

Results

When you look at the charted anomaly values for 2007–2017 (see Figure 3), the most striking result is for the 100-foot zone. This zone sees a clear decline in property values, starting in 2012, compared with the whole study area. This result is thought to be expected since the literature suggests that properties in this zone may not experience a positive impact from the garden given negative factors such as increases in traffic or noise. It is still unclear as to how beneficial a garden can be to homes in zone 1. The literature suggests that the zones that will experience the most impacts will be zones 2 and 4. A t-test: paired two sample for means indicates that out of zones 1–6, only zone 4 is significant with a p-value of $p<.05$. Zone 2 is not indicated as significant, but it does come rather close. An additional type of t-Test (two-sample assuming unequal variances) was conducted. This test reveals the same results, but in this case, zone 2 is also significant with a p-value of $p<.05$.

Anomaly values show steady trends before and after 2012. The lack of change (apart from the outlier changes that followed the 2008 market collapse) after 2012 would indicate that there are no significant impacts taking place. T-test results for both types of tests conducted on the first $n=6$ series, only show zone 4 as being significant. The second $n=6$ test shows that zone 4 is not significant, with p-values of $p>.05$. Both types of t-tests, however, show that zone 2 is rather close with a p-value of (.058). T-test results for the third test on $n=6$ show that there are no

zones that provide a significant enough p-value ($p < .05$). For both t-type tests, zone 2 does come close to being considered significant ($p < .05$).

The t-test: paired two sample for means results shows that zone 4 is significant with a p-value of $p < .05$. Zones 2 and 6 are not significant ($p > .05$), but they are near significance. The results for the second type of t-test show that zones 2 and 4 are both significant ($p < .05$) and that zone 6 is near significance. The most striking result is for zones 2 and 4, which would be consistent with the t-test results. These zones each show a clear change in property values, compared to the complete study area, starting in 2012.

The time periods before 2012 are not entirely linear. Zone 1 shows somewhat of a decline until 2011, an increase in 2012, a small decrease in 2013, and then consistent increases. Zone 6 increases after 2012, but then clearly declines after 2013. The t-test results do not indicate any zones with significance ($p < .05$) which is interesting to find. The t-test results for both test types do not show significance for the second test on sample size $n=14$. The first t-test type shows that zones 2, 5, and 6 are all near significance. The second t-test type changes those results to zones 4–6. The anomaly values for this test are generally linear and consistent before and after 2012. Consistent with the previous t-test results, zone 4 is significant ($p < .05$) in both t-test types. In both test types, zones 2 and 6 are near significance.

Discussion

In each test, zone 1 did not produce a significant enough value to be able to confidently say that there is a relationship between market values and the community garden. Perhaps there is more to the projected increase in noise and traffic.

The properties that are within 100 feet of the garden are also near a senior living center, city hall, a skate park, the North Ogden Police Department, and a county library. These locations often contribute to high traffic volumes on 2550 North, the road that also accesses the neighborhood. This could account for the uncertainty seen in zone 1.

What has been consistent through t-tests is the significant results of zone 4 and the occasional results of zone 2. Zone 2 rarely had a significant p-value ($p < .05$). Zone 2 (200-300 feet) is supposed to,

according to the literature, experience the greatest impact. This was not seen in the data analysis. It may be worth noting that even though zone 2 was rarely significant, it often was very near to reaching a p-value of $p < 0.05$. Zone 2 ceases to increase in value in 2012 and property values fluctuate up and down between 2012 and 2017. This could explain why zone 2 had different t-test results depending on the sample size. Additional research and investigation are needed to better understand the relationship between Common Ground and zone 2.

Occasionally, statistical analysis would show that zones 3, 5, and 6 were near significance, which was unexpected. The literature does not predict any impact on these zones; however, it could be consistent with the idea of distance decay. If zone 4 is experiencing a significant impact from the garden, and zone 2 shows potential to be doing the same, then it would make sense to have zones 3, 5, and 6 experience some sense of the garden's impact.

Conclusion

The focus of this study was to look at Common Ground and how it is impacting the surrounding properties. The initial study is unclear as to what direction the impact of the garden is having, positively or negatively. The literature would suggest that the impact is positive. The findings of the various t-tests in this study were consistent with Voicu and Been (2008) and Conway et al. (2010). It may be safe to suggest the same for the garden. However, additional research is needed to better understand this idea.

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Appendix



Figure 1.
Common Ground community garden site.
Photo credit: Andrew McDonald, taken Sept. 2018.

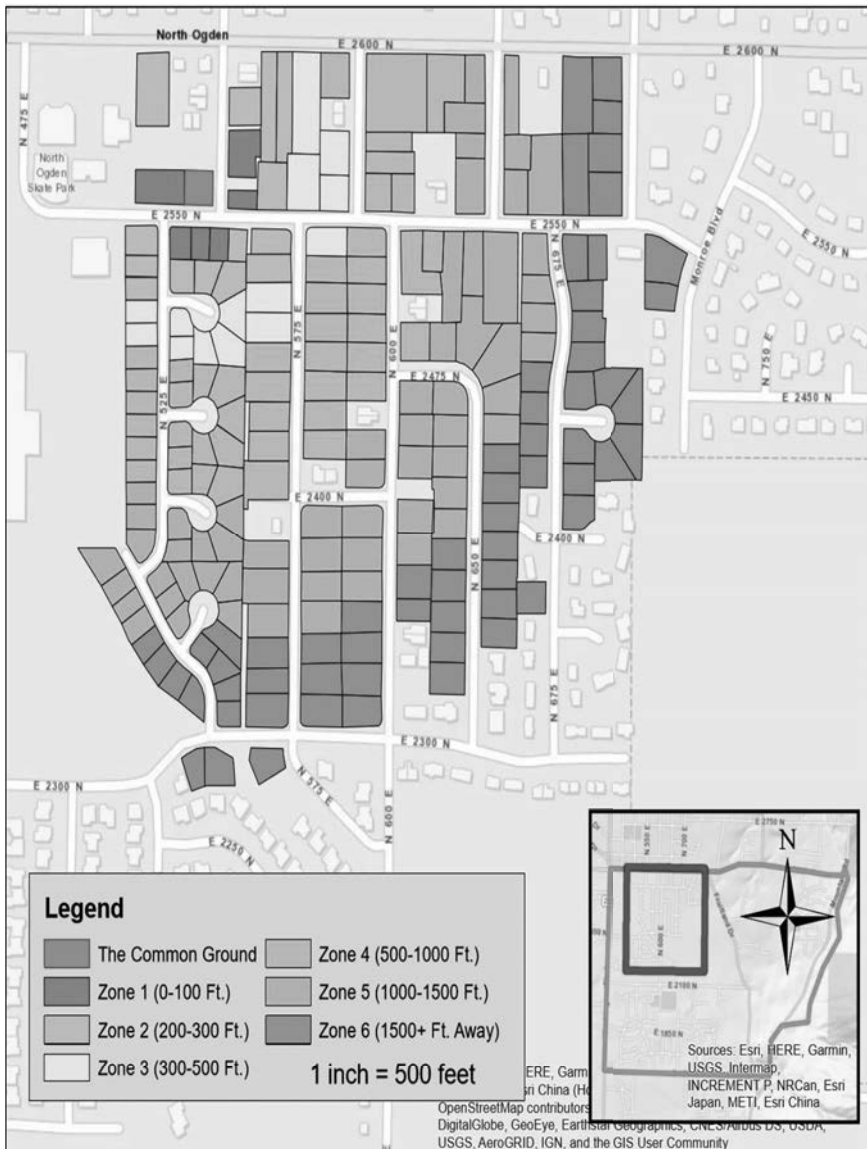


Figure 2. Map of the study. A locator map is included in the bottom right of the image showing the location of the garden in relation to the surrounding area within census tract 2102.04 shown in blue. Photo credit: Andrew McDonald, created using GIS Software.

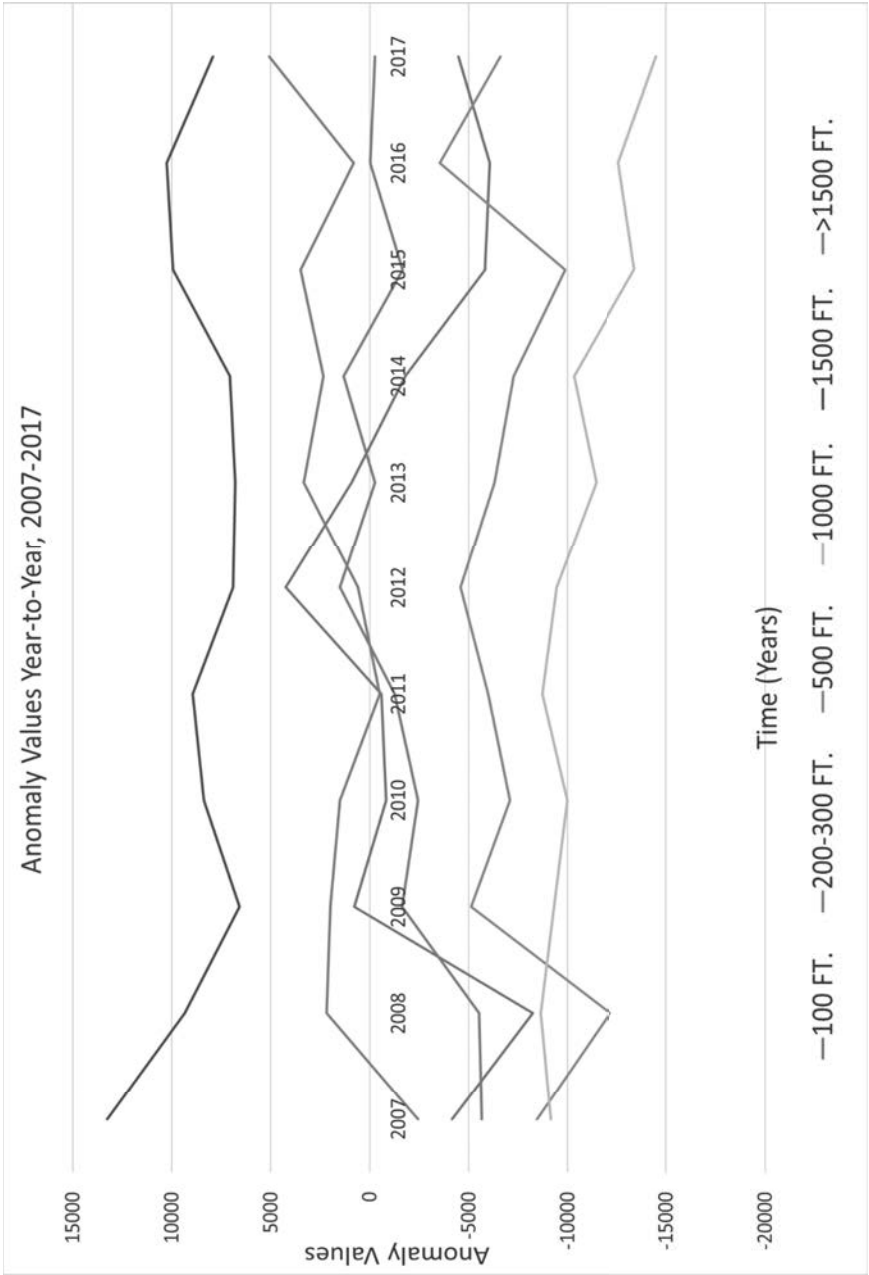
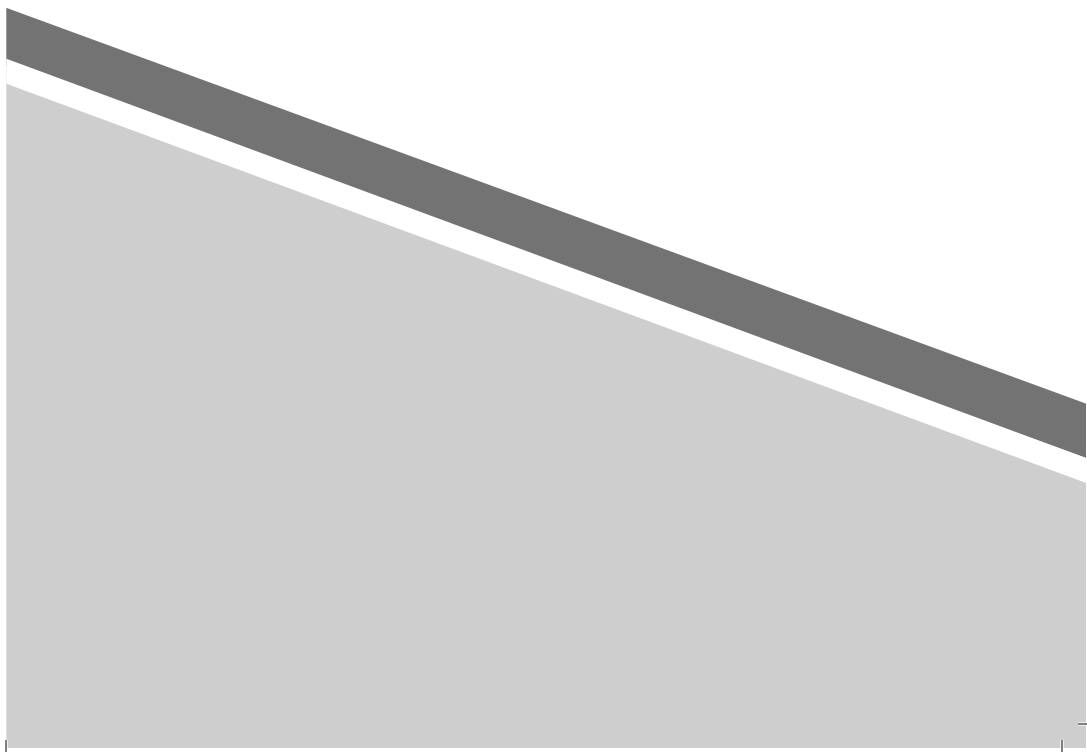


Figure 3. Average anomaly values by zone, calculated by subtracting the average of the year from the average of the zone in question.



Peeking Through The Palisades at Palmares: The Impact of Environment on History

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As a result of European dominance of the western hemisphere, the narratives commonly known about the history of the western hemisphere are decidedly European in terms of protagonists, dogma, and *weltanschauung*. Until the twentieth century, historians and their vocational cousins (such as archaeologists and anthropologists) almost exclusively covered the “rich and famous” of the Western tradition, such as Columbus, Cortez, Cabot, and Clark. In an apparent self-deception, the West coined the maxim “there are two sides to every story,” while simultaneously only engaging in one half of the historic Age of Discovery. Missing from the popular understanding of this monumentally important time in world history has been the other side of the story. These perspectives were often explicitly suppressed by their victorious contemporaries. The vanquished peoples of the new world underwent a dual tragedy: one destruction, physical; the other, cultural.

The result is a decidedly stilted view that silences the past—or at least broad swathes of it—in favor of the societies who conquered, utilized written language, or otherwise conformed to European ideals of strength or knowledge (Trouillot, 1995). It has only been in the last century that intrepid luminaries such as Charles Boxer, Stuart Schwartz, John Thornton, and Flávio Gomes began carving a new path through the dense overgrowth of historiography, and shining light on previously unexplored sections of the Age of Exploration. The resulting shifts in our current understanding of the early modern period have lent a voice to peoples who have been denied the opportunity to tell their own story.

One area of this period that still remains underexplored (in English especially) is the history of so-called maroon communities, or communities formed by those intrepid previously-enslaved people who threw off the chains of bondage and sought liberty in the dense jungles, vast plains, and other imposing terrains untouched by

European encroachment. The history of these communities offers a rich, yet challenging, avenue for historians seeking to improve our understanding of the period. Throughout the history of these communities, one in particular stands out in terms of its longevity, population, and ability to defy the multiple military expeditions sent by colonial authorities to destroy it. Known as Palmares in contemporary documents, this *mocambo*, or hideout, was actually a group of affiliated settlements, with its own collective economy, government, and societal institutions, including, paradoxically, the institution of slavery.

As historians are reliant on written documentation, Palmares presents a challenge for anyone seeking to illuminate its past. As a result of near-universal slave illiteracy, Palmares, like many maroon communities, exists in a fog of historical uncertainty. Historians cannot even agree on the year when Palmares was founded. All known primary sources dealing with Palmares come from the Portuguese or Dutch colonial authorities, the same authorities who vehemently sought Palmares' destruction. Because of the lack of documentary evidence, historians discussing Palmares have been forced to use broad strokes, unable to show in detail what Palmares was like. The challenge of documenting a society without its own documents has caused previous historians to either try filling in the missing spaces of our knowledge about Palmares, or remain silent on its inner workings. Perhaps new questions about Palmares could help overcome these obstacles and reveal new answers about a society which has largely been denied the opportunity to tell its own story.

Missing in the historical literature examining Palmares has been any examination of how its environment affected its development. Yet societies around the world have long had their development and culture shaped by their environment. Two environmental elements which had a significant effect on Palmares were those of distance, or rather, the large spaces common throughout colonial Brazil, and the harsh and severe terrain of northeastern Brazil. Understudied until now, distance and terrain had significant impacts on Palmares, from influencing the location chosen for Palmares, its governmental structure, and even guiding how Palmares managed to survive in such a hostile environment. In fact, examination of the role the physical environment surrounding Palmares played can help overcome the hurdles inherent in the challenge of documenting a people without their own documents. Through a critical

reading of available primary sources on Palmares, details emerge of how two environmental factors, distance and terrain, directly affected the development, survival, and eventual downfall of Palmares.

A better understanding of the ways in which distance and terrain affected Palmares can shed light on why the approximate location of Palmares was selected by its inhabitants. Logic dictates that escaped slaves would seek to flee as far away from their captors as possible, minimizing the chance of recapture. Yet Palmares remained relatively close to colonial centers of power along the Atlantic coast of Brazil. Why would former slaves choose to remain within striking distance of hostile colonial powers? In spite of the fact that most fugitive slaves desired to escape completely the vicinity of nearby plantations, the survival of these slave communities required critical goods that they could not manufacture themselves, such as firearms and gunpowder (Klein and Vinson, 2007, p.176). This dilemma left maroon communities with two choices to fill these needs: trading or raiding.

As a result, something like a maroon “Goldilocks zone” existed, allowing significant distance between the *mocambo* and colonial centers of control, such as port cities. At the same time, *mocambos* needed nearby farms, ranches, or settlements within a few days’ journey in order to have reliable access to manufactured goods, such as gunpowder and firearms. “Access” is a broad term, since the residents of *mocambos* would often trade with or violently raid (frequently doing both, in an effort to coerce cooperation from a farm owner or plantation overseer) in order to obtain materials needed for their defense (Gomes, 2008). In fact, the nearest village under *Palmarino* control was only a mile from a small colonial farming community (Relação, 2010, p. 226).

Because underground networks of trade were the lifeblood of any *mocambo* unable to produce important but difficult-to-manufacture items, such as gunpowder and firearms, Palmares, like many other *mocambos* in Brazil fostered the creation of strong—but clandestine—trade networks with nearby plantations (Gomes, 2008, p. 383). The documents show that Palmares would trade surplus goods produced by *Palmarino* residents (such as palm wine, palm oil, salt, and textiles) in exchange for goods from colonial merchants or their intermediaries (Relação, 2010, p. 220–33). In fact, the extent of Palmares’ trade network is lamented by colonial Governor Fernão de Souza Countinho

in a 1670 proclamation, which prohibits the trading of any firearms to “slaves, mulatos [sic]—free or slave—or any such person.” In the same proclamation the governor condemns the use of, transfer to, or possession of firearms by the above categories, “who use all types of firearms without any fear of God, nor respect and observation of . . . the laws of his highness” (Carneiro, 1958, p. 227–229). Obtaining these important items through trade would not have been possible without the resources found in the environment around Palmares.

Palmares was far from being the only *mocambo* to utilize trade. Both Flávio dos Santos Gomes as well as Stuart Schwartz demonstrate how several later *mocambos* in Brazil would develop trade networks to procure firearms for their defense. As Brazil was a land of vast mineralogical wealth, several later *mocambos* traded in gold panned from local alluvial deposits, in quantities sufficiently large enough to entice many *moradores* and merchants into risking their lives and liberty in order to profit from trade with *mocambos* (Schwartz, 1970). Although we do not have a specific source confirming the use of gold by *Palmarino* traders, the ubiquity of gold as an exchange commodity in later, better-documented *mocambos* makes it very likely that Palmares also participated in the gold trade. Regardless, the abundance of resources surrounding Palmares allowed the formation of strong clandestine networks of trade, representing another way in which environment played a significant role in the economy, as well as the survival, of Palmares.

The environment around Palmares helped significantly with its physical security. In fact, distance and terrain were by far the best defensive weapons in the *Palmarino* arsenal. As the leader of one expedition described, simply getting to Palmares was a formidable task:

It is a naturally difficult place, full of mountains and wildlands, seeded with every variety of trees, both known and unknown, as well as a density and confusion of paths, where in many places it is completely impenetrable to even light; the diversity of thorns and noxious, creeping trees impede each step and the passage of baggage (Relação, 2010, p. 221).

In order to reach the king’s palace in Palmares, soldiers laden with gear and supplies would have to navigate more than 187 miles of this terrain. Because of this, many military expeditions would fail before

even reaching Palmares, often running out of supplies and having to return to areas of colonial control. Students of military science will readily recognize that Palmares successfully deployed strategic depth as a fundamental strategy.

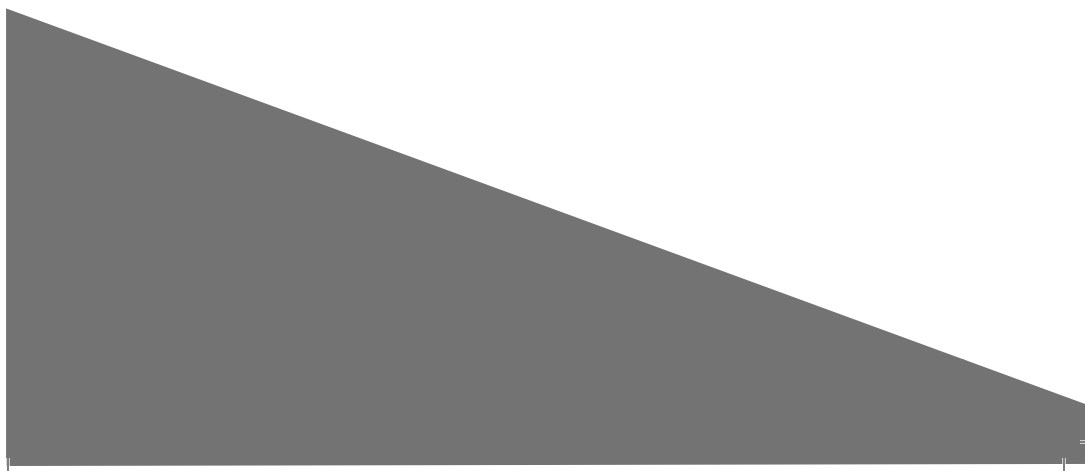
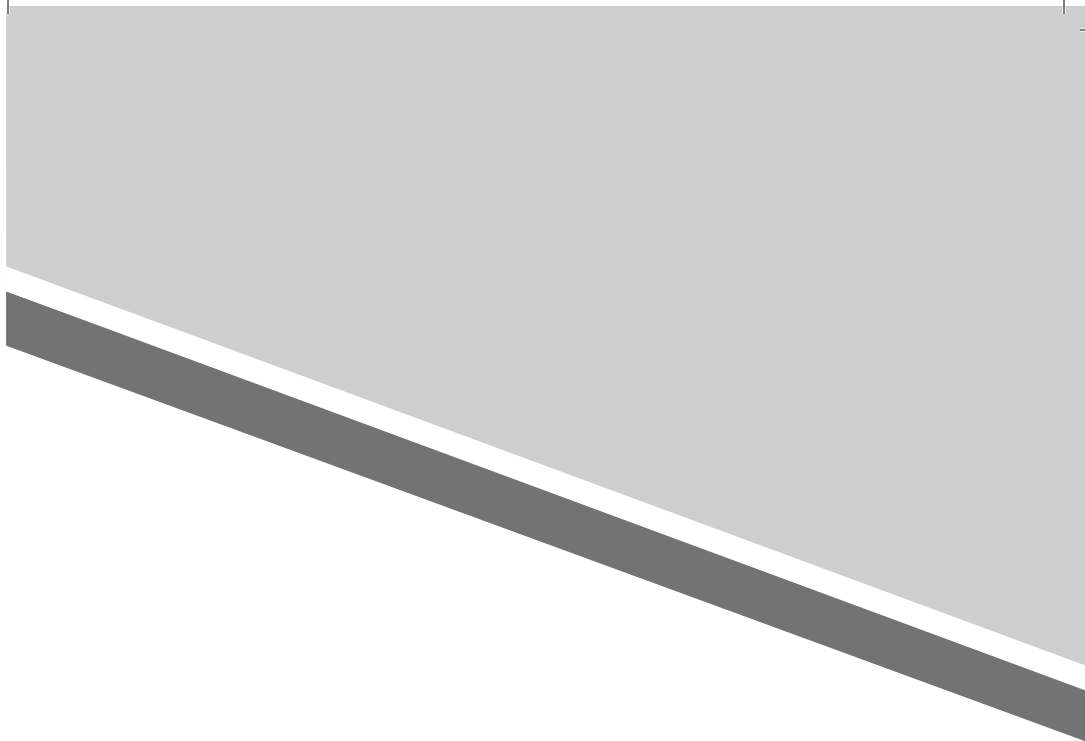
As for the government of Palmares, the large distances between villages making up Palmares supported the development of a governmental structure which favored autonomous local leaders, rather than an autocratic government. The utilization of autonomous local rulers was a departure from the governmental norms in the regions of West Africa most citizens of Palmares were originally from and most familiar with. The distance between the two most distant villages (Macaco and Zambi) was measured at 49 leagues, or 186 miles, with an average of 8.5 leagues between villages (Knobler, 2013). Over these long distances, rapid communication with a central authority would have been impossible. Thus, local leaders would require the authority to act in the leader's name when threats menaced the village, since waiting for royal authorization to call up citizens for defense would have meant certain destruction.

These effects were not only physical and political, but mental as well; distance also colored the way that colonial elites viewed Palmares. Colonists living nearest to Palmares complained to authorities about *Palmarino* raids seeking supplies and important commodities such as firearms and gunpowder. The colonist view of Palmares as a clear danger to their physical safety was heavily influenced by their close proximity to Palmares. Meanwhile, colonial authorities (safely far away from Palmares) were more concerned with the economic threat presented by Palmares, and elites came to the conclusion that Palmares' greatest danger came from the hope its existence gave enslaved workers. This view was expressed in a letter from Governor Aires de Sousa de Castro to the Prince Regent D. Pedro, listing his primary justification for seeking the destruction of Palmares in the following form: "they take away many slaves of the *moradores* nearby" (Aires de Sousa de Castro, 1680). Another way the same sentiment was expressed can be found in a 1676 recommendation from the Overseas Council, saying "the tyrannies of the rebellious blacks of Palmares . . . is one of the greatest damages inflicted upon the *moradores* of those captaincies, for [they] lack slaves to [do] their work" (Paracer, 2010).

As was the case in so many borderlands throughout the world, the physical environment surrounding Palmares exerted enormous influence over the polity. Specifically, the large distances and difficult terrain common throughout the unexplored territory of Brazil had a significant impact on how Palmares governed itself, selected its location, and even on its survival. In turn, these same large distances colored how colonial elites saw Palmares. The official posture on Palmares taken by colonial elites was very different than the way Palmares was seen by those who lived nearby, indicating the role distance played in how Palmares was perceived. Still, further work remains to be done on the ways in which environment affected the economy and society within Palmares, but a close examination of the environment around Palmares has broadened our understanding of this poorly-understood community.

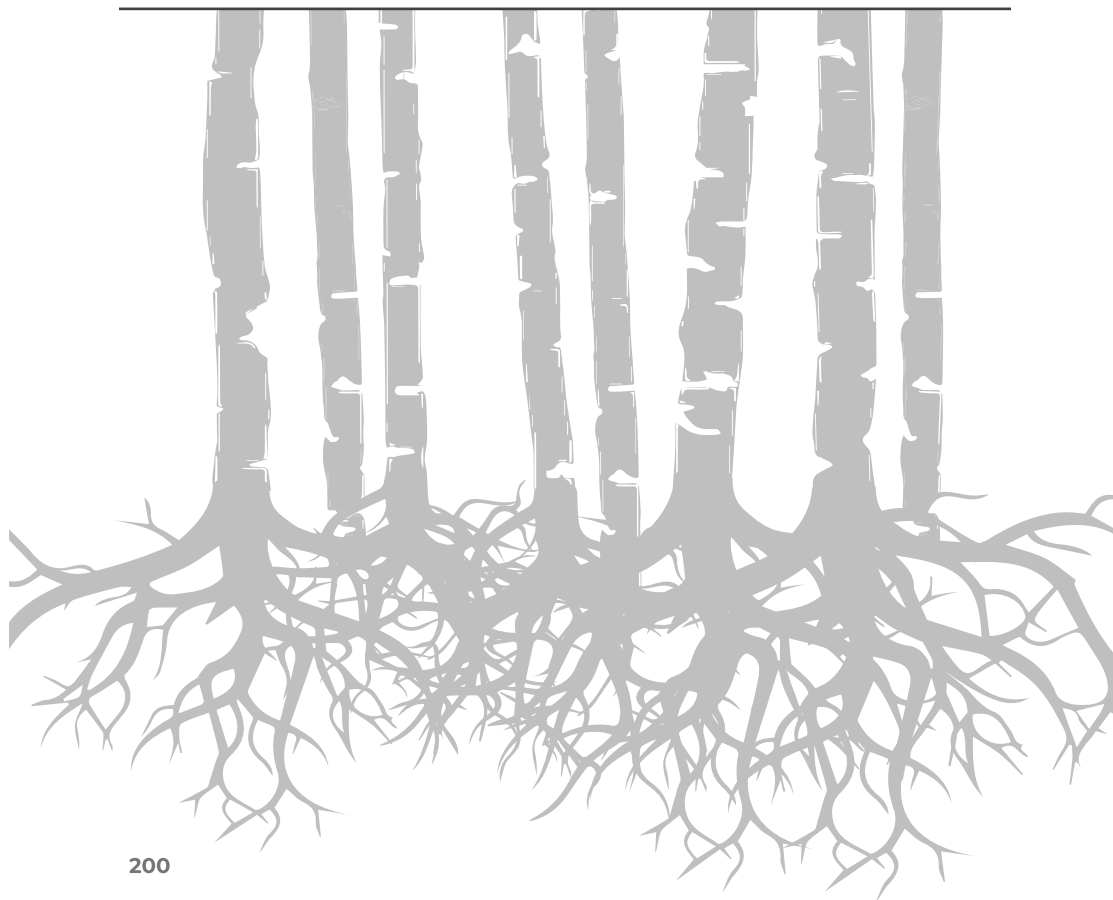
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Travel Abstracts



Proving People Wrong: Effects of Perspective and Presentation Modality on Overcoming Misconceptions

Author

Sabrina Badali

Mentor

Shannon McGillivray

Rocky Mountain Psychology Association 2018

Denver, Colorado | April 12–14, 2018

Abstract

Misconceptions about psychology are prevalent among the public, and while taking a psychology course is associated with a significant decrease in overall misconception endorsement, some misconceptions appear to be resistant to change (Bensley et al., 2015). However, if asked to think from a more scientific perspective, endorsement rate of some misconceptions may decrease (Amsel et al., 2009). Additionally, it has been found that teaching methods that activate a student's misconception before providing the correct alternative, known as refutational teaching methods, are more effective in dispelling misconceptions than standard teaching methods (Kowalski & Taylor, 2009).

The current study investigated the effectiveness of refutational videos versus refutational text, and perspective taking on overcoming misconceptions in psychology. Four misconceptions were chosen, and for each misconception, a refutational video and a text-based presentation was created. At pretest, participants completed questionnaires assessing endorsement of the misconceptions, as well as surveys assessing the real-life implications of belief in these misconceptions. Participants were then presented with the refutational teaching materials and were given the appropriate perspective-taking instructions. Two weeks later, students completed the misconception questionnaire again. It was hypothesized that the professor perspective condition and video format would be associated with the lowest misconception endorsement rates.



Meaning-making in Conscientious Neurotics

Author

Julia Blumenstein

Mentor

Cade Mansfield

Western Psychological Association

Portland, Oregon | April 25–29, 2018

Abstract

Research leads us to the conclusion that neurotic individuals respond to interpersonal stressors in maladaptive ways, such as secluding themselves or displaying animosity. They are also less likely to be supportive to peers that are involved and will more commonly reject the perspectives of others. They often view stressful life experiences as self-damaging and consider them a hindrance to their growth (Lee-Baggeley, Preece, & DeLongis, 2005; Lilgendahl, McLean, & Mansfield, 2013). Conscientious individuals, however, are more inclined to seek compromise, look for support, and try to develop solutions to a problem (Lee-Baggeley et al., 2005). This suggests that conscientiousness is the alternative that fosters positive, growth-promoting meaning-making when facing negative life events. Researchers even suggest that the combination of above average neuroticism and conscientiousness may enhance health benefits (Turiano, Mroczek, Moynihan, & Chapman, 2013), yet the literature is agnostic regarding how the conscientious neurotic makes meaning of negative life events in ways that benefit health. Due to this uncertainty, we sought to differentiate between high and low conscientious neurotics in regards to their process of meaning-making from remembered negative life events. In addition, we measured the extent to which these trait combinations impacted the subject's threat assessment for adaptive functioning. Study 1 utilized an undergraduate sample ($n = 125$; female = 84; $Mage = 22.46$; $SD = 4.10$). Participants recounted instances where they fell victim to interpersonal harm along with a time where they perpetrated interpersonal harm for two minutes. They then reported on adaptive functioning in several domains including, but not limited to: sense of empathy, feelings of loneliness, and self-alienation. Participants then wrote narratives of the negative events. Study 2 sought to extend Study 1 findings to a community sample ($n = 141$; Mean age = 43.32; $SD = 13.82$; Female = 75). Study 2 methods duplicated Study 1. Narratives are being reliably coded for self-blame, other-blame, insight-based meaning-making (McLean & Pratt, 2006), quantity and characteristics of emotion words, and cognitive words. We anticipate that our findings will ultimately uncover the relationship between how personality trait combinations and situations interact to result in different interpretations of their experiences.

The Human Cost of Mindfulness: Toward a More Nuanced Understanding of HRO Theory

Author

Benjamin Brandley

Mentor

Michael Ault

Prague, Czech Republic

Prague, Czech Republic | May 20–30, 2018

Abstract

Research into high reliability organization (HRO) theory has nearly always presented the principles of the theory in a positive way. This overly-optimistic portrayal of the benefits of HRO theory presents a skewed perspective that favors implementation of these principles without fully understanding the human costs to members of high reliability organizations associated with operating under the intense conditions the theory promotes. This paper is a first step toward understanding the adverse consequences associated with implementing HRO theory. Our findings posit that HRO members experience mental and emotional strain due to their membership. Constant comparative analysis identified five ways that this mental and emotional strain is present: paranoia, cost/benefit analyses of performing work tasks, mental fatigue, jadedness, and non-work relationship deterioration. Contrary to unequivocal calls from HRO researchers for all organizations to implement these principles, our findings advocate caution and mindfulness by organizational leaders when adopting these principles.

An Investigation of the Lysogenic Phage that Infects Halomonas Isolated from the Great Salt Lake

Author	Mentor
Carson Davis	Matthew Domek

American Society of Microbiology Tri-Branch meeting

Durango, Colorado | April 6–7, 2018

Abstract

It is known that bacterial populations in the Great Salt Lake (GSL) are regulated by bacteriophage which also act as vectors for gene transfer between species. Three similar *Halomonas* strains were isolated from the GSL; each displayed different growth patterns in culture but had similar and not identical 16s ribosomal DNA sequences. Bacteriophage LJ17 (previously isolated from the GSL) produced incomplete lysis or possibly lysogenic responses in the *Halomonas* isolates. This indicates the phage had incorporated its genome into the host's genome rather than overtaking the cell machinery until lysis or cell death occurs. We hypothesize the phage LJ17's genome can be found within the genome of this *Halomonas* organism. To test this, standard microbiological experiments will be conducted to see if lytic cycles can be induced from pure culture. The phage genome will be fully sequenced. Then, real-time PCR will be used to probe for the presence of LJ17 in the *Halomonas* genome. The phage sequence also has the potential to be used to probe other water samples for the presence of LJ17 or similar phage.

Effect of Organic Acids on Suppressing Growth of *Lactobacillus wasatchensis* Wdc04

Author

Ireland Green

Mentor

Craig Oberg

American Society for Microbiology Microbe 2018

Atlanta, Georgia | June 6–11, 2018

Abstract

Lactobacillus wasatchensis is a slow growing, non-starter lactic acid bacterium (NSLAB) causing late gas formation in aging cheese, which results in significant economic losses to the producer. During cheese aging, organic acids can be produced by other NSLAB cultures or purposefully added to cheese during manufacture. Organic acids are often used as preservatives, occur naturally in foods, generally don't affect flavor or product quality, and, under acidic conditions, enter bacterial cells altering the cell's proton motive force. Selected organic acids, in their natural concentration range (2.5-560 mM) in Cheddar cheese, were investigated for their ability to inhibit *Lb. wasatchensis* (WDC04). Five organic acids (lactic, formic, propionic, citric and acetic) that can be produced by NSLAB organisms were selected for this study, they were added at their minimum, median, and maximum concentrations as found naturally in aged Cheddar cheese to individual wells of a 48 well plate containing MRS broth with 1% ribose (MRS + R) inoculated with WDC04. Once interesting results were discovered in the initial MRS+R broth (pH 7.0) the tests were run again at pH 5.1 to determine if the chosen organic acids would be more effective at a pH similar to that found in aging Cheddar cheese. Growth rates were determined on a Tecan Infinite 200 PRO spectrophotometer over 40 hours with results graphed on Excel. Both formic and citric acid showed significant inhibition of *Lb. wasatchensis*. As formic acid concentrations increased, the inhibitory effect also increased. The maximum concentration (100 mM) showed the most inhibition, but the median concentration (63.15 mM) and the minimum concentration (26.3 mM) also caused observable inhibition. The addition of citric acid at the minimum (12 mM) and median (13.5 mM) concentrations showed similar inhibition. The use of selected organic acids at concentrations normally found in Cheddar cheese is a potential antimicrobial measure to prevent or reduce late blowing in aging cheese.

What it Means to Truly Listen

Author

Bryan Henrie

Mentor

Dan Hubler

International Association for Relationship

Fort Collins, Colorado | July 12–16, 2018

Abstract

Romantic relationship satisfaction is predicted by healthy communication between partners. This study explored the context and meaning of listening to partners within romantic relationships, using a qualitative analysis. We investigated a dyadic data-set of 27 couples (N Partners=54), with a mean age of 33.35 years (SD=10.59), who were relatively satisfied with their relationships (M=5.52 out of 6). These two questions were answered by respondents: “Describe a past situation in which you felt MOST listened to by your partner. How did you feel during this time?” | “Describe a past situation in which you felt LEAST listened to by your partner. How did you feel during this time?”

Overall themes emerged from responses, and they were organized into three categories (topic, partners’ actions, respondents’ feelings), showing that partners felt “most listened to” when venting about personal challenges, especially with challenges at work and with feelings of stress. Partners feeling “most listened to” described their experiences with words such as, “I felt valued and loved.” On the other hand, partners feeling “least listened to” reported on discussions about finances or sharing personal opinions. Also, some partners (n=6) in their early twenties reported their partners’ phone use as a reason their partners do not listen. When some of the partners did not listen, the other partners often reported feeling “invalidated and frustrated.” Interestingly, on a dyadic level, there were a few couples (n=4) who had matching feelings about when they felt “most and/or least listened to” (e.g. both partners felt most “listened to” when “talking about work issues,” or both partners felt “least listened to” when the other was “on [their] phone”). Finally, more partners reported feeling “most listened to” rather than “least listened to” (46 partners vs. 40 partners). Family educators and therapists using this information can help couples recognize recurring patterns and descriptions that both strengthen and hinder partners’ abilities to

effectively validate one another. This report also provides more description of what it means to truly listen to a partner. As listening skills are taught, couples are more equipped to enhance love, empathy, validation, and understanding, which may predict relationship satisfaction.

Does Xeriscaping Affect Surface Temperatures?

Author

Kyia Hill

Mentor

Daniel Bedford

American Association of Geographers Annual Meeting

New Orleans, Louisiana | April 9–15, 2018

Abstract

The arid and semi-arid climates of the western United States require constant attention to water conservation. Institutions of higher education have a special obligation to environmental sustainability writ large, of which water conservation is a part. One obvious approach to reducing a university's water consumption is through xeriscaping. However, xeriscape has the potential to exacerbate existing urban heat island effects as wet surfaces are replaced by dry ones, partitioning less energy into latent heat and more into sensible heat.

This study examines localized surface temperatures of several currently utilized landscaping materials at Weber State University in Ogden, Utah. These landscaping materials included grass, blacktop asphalt, light-colored river rock, and dark wood chips. Weather station measurements of incoming solar radiation were combined with albedo and infrared thermometer measurements of the various ground surfaces. These data were used to identify rates of heat absorption and sensible and latent heat partitioning for the different surfaces. The surface emitting the greatest amount of infrared heat was a dark-colored wood chip landscaping material. These findings were communicated to members of the university's facilities management team, and will be included in future decision making regarding landscaping materials. The ultimate objective is to ensure that water conservation measures are successfully implemented, without unduly raising temperatures across campus.

Rapid Methods for Measuring the Effect of Prebiotics on Probiotic Bacteria Growth

Author

Dana Hoffman

Mentor

Craig Oberg

American Society for Microbiology 2018

Atlanta, Georgia | June 7–11, 2018

Abstract

Prebiotics are used to stimulate probiotic bacterial growth in the gut to optimize their health benefits. A rapid method was developed to evaluate potential growth enhancement by prebiotics on probiotic bacteria using a programmable spectrophotometer, standard microtiter plates, and commercial media, with growth enhancement results ready in 12 hours. *Lactobacillus* strains were grown in MRS broth while *Bifidobacterium* strains were grown in MRS broth with L-cysteine. Each culture was back-diluted to an OD600 of 0.1, with the appropriate MRS broth then inoculated into wells (48 well plate) containing individual prebiotics. Plates were placed in a Tecan Infinite M200 spectrophotometer and incubated at 37°C with A600 readings taken for 12 h. Growth curves were done in triplicate with results compared to controls to determine extent of prebiotic growth enhancement. To optimize the method MRS concentrations of 20%, 35%, 50% and 100% were tested at selected pHs (7.0, 5.5, 5.0, 4.5, and 4.0) using 5 probiotic cultures. Addition of the bio-catalytic oxygen-reducing reagent oxyrase to test wells just prior to testing significantly enhanced growth of *Bifidobacterium* species and some *Lactobacilli* such as *Lb. acidophilus*. Results indicated a 25% MRS broth at pH 5.0 with 2% oxyrase addition optimized prebiotic growth enhancement. Using this method, the stimulatory effect of added prebiotics (2% v/v) FOS, GOS, and XOS was determined for *Bifidobacterium infantis* M-63, *Bifidobacterium longum* BB536, and *Bifidobacterium lactis* BL-04, *Lactobacillus rhamnosus* LR-32 and *Lactobacillus acidophilus* NCFM. All three significantly improved growth of M-63, but only FOS increased growth of BL-04. For BB536, just GOS enhanced growth. GOS and FOS slightly improved growth of NCFM but no oligosaccharides enhanced growth of LR-32. The method allows rapid testing of various inoculum levels, prebiotic concentrations, media pHs, and prebiotic combinations for any probiotic strain including *Bifidobacterium*. With multiple samples run concurrently, comparisons can readily be made. In addition, the method can determine optimum enhancement of individual prebiotics or prebiotic combinations for any probiotic strain.

Climate Confusion in the Classroom

Author

Tyler Hole

Mentor

Julie Rich

American Association of Geographers Annual Meeting

New Orleans, Louisiana | April 9–15, 2018

Abstract

In 2016, a nationwide study out of Pennsylvania State University surveyed 10,000 secondary education public school science teachers and found that “teachers’ knowledge and values can hinder climate education” (Plutzer, 2016). This groundbreaking study showed that a teacher’s knowledge of and belief in climate change affected what they taught in the classroom. A 2017 study, focusing on the northern Utah area, is an attempt to replicate and verify the above results. The study area, which includes some of the largest school districts in the nation, encompasses Weber, Ogden, and Davis school districts, which together serve approximately 113,000 students. To assess the views of the teachers in these districts, an email invitation to take the survey was sent directly to all associated junior high and high school science teachers that had publicly available email addresses, a total of 190 of the 220 listed science teachers in these districts. Survey participants were asked questions to assess their knowledge of climate change as well as their teaching practices in the classroom. These responses, as well as answers to demographic questions, were evaluated to determine the impact that their knowledge and beliefs had on their teaching. While the survey is ongoing, preliminary data analysis indicates that even in the context of a conservative state, teachers in northern Utah, in general, follow the same patterns shown in the nationwide Plutzer (2016) study. This strengthens the theory that teachers who lack a knowledge of or belief in climate change tend to convey that message to their students, thus clouding the scientific consensus on climate change.

Collaborative Process: How Law Enforcement Agencies And Universities Research Together

Author

Steiner Houston

Mentor

Monica Williams

Law and Society Annual Meeting

Toronto, Canada | June 6–11, 2018

Abstract

The current project is a collaboration between an official at a local law enforcement agency (LEA) and undergraduate students and faculty at a regional university. Although the main focus of the study is on disproportionate minority contact, this presentation will focus on the collaborative efforts of those involved in collecting and analyzing the data. In an effort to receive available grants and other funding, many LEAs have sought out partnerships with local institutes of higher education to conduct research on various topics. The willingness of LEAs to seek out such research shows a desire to look inside their organizations and proactively address any issues that may be discovered. As part of the current collaboration, the undergraduate students have maintained constant contact with the law enforcement official and have been given the opportunity to discuss the study with various record keeping and information technology personnel. Because previous research has rarely provided an in-depth look at the collaborative process, this study will be a great resource for future researchers and community partners who wish to enter into a collaborative process to study sociolegal topics, such as law enforcement's relationships with the communities they serve.

The Degradative Effects of Horseradish Peroxidase on Microbial Biofilms

Author

Samuel Kalis

Mentor

William Lorowitz

2018 Meeting for the Utah Academy of Sciences, Arts & Letters

Cedar City, Utah | April 6–7, 2018

Abstract

In the lab, bacteria are grown in suspension in liquid culture. But in nature, they grow attached to surfaces, often forming biofilms. These biofilms are often matrices of polysaccharides, proteins, and lipids, hosting a consortium of bacteria living together in a community safe from the environment at large. Biofilms not only grow in what is often thought of as nature (rocks, streams, ponds, etc.), but also in places or on objects where their presence is less than desirable (e.g., indwelling intravenous catheters, endoscopes, hulls of ships, and oil or gas pipelines). In this investigation the enzyme horseradish peroxidase was tested as a method of degrading and even eliminating biofilms formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*, species often implicated in medical contexts. Cultures of *S. aureus* and *P. aeruginosa* were grown in microwell plates to allow biofilms to form in the wells. The culture was then removed and the biofilms were treated with solutions of horseradish peroxidase and hydrogen peroxide, the substrate for horseradish peroxidase. Afterwards the biofilms were washed and stained with crystal violet. Staining solution was washed away and the crystal violet bound to the biofilm was dissolved in ethanol. Absorbance of the freed crystal violet was read in a plate reader. A range of concentrations and conditions were tested: time, temperature, concentrations of horseradish peroxidase, and concentrations of hydrogen peroxide. Also, the feasibility of reusing the enzyme solution was tested. Currently, no conditions generated results suggestive of clearance of biofilms superior to the positive control solution. Some combinations of enzyme and hydrogen peroxide concentrations yielded clearance, but no greater than the positive control. As of now, the results from this investigation suggest that under the conditions examined horseradish peroxidase was not effective for degrading microbial biofilms.

Adjunct Faculty: The Theory Behind Inclusion

Author	Mentor
Lesa Landrith	Kerry Kennedy

Utah Academy of Science, Arts & Letters

Cedar City, Utah | April 6–8, 2018

Abstract

Adjunct faculty are a large part of university life. Research about adjunct faculty is lacking whereas research about students, tenured faculty, and faculty on track for tenure is much more prolific. How can adjunct faculty be framed to be better understood? This research explores temporary worker satisfaction and part-time worker satisfaction in an effort to better understand adjunct faculty and their unique role in the university system. Using this framework and building on previous research from Weber State, results from a 25-question survey given to adjunct faculty will be presented within the frame of temporary workers in order to provide a deeper perspective to better understand this unique group.

Effects of Challenging Information, Religiosity, Superstitions on Belief of Psychology as a Science

Author

Raquel Lemos

Mentor

Shannon McGilivray

Rocky Mountain Psychological Association

Denver, Colorado | April 12–14, 2018

Abstract

There is an occasional conflict between scientific and religious beliefs that can cause tension (Dagher & Boujaoude, 1997) and lead people to feel they have to choose one belief over the other. Additionally, beliefs in popular paranormal phenomena have been shown to become even stronger when confronted with scientific evidence that challenges those beliefs (Ridolfo, Baxter, & Lucas, 2010). The current study investigated the relationship between religiosity, superstitious beliefs, and one's trust in psychology as a science. In addition, the study examined the effect of presentation of psychological findings that negatively portray those who endorse religious or superstitious beliefs on one's endorsement of psychology as a science. Participants in this study completed a survey that assessed their religiosity and belief in the paranormal. Following this, participants read several findings that negatively portrayed those who hold either religious or superstitious beliefs (or, as a control, neutral findings). Finally, participants completed a survey to evaluate their belief in psychology as a science. It was predicted that for those who indicated they were superstitious or religious, being presented with information that negatively portrayed those who hold such beliefs would be less likely to endorse psychology as a science, compared to a control condition.

The Tale of Peter Rabbit—Touring Performances

Author

Lynzee Linnarz

Mentor

Tamara Goldbogen

The Tale of Peter Rabbit – Touring Performances

Pittsburgh, PA and Charleston, SC | May 19–31, 2018

Abstract

Project Goals - My goal for this project is to create, perform, and tour an original theatre for young audiences piece in collaboration with professionals from the field and another WSU student.

Project Objectives - My objectives for this project are:

- Improve my understanding of theatre for young audiences
- Participate as a collaborator on bringing a new piece to life—from text to stage
- To develop and perform a live music soundscape utilizing my skills as a musician.
- Observe and learn from other TYA performances
- To bring together my passion for music, education, and young people

Project Outcomes - Outcomes for this project are:

- To create, develop, and perform an original musical soundscape
- To perform The Tale of Peter Rabbit as part of the Remake Learning Days in Pittsburgh, PA on May 23, 24, and 26 (with rehearsals on May 21 and 22)
- To perform The Tale of Peter Rabbit as part of the Piccolo Spoleto Festival in Charleston, SC on May 29
- At the conclusion of the project, I will write a reflection paper for my faculty mentor

Microbial Degradation of Art—Waste Solvents

Author

Gabriel McKay

Mentor

Craig Oberg

American Society for Microbiology 2018

Atlanta, Georgia | June 7–11, 2018

Abstract

Paint and solvents used in acrylic and oil painting generate wastes resistant to chemical breakdown, requiring expensive disposal fees and causing health hazards during storage. Containers holding these painting byproducts were found to have bacteria growing in them that could be degrading the waste. Microbial degradation of three solvents (linseed oil, bestine, and turpenoid) by bacteria isolated from paint waste containers was investigated. In addition, bacterial strains isolated from jet fuel–contaminated soil were tested for their ability to degrade paint waste. All bacterial isolates were propagated in M9 minimal media containing each solvent. Eight of the 16 isolates have been identified by 16S rRNA sequencing and taxonomic analysis of remaining individual isolates is still underway. Identified isolates that degrade paint wastes include *Pseudomonas zhao dongensis*, *Planococcus citreus*, and *Planococcus rifletensis*. Gas chromatography mass spectrometry (GC/MS) was used to measure microbial degradation of the solvents. GC/MS results indicate six bacterial isolates degrade both bestine and oleic acid, a selected component of turpenoid, as a number of new peaks (breakdown products) were detected and the initial solvent peak areas decreased over time. Results show that bacterial strains isolated from the paint waste and from jet fuel–contaminated soil have the ability to degrade paint waste solvents. Future work to optimize growth conditions (pH, oxygen, temperature) is currently underway to maximize solvent biodegradation. Once the most efficient bacterial strains and their optimum growth parameters are identified, they could be inoculated into waste containers to degrade paint waste, potentially reducing disposal fees and health risks.

Mental and Physical Health Effects on Stress Response and Cognitive Functioning

Author

Bailey Meibos

Mentor

Aaron Ashley

Rocky Mountain Psychological Association

Denver, Colorado | April 12–14, 2018

Abstract

The current study examined the relationship between overall general health and cognitive functioning. Participants completed three cognitive functioning tasks before completing several general and mental health questionnaires. Participants were then exposed to an ice bath stressor or a control group before completing working memory tasks again. Results show strong correlation.

Bio-Protective Lactic Acid Bacteria Cultures that Inhibit *Lactobacillus wasatchensis*

Author

Sophie Overbeck

Mentor

Craig Oberg

American society for Microbiology Microbe 2018

Atlanta, Georgia | June 6–11, 2018

Abstract

Lactobacillus wasatchensis, a novel non-starter lactic acid bacteria (NSLAB), has recently been identified as an important cause of late gas defect in aging cheese. Controlling growth of this unwanted NSLAB may be done by incorporation of bio-protective LAB cultures (BP-LAB) into the cheese during manufacture, inhibiting its growth during cheese aging. Previous research has shown that several BP-LAB cultures inhibit *Lb. wasatchensis* to varying degrees but the exact mode of inhibition has not been determined. Quantification of inhibition between BP-LAB cultures and *Lb. wasatchensis* was done using the spot test with the agar-flip method, then measuring inhibition zones in comparison to time incubated. In addition, potential synergistic quantification of inhibition by co-BP-LAB strains was tested by taking 1 mL of two different BP-LAB strains, growing them together, and then following the previous described protocol. The four most inhibitory BP-LAB cultures were *Lactobacillus paracasei* P-210, *Lactobacillus brevis* 13648, *Lactobacillus casei* F19, and *Lactobacillus paracasei* Lila. Four different co-cultures were tested with no significant increases in the inhibition zones observed when BP-LAB cultures were paired versus individual strains. Results confirmed that selected BP-LAB strains can inhibit growth of *Lb. wasatchensis*. Currently, we are trying to isolate the bacteriocins produced by these BP-LAB.

Influence of Meal Caloric Distribution in Metabolic Syndrome Parameters among College Students

Author

Ashley Petitta

Mentor

David Aguilar-Alvarez

Nutrition 2018

Boston, Massachusetts | June 9–12, 2018

Abstract

Purpose/hypothesis

We investigated the influence of meal calorie distribution on metabolic syndrome parameters in Weber State students. We hypothesize that variance in the percentage of calories eaten at each meal will affect MetS parameters.

Methodology

We assessed MetS parameters in 168 Weber State University student participants. Diet records for each participant were collected and analyzed. Participants were separated by gender (Male:53; Female:115) and by meal calorie distribution. Groups included high, medium and low percentage of calories in breakfast, lunch, dinner, and snacks. Mean differences in MetS parameters were analyzed.

Results

Women in the high calorie breakfast distribution group presented lower systolic blood pressure than their counterparts ($\mu = 107.3$ mm/Hg, $\mu = 113.3$ mm/Hg, $\mu = 115.01$ mm/Hg, $p = 0.05$). Men in the high snack consumer group presented higher HDL-C ($\mu = 41.3$ mg/dL, $\mu = 35.1$ mg/dL, $\mu = 32.3$ mg/dL, $p = 0.01$) and higher blood glucose ($\mu = 97.8$ mg/dL, $\mu = 92.1$ mg/dL, $\mu = 91.5$ mg/dL, $p = 0.05$) than the other groups.

Conclusion

Previous studies show that different meal calorie distribution patterns are associated with increased consumption of specific foods. These may account for the differences observed in MetS parameters among calorie distribution groups in this study.

The Influence of Learning Styles and the Testing Effect on Predictions of Memory Performance

Author

Joanna Reeder

Mentor

Shannon McGilivray

Rocky Mountain Psychological Association

Denver, Colorado | April 12–14, 2018

Abstract

The belief in learning styles is widespread. However, when people are presented with information in the teaching style that matches their learning style, their performance does not improve (Kratzig & Aruthnott, 2006). Conversely, the testing effect refers to the benefits of active retrieval practice (i.e., practicing testing oneself); this phenomenon shows a clear link to improved memory performance (Roediger & Karpicke, 2006). The current study examined the effect of learning styles and the testing effect. Four topics were chosen and for each topic, four versions of the presentation were created: visual with practice questions, visual without practice questions, verbal with practice questions, and verbal without practice questions. Participants' learning styles were assessed and they were informed of the results. Participants made global JOLs (i.e., prediction of their performance) as well as a prediction of ease judgement before receiving each type of lesson. After each lesson, participants completed a multiple-choice test on the material. It is predicted that participants' JOLs and judgments of ease will overestimate the benefits of learning styles, but will underestimate the benefits of testing effect, relative to its impact on actual memory performance.

Study Violin and Perform Solo and Chamber Music in Germany

Author	Mentor
Julee Reynolds	Shi-Hwa Wang

Sulzbach-Rosenberg International Music Festival

Sulzbach-Rosenberg Bavaria, Germany | July 31–August 12, 2018

Abstract

In August I will have the opportunity to attend the Interharmony Music Festival In Sulzbach-Rosenberg, Bavaria, Germany with my violin professor, Dr. Shi-Hwa Wang. While there, I will have a two-week intensive study of violin while participating in solo works, chamber groups, and orchestra. During my two weeks there, I will also have the chance to study with world-renowned musicians twice per week in private lessons. This will be a great opportunity for me to fine tune my skills on the violin and continue my education as I have just finished my Music Minor degree.

Ferritin Associations with Immune Cell Profile and Inflammatory Markers in Cross-Country Athletes

Author

Bess Bauer & Becky Rose

Mentor

David Aguilar-Alvarez

Nutrition 2018

Boston, Massachusetts | May 19–31, 2018

Abstract

Objective - We evaluated the effects of different levels of stored iron (ferritin) within normal ranges on immune cell group production and inflammation markers in cross-country athletes.

Research Methodology - Forty-one NCAA division 1 cross-country athletes, ages 18 to 25 years old, had blood drawn at the beginning of their season. Blood was analyzed by complete blood count (CBC) and enzymatic spectrophotometry was used to determine participant's ferritin levels. Participants were divided into two groups, high ferritin, and low ferritin. Cytokines IL-1 β , IL-6, IL-10, GM-CSF, IL-5, and IL-4 were measured at baseline by the magnetic multiplex panel for LuminexTM platform. Cytokines and CBC mean difference were compared between low and high ferritin groups. Pearson correlations were used to determine associations between cells groups and cytokines under low or high ferritin conditions. IBM SPSS statistics 22 software was used to analyze the collected data.

Results - Participants in the high ferritin group had higher levels of IL-1 β ($p=0.04$) and IL-5 ($p=0.05$) and eosinophils ($p=0.02$) when compared to the low ferritin counterparts. In contrast, IL-4 ($p=0.04$) was significantly higher in the low ferritin group. Moderate-strong correlations were found between eosinophils and cytokines; IL-1 β ($r=0.64$) and IL-5 ($r=0.59$) in the high ferritin group. Conversely, eosinophils from the low ferritin group correlated strongly with IL-4 ($r=0.86$).

Conclusions - Eosinophils correlated with different cytokines depending on the iron storage status. IL-1 β down-regulates ferroportin (FPN) by increasing hepcidin levels, which decreased iron absorption. As ferritin increases, the need to absorb iron decreases inversely to IL-1 β . Increases in IL-4 in the low ferritin group signaled an increase in iron uptake (absorption) and mobilization through transferrin (Tfn) expression. Eosinophils and IL-5 were higher in the high ferritin group. Increases in this biomarker may be linked to the increases in red blood cell production observed on the high ferritin group. Results indicate that Iron storage status measured by ferritin levels may modulate cytokine release and immune cell profile.

A Qualitative Analysis of First-Generation Faculty Experiences

Author

Jesus Saavedra

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Sarah Herrmann

Rocky Mountain Psychological Association

Denver, Colorado | April 12–14, 2018

Abstract

Past research has examined the experiences of first-generation college (FGC) students in order to predict performance and retention. This research reveals that FGC students have lower belonging, poorer performance, and higher dropout rates compared with continuing-generation college students. However, many FGC students persist and some even become faculty members themselves. The present study examined the experiences of first-generation faculty (i.e., first-generation college students who go on to be university faculty). A qualitative analysis of online biographies of 351 first-generation faculty from 29 universities revealed common categories including motivation to attend college, challenges, and advice for students. Specifically, we investigate how the theme of mentorship emerged in first-generation faculty stories, and how it relates to challenges in college (e.g., financial, familial, and school related). We hypothesize that participants who experienced formal or informal mentorship by a faculty member would report facing fewer challenges than those who did not. We additionally investigate whether having a first-generation versus continuing-generation faculty member impacted reported challenges. Implications of having first-generation faculty “out” themselves to students (i.e., make their social class identity visible) are discussed.

Inhibition of Pathogens by Probiotic Strains

Author	Mentor
Ammon Smart	Karen Nakaoka

Utah Academy of Science, Art and Letters

Cedar City, Utah | April 6–8, 2018

Abstract

Many studies have characterized lactic acid bacteria (LABs), noting their potential health-promoting features and encouraging their use as probiotics. This study’s purpose was to identify five strains of LABs, isolated from probiotic products, by DNA analysis and to test their ability to inhibit bacterial pathogens. LABs were identified by 16S rRNA gene analysis that indicated three isolates are *Lactobacillus rhamnosus* and two are *Lactobacillus plantarum*. Two assays, the flip agar and the agar overlay assay, were used to compare the ability of LAB colonies to inhibit five pathogens (*Escherichia coli*, *Salmonella Typhimurium*, *Shigella sonnei*, *Enterococcus faecalis* and methicillin resistant *Staphylococcus aureus* (MRSA), an important pathogen that infects wounds. The agar overlay assay indicated that all five LABs inhibited all five pathogens. In contrast, the flip agar assay showed only minimal inhibition of MRSA by three of the LAB cultures. To determine if the inhibitory activity was present in the LAB culture supernatants (CSs), LABs were grown in MRS broth for six days of incubation (pH 3.5). The CSs were centrifuged, filter sterilized and tested for their ability to inhibit the five pathogens using a well diffusion assay. All five LABs inhibited all five pathogens as shown by zones of inhibition around the wells containing CSs. However, when these same supernatants were adjusted to pH 6.0, they lost all inhibitory activity. Importantly, non-inoculated MRS broths at pH 6 and pH 3.5 were not inhibitory to the pathogens, indicating that the inhibitory activity was due to LAB metabolites. Further studies are underway to determine if the inhibitory activity is due to organic acids or some other LAB metabolite.

Disproportionate Minority Contact in Policing: Where Does the Bias Lie?

Author

Carrie Stone

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Monica Williams

Law and Society Annual Meeting

Toronto, Canada | June 6–11, 2018

Abstract

The current project involves a collaboration between a local police department and undergraduate students and faculty at a regional university to examine a heretofore underexplored explanation for disproportionate minority contact (DMC). Previous researchers have explained DMC by utilizing two hypotheses. The first, differential treatment, is the theory that variations exist in policing whereby officers disproportionately focus on minority groups. The second, differential offense, is the theory that variations exist in patterns of offending whereby minority groups disproportionately place themselves within police focus. Utilizing reports provided by a local police department, we analyze a third hypothesis that we call differential civilian response. Differential civilian response is the theory that civilians disproportionately place minorities within police focus. After controlling for offense rates, we will examine the extent to which biases within police departments and among civilians contribute to DMC between officers and community members. By examining an underexplored explanation for DMC rates, findings from this study have the potential to enhance community education efforts, influence police training practices, and aid future researchers in understanding how civilian bias impacts rates of DMC within communities.

Can Policies Mitigate Barriers to Healthcare among African Americans?

Author

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Law and Society Annual Meeting

Toronto, Canada | June 6–11, 2018

Abstract

Health disparities in African American communities compared to other racial and ethnic groups remains high. This research explores why certain healthcare facilities and resources remain underutilized in African American communities in a predominantly white state, and will inform discussions on policy changes and other solutions. Previous research shows some of the barriers to accessing healthcare are limited access, mistrust, and socioeconomic factors. While health disparities are not improving among younger generations, no previous research has examined the persistence of disparities in access to health care among older and younger populations of African Americans. In collaboration with Project Success Coalition, a non-profit organization that has worked with African American communities in Utah since 1989, the current study uses a series of four focus groups with both younger and older African American individuals to compare the responses of the different age groups to various health-related questions. Preliminary analysis of data from the focus groups has revealed themes not mentioned in previous studies, such as cultural factors and food deserts. By helping explain how each generation encounters barriers to health care, the findings from this study will inform discussions on policy changes and other solutions to the problem of undesirable health outcomes within African American communities.

Inhibition of Pathogens by Probiotic Strains of Lactobacilli

Author

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Tri-Branch American Society for Microbiology

Durango, Colorado | April 6–8, 2018

Abstract

Many studies have characterized lactic acid bacteria (LABs), noting their potential health promoting features and encouraging their use as probiotics. This study's purpose was to identify five strains of LABs, isolated from probiotic products, by DNA analysis and to test their ability to inhibit bacterial pathogens. LABs were identified by 16S rRNA gene analysis that indicated three isolates are *Lactobacillus rhamnosus* and two are *Lactobacillus plantarum*. Two assays, the flip agar and the agar overlay assay, were used to compare the ability of LAB colonies to inhibit five pathogens (*Escherichia coli*, *Salmonella Typhimurium*, *Shigella sonnei*, *Enterococcus faecalis* and methicillin resistant *Staphylococcus aureus* (MRSA), an important pathogen that infects wounds. The agar overlay assay indicated that all five LABs inhibited all five pathogens. In contrast, the flip agar assay showed only minimal inhibition of MRSA by three of the LAB cultures. To determine if the inhibitory activity was present in the LAB culture supernatants (CSs), LABs were grown in MRS broth for six days of incubation (pH 3.5). The CSs were centrifuged, filter sterilized and tested for their ability to inhibit the five pathogens using a well diffusion assay. All five LABs inhibited all five pathogens as shown by zones of inhibition around the wells containing CSs. However, when these same supernatants were adjusted to pH 6.0, they lost all inhibitory activity. Importantly, non-inoculated MRS broths at pH 6 and pH 3.5 were not inhibitory to the pathogens, indicating that the inhibitory activity was due to LAB metabolites. Further studies are underway to determine if the inhibitory activity is due to organic acids or some other LAB metabolite.

The Impact of Taste on Judgments of Emotional Expression

Author

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Rocky Mountain Psychological Association

Denver, Colorado | April 12–14, 2018

Abstract

Previous research in embodied cognition has examined the ways that the perceptual system influences other cognitive processes. Recently, there has been growing interest in investigating how tastes impact cognition and behavior. For example, it has been found that bitter taste increases hostility (Saglioglou & Greitemeyer, 2014) and harsh moral judgments (Eskine et al., 2010), compared to participants who tasted something sweet or control groups. Although several conceptual frameworks have been proposed to explain the dynamics of how bitter taste affects judgment, little research has examined the impact of sour tastes on cognition or behavior. The present research compares the effect of sour, bitter, sweet, and no taste on social judgment of emotional expression. The study will examine the extent to which the taste experienced by participants influences the ability to detect specific positive and negative emotions in faces displaying neutral, subtle, and apparent emotional expressions (e.g., the degree to which bitterness or sourness influences the detection of disgust in faces, compared to sweetness or no taste). The current study will also examine the effect of taste on participants' rating of the intensity of the emotion expression, as well as the likability of the target faces.

The Role of Auxin in Growth Promotion of Rice by *Rhizobium* sp. IRBG74

Author

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Tri-Branch American Society for Microbiology

Durango, Colorado | April 6–8, 2018

Abstract

Auxin is a known growth hormone for plants and many plant-beneficial bacteria are known to produce it. It has previously been shown that *Rhizobium* sp. IRBG74 promotes growth of rice, but the mechanism is unknown. We hypothesized that *Rhizobium* sp. IRBG74 accomplishes this by production and secretion of auxin. To address this hypothesis, we first performed a bioinformatic analysis to identify putative auxin biosynthesis genes in the genome of IRBG74 using BLAST with known auxin biosynthesis genes as queries. To test whether the genes identified by BLAST play a role in promoting growth of rice, we are making in-frame deletions of each one. Briefly, we use overlap-extension PCR to create and stitch together deletion fragments and then we clone these fragments into the *sacB* deletion vector pJQ200SK. The target genes are then disrupted by homologous recombination and then deintegration is selected for with sucrose. After this is done we test the mutant to verify the deletion occurred by PCR. Once the deletion mutants are verified, each one is tested on rice seedlings and compared to wild-type IRBG74. Rice growth is quantified by shoot dry weight and by root branching, as measured using ImageJ.

United Way of Salt Lake 2-1-1: Evaluating the Effectiveness of Housing Referrals

Author

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Mentor

Kerry Kennedy

Utah Academy of Science, Art and Letters

Cedar City, Utah | April 6–8, 2018

Abstract

The United Way of Salt Lake's 2-1-1 provides a statewide information and referral service for Utah residents looking for assistance. One of the most common requests they receive is referrals for affordable housing. In assessing ways that 2-1-1 can improve its services, a study was proposed to offer callers who needed assistance with housing or rent additional services for utilities, food shelters, healthcare, and job assistance. The goal was to determine if proactively offering comprehensive referrals impacts the likelihood that the caller's overall situation will be improved. Callers who requested housing referrals within a 75-day period received a follow-up call one month later to assess problem resolution, referral helpfulness, and situation change. Out of 125 callers, 45.6% participated in the survey ($n=57$), of which 75% were female, 41% had children, 37.5% had a disability, and 64.3% were Caucasian/non-Hispanic. In both groups, only about one-third of participants claimed to have their problem resolved. When asked, 15.6% of participants who received only housing referrals reported the agencies as extremely helpful, compared to 32.8% of participants who received multiple referrals. Additionally, 42.8% of participants who received only housing referrals reported their situation as much worse, whereas 42.3% of participants who received multiple referrals reported their situation to be the same. Overall, participants who received multiple referrals were 26.7% more likely to have their situation improve, even if their initial problem of housing was unresolved. This data reflects a need for more research in the effectiveness of offering additional resources to individuals in crisis situations to better improve their overall well-being.

Characterization of Hemolytic Lactobacilli

Author

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Utah Academy of Science, Art and Letters

Cedar City, Utah | April 6–8, 2018

Abstract

Lactobacilli are valued for their health-promoting aspects and their use as probiotics. However, we observed in our lab that seven probiotic lactobacilli (PLBs) lysed sheep blood agar (SBA), a characteristic of pathogens. This study's goal was to characterize this hemolysis. Initially, PLBs were inoculated onto SBA, incubated at 25, 30 and 37 °C, aerobically and anaerobically. All seven PLBs lysed SBA after 2–4 days of incubation under all conditions. PLBs were then grown in broth for 2 and 6 days to obtain culture supernatants (CSs). CSs of the seven PLBs were filter sterilized and pipetted onto SBA plates. After 24 hours of incubation, the SBA was lysed by all CSs, with day 6 CSs causing larger zones of hemolysis than day 2 CSs. The pH of CSs and non-cultured broth was adjusted to pH 6. These were tested along with the original CSs (pH 3.4–3.5) on SBA. All pH 3.4–3.5 CSs lysed SBA but none of pH 6 CSs lysed the SBA. Importantly, non-cultured broth did not lyse SBA at either pH, indicating that metabolites in the CSs were responsible for hemolysis. Studies are underway to characterize the chemical nature of the hemolytic activity of cultured PLBs.

Analysis of Enterovirus Mutations Reveals Changes Responsible for Neuroinvasive Disease

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Tri-Branch American Society for Microbiology

Durango, Colorado | April 6–8, 2018

Abstract

Enterovirus A71 (EV71) and Coxsackievirus A16 (CA16) both cause Hand, Foot & Mouth disease (HFMD), but EV71 can also cause a neuroinvasive disease which may be fatal. In an effort to discover what part of the Enterovirus genome is most likely to mutate and cause this neuroinvasive disease, we mapped conservation across the genome of Enterovirus, Coxsackievirus, and Poliovirus (PV1) which is also known to be neuroinvasive. We used available GenBank records and performed an alignment and conservation analysis to generate a map of viral mutants. This map was superimposed on viral proteins, including models of secondary protein structures. A comparison of protein mutations and their change in function was carried out in an attempt to broaden the data on relations between these mutations and neuroinvasive properties in EV71. Evaluating mutations present in EV71 viral proteins has shown that most known mutations are in the P1 region, which is primarily structural proteins. Evaluating mutations present in several picornaviruses has shown that mutations overlap among species and exist in hotspots or instability islands. The P1 region contains structural proteins involved in the outer structure of the virus which is involved in attachment and entry into cells. P1 also contains mutations associated with paralytic polio in PV1. These findings indicate that for poliovirus, any change from enteric disease to a nervous system disease occurs at the level of entry into host cells. None of these mutations found in the EV71 P1 region were present in CA16, further validating our hypothesis that these structural mutations contribute to the virus' ability to penetrate the central nervous system. Because alpha helices are in general found in functional rather than structural proteins, EV71 tropism change from enteric to nervous system disease is likely due to virus compatibility within cells instead of entry into cells. These findings provide insights into the role of mutations in EV71 and will allow for targeting specific proteins involved in neuroinvasive disease.







