W
I
N
G
A
T
E

wingate.edu

TABLE OF CONTENTS

EDITOR'S INTRODUCTION

The nine essays in this journal were all written by undergraduates during the 2021-2022 school year at Wingate University. The essays illustrate the brilliant scholarship that our students are undertaking and the interesting directions of scholarship that is happening at Wingate. Crossing four academic disciplines, this journal is truly interdisciplinary; many of the articles cross disciplinary bounds as well. The students published in this edition of the *Wingate Research Review* should be exceptionally proud of their work, just as we are proud of them.

But these students were guided in their research through several devoted faculty mentors, to which we owe a great deal of gratitude. The faculty who sponsored student publications in this issue include: Taura Napier, Christy Cobb, Acchia Albury, Shem D. Unger, Mark Rollins, Debra A Davis, and Kristen Stowe. Some of these faculty sponsored multiple papers as well, including Dr. Stowe who worked with students on three of the following articles and Dr. Albury who worked with a total of five students on two published articles. The faculty at Wingate University is outstanding and often go above and beyond in their support of students. The essays included in this journal are a testament to the dedication of these faculty members.

Christy Cobb, PhD, Editor of the *Wingate Research Review*

Mallory Challis, Assistant Editor of the *Wingate Research Review*

June 2022

Common Ground for Going to Church

Hannah Haigler

Faculty Mentor: Dr. Taura Napier, Professor of English

ABSTRACT

All major religions, place importance on sacred spaces in which worship can occur. However, many of these sacred spaces have become vacant and are continuing to diminish with each new generation. Philip Larkin's poem "Church-going" elaborates the view of a sojourner struggling to find his place in the world and questioning the future impact of the church building. Larkin proposes hypothetical solutions for the church's future, such as a last resort for the sick or a place for Christmas events to occur. Similarly, the architect Rudolf Schwarz conceived the sacred space with the sole purpose of the spaces's longevity. Within the twenty-first century, organizations such as the Greater Church Network in the United Kingdom have devised plans to formulate church growth, yet many of these plans disregard the church as a physical space that is designed to be sacred. As the speaker in "Church-going" contemplates his personal belonging within the church, the poem illustrates the importance of creating a space in which the community feels welcome and can respect. This paper seeks to examine Larkin's "Church-going" in conjunction with concepts and plans from Rudolf Schwarz, the Greater Church Network, and other archeological ideologies to reflect the overarching goal proposed by Larkin: to institute a space that the community can regard as sacred and respect at in any spiritual stage of life.

Crush, depress, uplift, enlighten, hover, breathe, grow. Verbs assist us as we attempt to understand the world around us. Verbs possess the power to describe a specific space and the emotions it evokes in those who enter or exit; as Gerald Robinson writes, "This correspondence between the configuration of space and our bodily sensations makes it possible for space to express emotion" (4). Within the poem "Church Going," Philip Larkin uses verbal descriptions of a sacred space that convicts and redeems yet also stifles and gasps for life. His technique throughout the poem expresses the conflict he feels in being removed from the community and religion and questions the church's purpose as its attendance during his lifetime rapidly dissolves. Seeking some kind of revelation for his soul, throughout the poem Larkin initiates

hypothetical solutions to the building of the church, such as a last resort for the sick or a place for Christmas events to occur, in an attempt to resolve his personal spiritual conflict. In the shift from the twentieth to the twenty-first century, resolutions similar to Larkin's are being considered through better definitions of sacred space, possibilities for declining sacred spaces, and construction plans for the best utilization and prioritization of space, worship, and ministry, as "...space through its worship, assists us in our worship" (Gerald 4). Although Larkin doesn't seem to find personal spiritual revelation and assistance in worship within the church, in "Church Going," he recognizes the church as a sacred space needing revitalization, just like the Greater Church Network recognizes in the twenty-first century. This paper seeks to examine Larkin's "Church-going" in conjunction with concepts and plans from Rudolf Schwarz, the Greater Church Network, and other archeological ideologies to reflect the overarching goal proposed by Larkin: to institute a space that the community can regard as sacred and respect at in any spiritual stage of life.

While interior space is commonly described as an area free of excess occupation, in his article "Liturgical Architecture: Creating Space for Worship," Gerald Robinson disagrees, saying that this method makes it "hard to imagine space doing something exact" (3). Robinson describes six plans for worship spaces created in 1938 by German architect and mystic, Rudolf Schwarz (4). While none of these plans were specifically followed through in construction, they created a foundation for further inspiration and expression of space for these six specified ministries: contemplation, pastoral care, witness, dedication, evangelism, and justice (26). Each architectural plan seeks to create an atmosphere supportive of said specific ministry whether that be in light exposure, acoustics, expansions for sacraments, or circular areas for one's community. Similarly, Larkin's "Church Going" begins with a physical description of the church that includes a false

stiffness and a separation from the outside world that creates "a tense, musty, unignorable silence" (line 7). The speaker holds an anthropological outlook on the sacramental objects at the front of the church, as seen in this stanza of the poem:

...matting, seats, and stones

And little books; sprawlings of flowers, cut

For Sunday, brownish now; some brass and stuff

Up at the holy end; the small neat organ (Larkin lines 3-6).

All of these objects allow him to observe the culture of the space that continually draws him back. As he feels an impulse to go into the church, the speaker unknowingly desires a resolution for his compulsions and lack of hope. He never leaves feeling satisfied, saying "Yet stop I did: in fact I often do, / And always end much at a loss like this" (lines 19-20).

Examining Larkin's description of a church through Schwarz's architectural examples, this sacred space aligns the most with the fifth path of ministry: evangelism. This fifth architectural type is based on creating an open and welcoming entrance and space for travelers to find a safe harbor and place to rest, not a permanent home. Its centrality is the ability for people to come and go as needed in order for them to find their desired sacred space, and the poem illustrates this idea as the speaker impulsively comes into the church and then departs. While this space is intended for "missionaries, teachers, and greeters, and those who take Christ's sacrament seriously to grow the church," the idea that the church is dying out creates a layer of conflict (Gerald 19). The speaker's "awkward reverence" shows his interest, and even his seriousness toward Christ's sacraments by entering without his hat, removing his cycle clips, and donating a worthless Irish sixpence (Larkin lines 8-9, 15). All of these actions allude to the alien feelings of

6

the speaker while in the sacred space. The speaker's description of the church connects the absence within the church to one within himself. Robinson notes the common definition, not his own, for space as "what's left over after everything else in the universe has been eliminated", and as Larkin's church is dying out, the most significant absence from this building is the congregation (3). Standing at the front of the church, the speaker vocalizes verses much louder than he expects, producing echoes that attempt to fill the space. The jeering echoes amplify the lack of congregation and propel the speaker into questioning the use of the church when the congregation completely disappears.

In his article "'Here's the Church, Here's the Steeple': Robert Morgan, Philip Larkin, and the Emptiness of Sacred Space," Robert West, known for his focus on Southern and Appalachia poetry, creates a comparison between Larkin's "Church Going," and the children's rhyme "Here's the Church, Here's the Steeple." In this comparison, West explains that at first, the speaker of "Church Going" illustrates an empty church like children create with their hands and fingers in the first line of the rhyme, "Here's the church, here's the steeple. / Open the doors: where are the people?" (West 94). However, in Larkin's proposed solutions, the congregation can be found just like they can in the second stanza of the rhyme that says, "Here's the church, here's the steeple. / Open the doors: here are the people." (94) The speaker of "Church Going" understands that people are disappearing, but he also understands the presence of longing and wandering to enter the church and the impetus to attend, seeing the "sprawlings of flowers, cut / For Sunday, brownish now" (Larkin lines 4-5).

The speaker wonders what will become of the people who bring in the flowers and hold service in the church "when churches fall completely out of use" (line 22). Creating personas for the future congregation, his speculations on their forthcoming identities allude to the questions

7

he wrestles with concerning religion. "Dubious women," sick children, and those close to death are people that he imagines creating new purposes for the church but are also characteristics that the speaker embodies (line 28). Doubts entangle his mind as he walks into the church trying to find healing for his soul from the guilt that makes him a sick child that is close to spiritual death. These dubious and sick people desire to go to church to gain any superstitious amount of hope and connection to divine power, but the speaker understands the gravity of visiting a sacred space. The seriousness of visiting a church to find relief should not be removed by superstition "in games, in riddles, seemingly at random; / But superstition, like belief, must die" (lines 33-24). Visiting the church is also not about observing it through the lens of an antiquarian, as West writes, holding reverence for the church based on the sacrament, or its connection to Christmas and other church events (West 93). The purpose of the sacred space and church is to find community and a remedy for human compulsions, although finding a place to belong in a congregation can often be a difficult task. Judith Muskett explores a symbolic idea by J. F. Hopewell, that finding a church is synonymous with finding a home. As people desire to feel they belong, searching for their congregational home includes questioning the purpose of the space and one's relation to it (26).

In the twenty-first century, action is being taken for the churches that are beginning to die out. For example, the Greater Church Network in the United Kingdom provides funding to non-cathedral churches to ensure their longevity and seeks to promote community events and restorative grants and practices for the buildings to maintain a sacred space. Created in 1991, the network began with Larkin's mindset questioning what should happen to the church buildings that were empty and not fulfilling their purpose. Churches within this network share five characteristics: building size, visitor number, additional paid staff, open doors throughout the

8

week, and working towards a greater mission for the community (Muskett 24). With the main goal being to find the best way to use the building to reach members of the community, evangelism is the ministry of the Greater Church Network, just as it is of Larkin's described church. Muskett provides an example of an attempt made at St. Wulfram's in Grantham, England by Father Stuart Cradduck and the Greater Church Network.

In 2015, St. Wulfram's hosted a six-day Christmas Tree Festival inside of the church with indoor ice skating, one hundred five Christmas trees, extravagant nativity scenes, among other decorations; and refreshments served throughout the festival. The festival only occurred for one week, Wednesday through Sunday, and employed clergymen to collect entrance and ice-skating fees and talk to community members, both of which assist the church in aligning with two of the Greater Church Network's criteria: "being open on most days, all day" and having "fulfilling a wider ministry than a regular parish church" (Muskett 6). Throughout the festival's short period, over ten thousand people visited St. Wulfram's, many for the first time. And as the Lady Chapel was also open during the festival, many people encountered information about prayer, religion, and the Bible. Additionally, they were able to hang prayers on a prayer tree which, at the end of the festival, amounted to 682 prayers (30). While people were being exposed to God and the church's beliefs, was this the best plan for the people to experience the church's sacredness? Were they experiencing sacredness at all?

In comparison with Larkin, St. Wulfram's festival fulfills the speaker's expectation that the church would be used for a "Christmas addict, counting on a whiff / Of gown-and-bands and organ-pipes and myrrh" (Larkin lines 43-44). The church is merely being used as a stage or setting for a production that dissolves its sacredness. The church, he would say, as he does in the poem, is becoming "less recognizable each week / A purpose more obscure..." hosting festivals

9

rather than being the sacred space for wisdom and revelations (lines 37-38). Many members of the church seem to agree with Larkin, expressing that the church should not be seen as a "perpetual funfair" (Muskett 32). However, Father Stuart reminds them of the Medieval purpose of the church to be a common ground for the community. But is the redirection of this sacred space really a serious place for humanity to find a solution to their guilt and connection to Christ and community? Larkin describes the church as "A serious house on serious earth it is, / In whose blent air all our compulsions meet, / Are recognised, and robed as destinies" (Larkin lines 55-57). While not finding personal resolution, Larkin seems to acknowledge the church as a sacred space. His admittance of its ability to draw him inside repeatedly illustrates that although he is not aware of its purpose he knows one of seriousness and solution exists. It provides a solution for the compulsions of guilt, temptation, shame, and anger as it recognizes and sympathizes with the weight of emotions and purloins them to give humanity a different future.

In meeting to find a solution for one's compulsions, the church then takes on a personal experience rather than one of a community. The Greater Church Network's goal of creating a community-oriented church aligns with biblical ideology for the church to be the body of Christ. There has to be an individualized community within the church so that members may be able to have their compulsions met, as Larkin desires, and be a "sacred space for common ground" (Muskett 23). The idea of the individualized community closely aligns with Scripture; as Romans 12:4-5 says, "For as in one body we have many members, and the members do not all have the same function, so we, though many, are one body in Christ, and individually members one of another" (ESV). The body of Christ ultimately makes up the church, not the building in which they assemble. But would the church Larkin writes about be noticed by the Greater Church Network as a church in need of help? Bringing events and funding to Larkin's church

10

creates a possibility for its revitalization, but it also loses its purpose of bringing in community members, like the speaker, who do not feel compelled to come into the church otherwise. Church growth is a gratifying occurrence, yet when the growth of a church overrides its purpose, then its growth hinders and withers the community's souls, instead of feeding them.

Larkin's honesty in the poem creates a haven for those who have experienced religious dissension and separation from the Church. His tone reflects confusion yet fascination and a feeling of necessity to visit. The church building remains for him a "special shell" where "it pleases me to stand in silence here" (line 52, 54). At the end of the poem, Larkin seems to find a resolution or peace with the church as simply being in the building creates a reverence, although awkward, within him. A reverence and continual return to the church then illustrate that Larkin observes the church as a sacred space whether he finds spiritual revitalization or not. Preserving this feeling is crucial for the church's sacredness to persevere those both on the inside and out. To some extent, the Greater Church Network holds satisfactory ideas for church growth in creating new services and events, participating in more community outreach, and becoming more diverse with member populations (Muskett 8). However, in some ways, it also appears similar to a false advertisement in creating elaborate and extravagant events, like indoor ice-skating rinks, simply to draw people into the church. Father Stuart is correct in his argument of a communal church, like those of the Medieval period, yet for those who visit the church in solemnity and silence for personal reflection, as Larkin does, this goal of the church offers nothing. For the church to embody community, its leaders need to discern the church's main goals of ministry in its relation to the space and its parameters in order to best serve the space, the congregation, and, most importantly, God who is being worshiped.

WORKS CITED

Larkin, Philip. "Church Going." In *The Norton Anthology of English Literature.* Gen ed.

Stephen Greenblatt. 10th edition. Volume F: The Twentieth and Twenty-First Centuries.

Ed. Jahan Ramazani. New York: W. W. Norton, 2018. 924-25.

Muskett, Judith A. "The Greater Church as 'Sacred Space, Common Ground': A Narrative Case

Study Within a Rural Diocese." *Rural Theology*, vol. 15, no. 1, May 2017, pp. 22–38.

Academic Search Ultimate, doi:10.1080/14704994.2017.1298227.

Robinson, Gerald. "Liturgical Architecture: Creating Space for Worship." *Toronto Journal of*

Theology, vol. 28, no. 1, 2012, pp. 3–27. *MLA International Bibliography*,

doi:10.3138/tjt.28.1.3.

West, Robert. "'Here's the Church, Here's the Steeple': Robert Morgan, Philip Larkin, and the

Emptiness of Sacred Space." *The Southern Quarterly: A Journal of the Arts in the South*,

vol. 47, no. 3, 2010, pp. 91–97. *ProQuest*,

go.openathens.net/redirector/wingate.edu?url=https://www.proquest.com/scholarly-

journals/heres-church-steeple-robert-morgan-philip-larkin/docview/670184989/se-

2?accountid=15065

Space, Time, and Magic: Bakhtinian Chronotope in *Acts*

Allison Barbee

Faculty Mentor: Dr. Christy Cobb, Assistant Professor of Religion

ABSTRACT

Acts is the one of few biblical books that mentions magicians and the only example of a New Testament text incorporating magician characters into the narrative structure. While magicians are mentioned elsewhere in New Testament and early Christian literature, Acts presents one of the only instances where the magicians play an important role in the narrative and have their words and deeds recorded. While the purpose of including these magicians in the narrative alone can be ambiguous, the distortion of time and space in these episodes presents further interesting angles from which to approach the text. How does one's reading of the text change when paying attention to the time and space in which the narrative takes place? What rhetorical purpose does the distortion of the narrative setting seek to accomplish? In this paper, I will read the magician stories in Acts through the lens of Bakhtinian chronotopic theory, arguing that chronotope functions as a rhetorical device to reject practices of magic and the magicians that were present in contemporary society. These stories stretch the boundaries of time and space within the narrative to make room for the Jesus movement.

The characters of Simon Magus and Bar-Jesus in *Acts* serve as the only examples of incorporating magicians into the narrative structure of the New Testament literature. While magicians are mentioned elsewhere in New Testament and early Christian literature, Acts presents one of the only instances where the magicians play an important role in the narrative and have their words and deeds recorded. Simon Magus, whose story is told in *Acts 8*, is the first magician to appear when Philip and the rest of the apostles travel to Samaria. This magician hears the apostles' teaching and confesses his belief in Jesus, becoming a baptized member of the Jesus movement. However, after offering money to Peter and John in exchange for the power of the Holy Spirit, Simon Magus is chastised, called wicked, and never mentioned again in *Acts*. The second magician in *Acts 13*, Bar-Jesus, meets a similar fate as Simon Magus, though he is

physically injured as a result of his deeds. After attempting to turn the proconsul, Sergius Paulus, away from the Jesus movement and being charged with "false prophecy," Bar-Jesus is blinded, then he too disappears from the narrative.

Since neither of these stories ends "happily" with repentance and conversion, the reasons for including these characters in the greater text of *Acts* can be ambiguous. Additionally, the timing of these narratives is a bit hard to place: Simon Magus goes from being a popular magician, to a baptized follower of the Jesus movement, to being chastised and rebuked for his request for power in a matter of verses. Likewise, Bar-Jesus is blinded and then forgotten, with no indication of how long "a little while" lasts when one becomes blind by the power of God. It is because of this disruption of time within these narratives that I suggest Bakhtin's theory of the chronotope can provide a helpful lens through which one may read and understand these passages. The boundaries of time and space are stretched and reimagined throughout these narratives. Through this reading of *Acts* through the lens of Bakhtinian chronotopic theory, I argue that chronotope functions as a rhetorical device to reject practices of magic and the magicians that were present in contemporary society. These stories stretch the boundaries of time and space within the narrative to make room for the Jesus movement. In this paper, I will examine various aspects of the chronotope in the genre of the Greek novel, namely, the concepts of adventure-time, and the author's involvement in employing this strategy of chronotope to achieve their rhetorical goals.

Before turning to the text of Acts, I will define and describe the term chronotope as defined by Bakhtin. At its most basic, the chronotope is defined as the "intrinsic connectedness

of spatial and temporal relationships."[1] Rather than viewing time and space as separate pieces of background information, Bakhtin would argue that these aspects are essential to understanding the narrative and placing the narrative in conversation with the author, reader, and the contemporary world that exists behind the physical words of the text. He also would argue that time and space cannot be separated to understand the narrative, but rather that: "Spatial and temporal indicators are fused into one carefully thought-out, concrete whole. Time, as it were, thickens, takes on flesh, becomes artistically visible; likewise, space becomes charged and responsive to the movements of time, plot and history."[2] Thus, in order to understand the events in this narrative, and any other work of novelistic literature for that matter, we must first identify and understand the literary chronotope that exists within this narrative. It is purely because of this interconnectedness of time and space and the "tying and untying" of narrative knots which results from the chronotope that we can seek to truly understand the narrative before us.[3] By understanding this idea of the chronotope, the narrative events are made "concrete, [made to] take on flesh, and causes blood to flow in their veins."[4] It is through the understanding of the chronotope of the novel that we can make the narrative come to life, which is what I intend to prove with my investigation of the magicians in *Acts*.

To understand why the presence of Simon Magus and other magicians are significant in the narrative of *Acts*, we must first discuss two things: the purpose of the book of *Acts* and the

---

[1] Mikhail Bakhtin, "Forms of Time and of the Chronotope in the Novel," in *The Dialogic Imagination: Four Essays*, ed. Michael Holquist, trans. Michael Holquist and Caryl Emerson, University of Texas Press Slavic Series 1 (Austin: University of Texas Press, 1981), 84.

[2] Bakhtin, 84.

[3] "Chronotope is the place where the knots of the narrative are tied and untied." Bakhtin, 250.

[4] Bakhtin, 250.

prohibition of magic that was ingrained into Jewish culture and belief. While scholars have proposed various genres and purposes for the writing of *Acts* for decades, the suggestion that *Acts* utilizes rhetorical strategies meant to persuade its audience is certainly compelling to me personally. Shelly Matthews, when describing the degree to which *Acts* paints a completely factual historical narrative, claims that *Acts* (and other ancient histories) relies on a combination of "historical events, invented stories, and persuasive artifice."[5] I propose that these invented stories of magicians are included and used in *Acts* as part of this persuasive artifice: they are included as a rhetorical device in order to distort time and space to make room for the Jesus movement while simultaneously destroying the space that magician previously occupied. While here the word "space" refers to space in the narrative, it is composed and included as a way of representing the contemporary society in which this text was written.

Furthermore, other scholars of religion and literature have suggested that indeed many forms of ancient literature function in a chronotopic way, especially the ancient Greek novel and the books of *Luke* and *Acts*. Roderick Beaton suggests that the world of the ancient novel "is distanced from that of its authors and first readers in terms of *political*, though not spatial, geography (the suppression of the reality of Roman rule) and of historical time."[6] Essentially, the association of one with a particular political empire was far more important to the narrative structure than indications of time or space. Virginia Burrus echoes this sentiment in her chapter of the *Postcolonial Commentary on the New Testament Writings* by arguing that the

---

[5] Shelly Matthews, *The Acts of the Apostles: An Introduction and Study Guide: Taming the Tongues of Fire*, (United Kingdom: Bloomsbury Academic, 2017), 12.

[6] Roderick Beaton, "Historical Poetics: Chronotopes in Leucippe and Clitophon and Tom Jones," in *Bakhtin's Theory of the Literary Chronotope: Reflections, Applications, and Perspectives*, ed. Nele Bemong et al. (Gent: Academia Press, 2010), 72.

"transcultural" nature of *Acts* makes it "extraordinarily difficult to tie its author to a specific social or geographic location."[7] Further, Burris writes that the "advent of the Roman empire and the consequent experience of cultural and political dislocation largely accounts for the prominence of novelistic writing in this period as well as for the blurring of boundaries between history and fiction that characterizes such writing."[8] Finally, my argument is similar to the one made by Judith Perkins using the *Apocryphal Acts of the Apostles*, who asserts that "Christians" attempted to "reconstitute contemporary power relation through their narrative reordering of some of the dominant spatial categories of their culture."[9] Thus, as I have shown, the application of such theories to New Testament literature such as *Acts* has already been occurring in biblical and literary scholarship and that this argument is similar to those being made by contemporary scholars.

The later dating of *Acts* suggested by Matthews provides further evidence for the use of chronotope as a rhetorical strategy. Placing the completion of *Acts* sometime during the early second century means that readers of *Acts* would have been familiar with the "issues that plagued the church in the second century" such as the divisions between Christians and Jews and the surrounding beliefs that the disciples were placed in competition with when they tried to spread the Jesus movement.[10] Here, it is possible that one of the many issues plaguing early Christians

---

[7] Virginia Burrus, "The Gospel of Luke and the *Acts* of the Apostles," in *A Postcolonial Commentary on the New Testament Writings*, ed. Fernando F. Segovia, vol. 13, Bible and Postcolonialism (London; New York: T & T Clark, 2009), 133.

[8] Burrus, 145.

[9] Judith Perkins, "Social Geography in the Apocryphal *Acts* of the Apostles," in *Space in the Ancient Novel*, ed. Michael Paschalis and Stavros Frangoulidis, Ancient Novel Supplementum 1 (Groningen: Barkhius Publishing, 2002), 118.

[10] Matthews, 22.

was the existence of magicians and other miracle-workers in their sphere. As Beverly Gaventa points out in her commentary on the story of Simon Magus, Jews are "strictly forbidden from any involvement in magical practices" because magic is deceptive and "associated with idolatry and with the demonic."[11] However, as Gideon Bohak and other scholars of Second Temple Judaism have shown, there were certainly practitioners of magic that also considered themselves Jewish and even some Talmudic passages that refer to Rabbis as magicians.[12] It is evident that laws regarding whether or not Jews could perform magic or miracles were ambiguous, but those who did could become very popular and prominent members in society. This provides only further evidence for my argument that *Acts* was attempting to restrict magic and magicians from the early Christian movement.

Historically, this argument makes sense due to the prominence of "religious anxiety" during the first century. New Testament scholar Daniel Marguerat has argued that the prominence of religious anxiety in the first century stemmed from the collapse of traditional cult and can explain both the rise of religions such as the cult of Isis and "the multiplication of occult, magic, and salvific offers."[13] This religious anxiety could also speak to the need of the author of

---

[11] Beverly Roberts Gaventa, *Abingdon New Testament Commentaries: Acts*, (United States: Abingdon Press, 2003), 137.

[12] Gideon Bohak, *Ancient Jewish Magic: A History*, (Cambridge, UK: Cambridge University Press, 2008); Shamma Friedman, "Now You See It, Now You Don't: Can Source-Criticism Perform Magic on Talmudic Passages about Sorcery?" in *Rabbinic Traditions Between Palestine and Babylonia,* eds. Ronit Nikolsky and Tal Ilan, (Leiden: BRILL, 2014), 32-83.

[13] Daniel Marguerat, "Magic and Miracles in the *Acts* of the Apostles," in *Magic in the Biblical World: From the Rod of Aaron to the Ring of Solomon*, ed. Todd Klutz, 1st ed., vol. 245, The Library of New Testament Studies (London, United Kingdom: Bloomsbury Publishing Plc, 2004), 101. The cult of Isis involved the worship of the Egyptian goddess Isis, who was said to represent life. During the Greco-Roman period, scholars have determined that Isis became a "universalized goddess" and was worshipped as protector, healer, and "mistress of the universe," among other things. For more on the cult of Isis during the Greco Roman period, see Jodi Magness, "The Cults of Isis and Kore at Samaria-Sebaste in the Hellenistic and Roman Periods." *Harvard Theological Review* 94, no. 2 (April 2001): 157-177.

Acts to provide numerous and extensive examples of rhetoric to persuade readers to support the Jesus movement - one of these being chronotope.

Bakhtin's conception of the chronotope is a helpful theory to utilize when examining the rhetorical strategies employed by the author for some sort of persuasive purpose. Bakhtin highlights the intimate connection between the author, their work, and the chronotope present within the work by writing, "We find the author *outside* the work as a human being living his own biographical life. But we also meet him as the creator of the work itself, although he is located outside the chronotopes represented in his work, he is as it were tangential to them."[14] Even though the author exists in their own time and space separate from the one they create within the narrative, Bakhtin explains that "we sense the chronotope of the represented world as well as the chronotope of the readers and creators of the work."[15] This relates to *Acts* in that readers can sense the time, space, and contemporary issues of first and second century Greco-Roman society. Some of these center around the presence of magicians and beliefs present in the societies in which the disciples are trying to convince. It is precisely because of this chronotopic nature of the magician pericopes that allows the author of *Acts* to reflect and insert contemporary dilemmas and beliefs into the narrative from the narrator's point of view. Though the author is not present in the literature's chronotope, they can employ this as a rhetorical device to insert pieces of their chronotope into the written story.

The character of Simon Magus is the first magician to appear in the narrative and his story certainly speaks to the making and breaking of boundaries of time and space. The disciples

---

[14] Bakhtin, 254. I choose to use gender neutral language here when discussing the author rather than the masculine pronouns used by Bakhtin.

[15] Bakhtin, 255.

first encounter Simon in Samaria, where he is described as "a certain man who had previously practiced magic in the city."[16] Already, the interconnectedness of time and space has appeared and is essential to a reader's understanding of these events. Gaventa highlights in her commentary on *Acts* that this location is a bit strange for the Jesus movement to travel to preach and convert - Samaritans had long been in contention with Jewish populations and it was considered extremely dangerous for Jews to travel there.[17] However, the exact location of the disciples in Samaria is disputed. Some translations of Acts 8:5 refer to it as "a city," which could be anywhere, and others as "the city" which would indicate the capital or main city of Samaria - which in the first century would be the Gentile city of Sebaste.[18] This movement into the region of Samaria already shows the fluidity of boundaries that the concept of chronotope speaks to. The Jewish disciples enter into an area that has been traditionally hostile to Jews and the physical boundaries of the area of Samaria/Sebaste are muddled due to the lack of specificity.

Not only are the physical boundaries of land and space bent and reimagined in the exposition to this narrative, but the readers' conception of time is unclear, as well. Simon is described as having "previously" practiced magic in the city and having the citizens eagerly listen to him "for a long time" because of his magic.[19] While this introduction employs the use of temporal language, it provides no real indication of how much time passes between Simon practicing magic and the disciples visiting Samaria. Simon could have practiced magic days or even years before the disciples came - the reader has no way of knowing. As time is intrinsically

---

[16]*Acts* 8:9.

[17] Gaventa, 135–36.

[18] Gaventa, 136.

[19] *Acts* 8:9, 11.

connected to space through the concept of chronotope, it only makes sense that a lack of specific spatial boundaries would be accompanied by a lack of specific temporal progression.

Though Simon eventually believes in the message of the disciples and is baptized, there continues to be a lack of temporal and spatial indications in the story. After he is baptized, Simon is said to have "stayed constantly with Philip" and the rest of the disciples until Peter and John arrive in Samaria (Acts 8:13). Once again, no temporal indications are given here. It is not known how long Simon hears the message that Philip is preaching before believing, nor how long it takes him to be baptized following his confession of belief. Moreover, there is no indication of how long the message of the conversions in Samaria takes to reach the apostles in Jerusalem, nor how long it takes them to travel there after hearing these messages and deciding to travel to Samaria. While modern technology can now provide us with tools to investigate the distances between these two areas and the amount of time it might have taken to travel between the two, the text gives no such indication of the temporal course of events nor specific spatial details that are necessary for reconstructing important aspects of the text.

The above example shows the use of Greek adventure-time in the construction of the pericope in *Acts* featuring Simon Magus. As described and explained by Bakhtin, adventure-time "lacks any natural, everyday cyclicity…" and contains "absolutely no indications of historical time, no identifying traces of the era."[20] While I am unconvinced that there are absolutely *no* indications of historical time contained in this narrative, they are certainly lacking throughout the text and can be significantly impacted by the removal of just one word (such as with my earlier discussion of manuscripts that contain "a city" compared to "the city"). Therefore, I argue that

---

[20] Bakhtin, 91.

the narrative featuring Simon Magus falls into the category "adventure-time" in which the timing of specific events is muddled, with one short event following the other in rapid succession. Adventure-time seeks to expedite the events of the magicians who oppose the Jesus movement to create more space for the speech and events of the disciples and members of the Jesus movement.

Similar distortions of time and place can be seen in the pericope featuring Bar-Jesus in *Acts 13*. Additional tropes found in this story only further highlight the rhetorical connections between *Acts* and other ancient Greek adventure novels, namely, travel by sea. Saul and Barnabas are said to sail to Cyprus (home of Bar-Jesus) from Seleucia. While there are some spatial indications in this text, there are no temporal ones. We are not told how long the journey by sea from Salamis to Cyprus takes. However, we do know that Saul and Barnabas land in the city of Salami and travel throughout the rest of the island, but do not receive another spatial indication until they reach Paphos. Again, the author provides no temporal indication for how long it takes for them to travel through the island.

It is at this point in the narrative that they meet Bar-Jesus and all spatial indications disappear. We know that Sergius Paulus, the proconsul whom Bar-Jesus was with is the man who summoned the disciples, but we do not know when he did this summoning or how long it took for them to come to him. We also know that Bar-Jesus is said to have opposed the message of the disciples and tried to turn Sergius Paulus away from his faith, but again, we have no temporal indication about the course of events. Perhaps Bar-Jesus opposed the message of the disciples before hearing it from them directly and tried to turn Sergius Paulus away from his faith before they arrived. Or, perhaps he went along with their message at first, then determined he opposed it and tried to turn Sergius away from them.

Finally, the most puzzling aspect of the lack of temporal indications in the narrative accompanies the blinding of Bar-Jesus. As punishment for his false prophecy and attempt to turn Sergius Paulus away from the Jesus movement, Saul curses Bar-Jesus, saying, "And now listen—the hand of the Lord is against you, and you will be blind for some time, unable to see the sun." Immediately mist and darkness came over him, and he searched about for someone to lead him by the hand," (Acts 13:11). Here, the lack of temporal indication can be seen through Saul's charge that Bar-Jesus will become blind for "some time," a phrase which provides little to no indication of how long this blindness will last. We do receive some indication that the curse of blindness is effective, as mist and darkness cover Bar-Jesus and he goes about "searching for someone to lead him by the hand" (Acts 13:11). After this, Bar-Jesus completely disappears from the narrative. There is no indication of how long he is blinded or if he ever regains his sight at all.

The phrase in the Greek that I chose to translate "some time" literally means "a time," which provides even less of a temporal indication. Some translations use this phrase to mean "a season;" however, the word here used to mean time (καιρος) reflects connotations of being the "right" or "opportune" time, rather than the precise nature of the other word for time in Greek, χρόνος. The only other time this phrase is used in the New Testament is to describe the tempting of Jesus by Satan in Luke chapter 4. When Satan finishes his testing, he departs from him until "an opportune time" (Luke 4:13, NRSVUE). This instance provides about the same amount of temporal indication as the phrase in *Acts*, since Satan disappears from the narrative following his testing and does not appear again until he enters Judas Iscariot in chapter 22. Since the author provides no such indication of what the "right" or "opportune" time is for Bar-Jesus to regain his sight, the reader is left to piece together what becomes of both Bar-Jesus and his sight. However,

while there is no detail explaining what happened to Bar-Jesus after this encounter, the narrative does include the fact that the proconsul Sergius Paulus believes and is astonished at the teaching of Jesus after witnessing this event. Thus, it is precisely this lack of detail regarding the fate of Bar-Jesus which allows the author to focus on the main purpose of their writing - furthering the Jesus movement. The fate of Bar-Jesus does not matter, because he represents the opposition to the Jesus movement in contemporary society. Therefore, the author distorts and minimizes the impact of Bar-Jesus within the narrative's time and space to create space for the story and future of the Jesus movement.

Thus, in both the stories of Simon Magus and Bar-Jesus found in Acts, the boundaries of time and space are fluid, stretched, and distorted. They are ultimately glossed over in an attempt to eliminate magic from the Jesus movement. Through the rhetorical use of Greek adventure time and lack of temporal and spatial indications, the author incorporates his views on magic into the chronotope of *Acts* to make an argument about contemporary society. To create space for the Jesus movement, the belief systems and popular leaders of their contemporaries must be removed from the text, which is exactly what the use of the chronotope in this narrative aims to do.

Observation of Insect Immunity

in Response to Priming with LPS in the House Cricket, *Acheta domesticus*

Niki Nicole Apostolou, Ian Baranyk, Jonathan Cornejo, and Nelly Herrera Hernandez

Faculty Mentor: Dr. Acchia Albury, Associate Professor of Biology Department

ABSTRACT

Most insects are capable of producing innate responses such as melanization and encapsulation, along with changes in hemocyte count, and the production of phenoloxidase when exposed to pathogens. After birth and into adulthood, if they are exposed to any foreign pathogen, their immune system will take measures to combat them. Previous studies have shown that insects may be capable of specificity in protection through immune defenses for selective pressures over evolutionary time. Therefore, this study aims to investigate if the house cricket, *Acheta domesticus*, could produce a stronger immune response to a secondary exposure to LPS after immune priming by measuring immune function through encapsulation activity and hemocyte count.

Crickets were maintained in a colony until adult emergence. Newly emerged adults were designated as Day 0 and then held in isolated colonies by age (Day 1, 2, 3, etc. - 24, 48, 96, etc., hours respectively after adult emergence). The crickets were separated into three groups: Control group, PBS group, and LPS group. Immune function was evaluated by measuring encapsulation activity and hemocyte count.

When analyzing encapsulation activity, the LPS group demonstrated a greater mean scale percentage than the Control and PBS groups. In addition, crickets exposed to LPS had a greater hemocyte load than the Control and PBS groups. This indicates that crickets previously exposed to LPS had a greater immune response after immune priming. This aligns with other studies that suggest that the innate immune responses of insects and other invertebrates are capable of some adaptive traits.

INTRODUCTION

Immunity refers to the body's ability to prevent the invasion of pathogens, such as bacteria and viruses. An immune response is the body's defense system to fight against pathogens; thus, possessing an immune system is vital to combat foreign invaders that may interfere with our health.

25

There are several types of immunity, including adaptive immunity and innate immunity (Chaplin, 2010). Adaptive immunity consists of specialized cells that target specific pathogens, whereas innate immunity consists of physical barriers and mucus membranes to gastric juice and phagocytic cells (Chaplin, 2010).

Humans possess immunological memory cells that provide beneficial protection against bacterial infections and viruses after initial exposure. This guarantees that if there is a second exposure, the specialized immune cells will have a memory of the initial exposure and be able to mount an attack on the foreign object at a faster rate. Insects do not have specialized memory cells; however, several studies have reported that insects are capable of producing immune responses that possess adaptive traits when the immune system is primed. During immune priming, a non-lethal dose of a pathogen is used to provide protection upon a second exposure. Usually, the protection offered by immune priming is not specific; instead, it is a broad form of protection against multiple pathogens (Hillyer, 2016).

Insects carry out multiple general immune responses in response to pathogens. One response is to increase the density of circulating hemocytes in insect hemolymph (Butolo et al., 2020). Hemocytes are the immune cells of insects and can be either sessile or circulatory (Hillyer, 2016). Another response is encapsulation, which occurs when the pathogen is too large and cannot be phagocytosed (Hillyer, 2016). During this process, granulocytes attach to the pathogen's surface and form a layer (Hillyer, 2016). Encapsulation will restrict the growth and movement of the invader, thus leading to death by asphyxiation or the release of cytotoxic products (Ratner & Vinson, 1983). Melanization occurs for several reasons, such as an immune response, wound healing, and cuticle hardening (Hillyer, 2016). During melanization, tyrosine is converted to melanin precursors; after proteins undergo cross-linking, this forms a melanin barrier around the

26

pathogen that sequesters it (Hillyer, 2016). Melanin also plays a role in darkening these capsules (Tabunoki et al., 2019). Therefore, this study aims to investigate if the house cricket, *Acheta domesticus*, could produce a stronger immune response to a secondary exposure to LPS (lipopolysaccharides), an immune elicitor of *Escherichia coli*, after immune priming by measuring immune function through encapsulation activity and hemocyte count.

METHODS

Animal Care

Juvenile crickets were obtained from Armstrong Cricket Farm, Georgia (Glenville, GA). Crickets were maintained at a temperature ranging from 22-28°C, with a light cycle of 14 hours of light and 10 hours of dark photoperiod. Crickets were maintained in a colony until adult emergence. Newly emerged adults were designated as Day 0 and then maintained in isolated colonies by age (Day 1, 2, 3, etc. - 24, 48, 96, etc., hours respectively after adult emergence). Crickets were given food and distilled water daily, and their habitats were cleaned as needed.

Protocol

The crickets were separated into three groups: Control group, PBS group–experimental control injected with PBS (0.01M phosphate-buffered saline; pH 7.4) only, and LPS group–experimental group injected with LPS (1 mg/ml amount of LPS was dissolved in PBS). These groups remained separated throughout the entire experiment. Individuals in the control group were left untreated. Day 0 crickets from the PBS group were anesthetized on ice for 10 minutes and injected with 1μl of PBS. Crickets in the LPS group were treated similarly to those in the PBS group; however, this group was injected with 1μl of LPS. Next, Day 4 crickets from the PBS and LPS groups were anesthetized on ice for 10 minutes, then the crickets were injected with a second

dose of PBS or LPS according to their respective group. All injections were administered between the insect's second and third abdominal sternite. Immune function was then measured for all groups.

Encapsulation Activity

Nylon monofilaments were cut into 5-mm pieces, roughened, and sterilized with 70% ethanol. Day 6 crickets were anesthetized on ice, then using a sterilized needle the abdomen was punctured between the second and third sternite. Next, the monofilament was inserted underneath the exoskeleton. The monofilaments were removed after 24 hours, photographed, and the mean grayscale values were analyzed using ImageJ.

Hemocyte Count

Day 6 crickets were obtained and anesthetized on ice, and 10μl of hemolymph was collected between the cuticle of the first and second prolegs of individual crickets with a 20-gauge needle. The samples were added to a chilled microcentrifuge tube, mixed 1:1 in PBS, and placed back on ice. Next, the hemolymph and PBS mixture were stained using 1:1 0.4% trypan blue. Finally, 10μl of the mixture was added to a hemocytometer (SKC, Inc. DHCN012), and hemocytes were counted using a light microscope (Wolfe Beta Elite Binocular Microscope Model).

Statistical Analysis

Results were reported as means ± standard error. Data were analyzed using a single factor ANOVA, and POST HOC T-Test was performed to compare differences between the groups using Excel. Statistical significance was determined if $p \leq 0.05$.

RESULTS

Encapsulation Activity
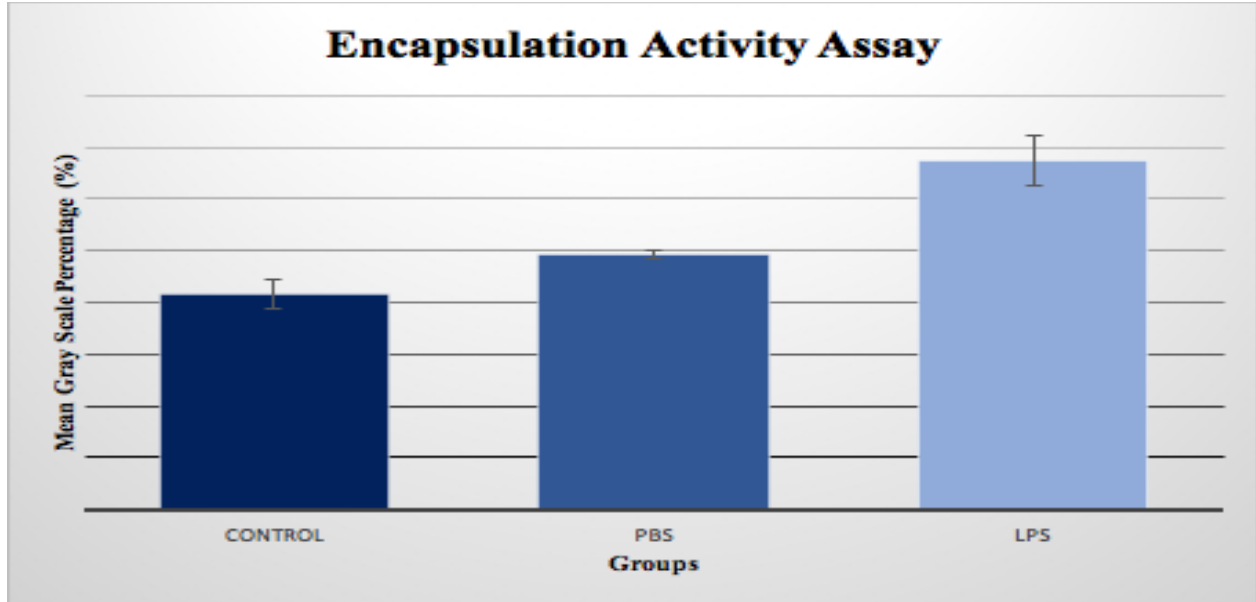
Figure 1: An ANOVA confirmed significant differences in mean grayscale percentage between the groups [F (2, 10)=13.3725; p=0.0015]. The mean grayscale percentage was higher in the LPS group than in the Control and PBS groups. The Control group had a mean grayscale percentage of 41.64% ± 2.98, the PBS group had a mean grayscale percentage of 49.18% ± 0.9049, and the LPS group had a mean grayscale percentage of 67.29% ± 4.778.

Groups	P-value (T test)	Significant
Control vs LPS	0.002468948	Yes
Control vs PBS	0.110351452	No
PBS vs LPS	0.065927688	No

Table 1: Encapsulation activity was statistically significant between the Control and LPS groups. However, no statistical significance was found between the Control and PBS groups or the PBS and LPS groups in terms of encapsulation activity.

In response to LPS, encapsulation activity measured by mean grayscale percentage was found to be significantly higher (p=0.002468948) in the LPS group (67.29% ± 4.778) compared to the Control group (41.64% ± 2.98). Encapsulation activity was also noticeably higher in the LPS group compared to that of the PBS group (49.18% ± 0.9049); however, these findings were not statistically significant (p=0.065927688). On the other hand, encapsulation activity for the PBS group was similar to that of the Control group (p=0.110351452).

Hemocyte Count
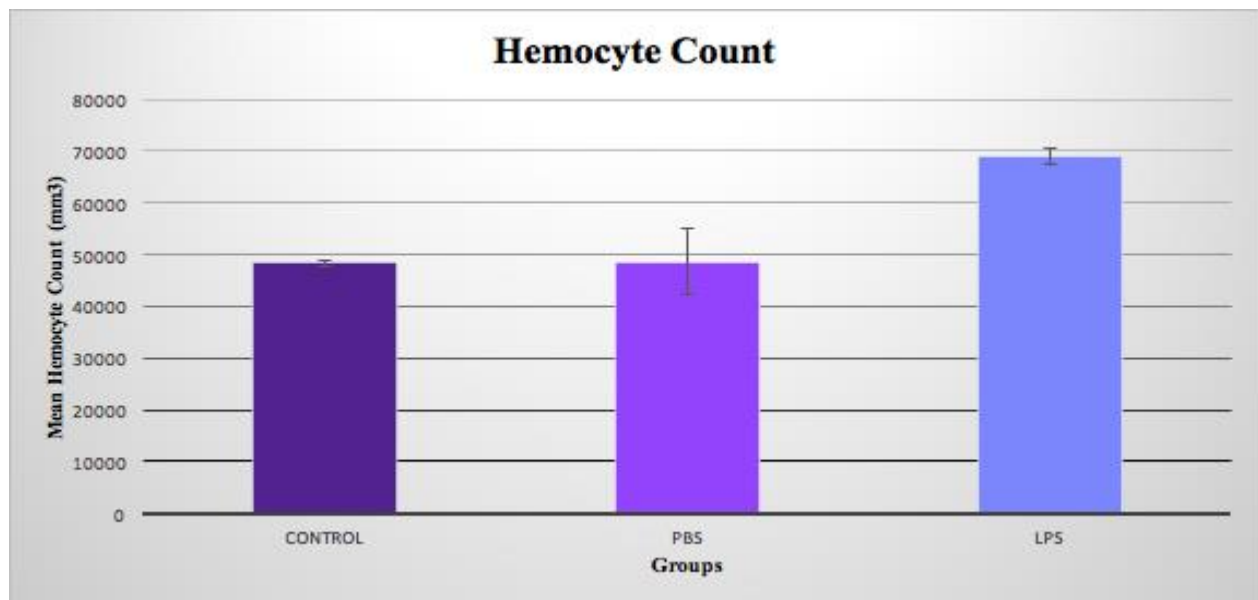
Figure 2: An ANOVA confirmed significant differences in hemocyte count between the groups [$F_{(2, 13)}$=17.0259; p=0.0002]. The mean hemocyte count was higher in the LPS group compared

to both the PBS and the Control groups. The Control group had a mean hemocyte count of 48368.83 mm3 ± 704.37; the PBS group had a mean hemocyte count of 48644.75 mm3 ± 6516.27; and the LPS group had a mean hemocyte count of 69071.17 mm3 ± 1660.33.

Groups	P-value (T test)	Significant
Control vs LPS	0.00000044	Yes
Control vs PBS	0.95919037	No
PBS vs LPS	0.00623853	Yes

Table 2: Hemocyte counts were statistically significant between the Control and LPS groups and the PBS and LPS groups. There was no statistical significance between the Control and PBS groups when examining hemocyte counts.

In response to LPS, the density of hemocytes was found to be significantly greater in the LPS group (69071.17 mm3 ± 1660.33) compared to the Control (48368.83 mm3 ± 704.37) and that of PBS groups (48644.75 mm3 ± 6516.27) (p=0.00000044 and p=0.00623853 respectively). It was reported that there was no significant difference between the Control and PBS groups (p=0.95919037).

DISCUSSION

Immune priming is a phenomenon that shows that insects might possess some adaptive immune traits associated with their innate immune responses. During immune priming, insects are

exposed to a non-lethal dose of a pathogen, and upon second exposure reports show that a stronger immune response is produced as a result. This phenomenon has been researched in many other organisms, such as scorpions (Gálvez et al., 2020), but not in house crickets. Previous studies have suggested that increases in encapsulation activity and hemocyte count occur when hemocytes are actively surrounding foreign pathogens, resulting in hemolytic capsules asphyxiating and releasing cytotoxic products to kill the invading matter (Ratner & Vinson, 1983). Therefore, this study aims to investigate if the house cricket, *Acheta domesticus*, could produce a stronger immune response to secondary exposure to LPS after immune priming by measuring immune function through encapsulation activity and hemocyte count. Upon reviewing the results for encapsulation activity, the LPS group was found to have a higher mean grayscale percentage than the Control and PBS groups. This can be attributed to the LPS stimulating an immune challenge. It has been reported that granulocytes are called in to encapsulate the foreign pathogen. Then plasmatocytes form a second layer around the pathogen to prevent the movement and spread of the infection in similar studies (Hillyer, 2016). When reviewing the results for the hemocyte counts, the LPS group had the highest density of hemocytes compared to the Control and PBS groups. This increase in number is due to the immune response elicited by the granulocytes to adhere to and capture the foreign pathogen to prevent it from proliferating (Li et al., 2019).

The data from this experiment shows that the house cricket, *Acheta domesticus*, has the ability to produce a strong immune response after priming. Previous studies have shown that other insects, such as the mealworm beetle (Moret & Siva-Jothy, 2003) and the fruit fly (Pham et al., 2007), can produce stronger immune responses after priming. This shows that insects, and other invertebrates, could possess a form of immunological memory, a trait of adaptive immunity.

CONCLUSION

Crickets exposed to LPS had a greater mean grayscale percentage than the Control group and PBS group. This indicates that the crickets exposed to LPS had a greater encapsulation activity after immune priming. The same was observed with hemocyte count, where crickets exposed to LPS had a greater hemocyte load than the control and PBS groups. Previous studies have suggested that increases in hemocyte count and encapsulation activity occur when hemocytes are actively surrounding foreign pathogens, resulting in hemolytic capsules asphyxiating and releasing cytotoxic products to kill the invading matter (Ratner & Vinson, 1983). Due to the significant increase in encapsulation and hemocyte count in the LPS group when compared to the Control and PBS groups, this research aligns with other studies that have shown evidence that innate immune responses of insects and other invertebrates are capable of some adaptive traits.

REFERENCES

Butolo NP, Azevedo P, de Alencar LD, Domingues CEC, Miotelo L, Malaspina O, Nocelli RCF. 2020. A high quality method for hemolymph collection from honeybee larvae. PLoS ONE. 15(6): e0234637.

Chaplin DD. 2010 Feb. Overview of the immune response. The Journal of allergy and clinical immunology. 125(2): S3-S23.

Gálvez D, Añino Y, Vega C, Bonilla E. 2020. Immune priming against bacteria in spiders and scorpions? PeerJ 8: e9285.

Hillyer JF. 2016 May. Insect immunology and hematopoiesis. Developmental and comparative immunology. 58: 102-118.

Li T, Yan D, Wang X, Zhang L, Chen P. 2019. Hemocyte Changes During Immune Melanization in *Bombyx mori* Infected with *Escherichia coli*. Insects.10: 301.

Moret Y, Siva-Jothy MT. 2003. Adaptive innate immunity? responsive-mode prophylaxis in the mealworm beetle, *Tenebrio Molitor*. Proceedings of the Royal Society of London. Series B: Biological Sciences. 270: 2475–2480.

Pham LN, Dionne MS, Shirasu-Hiza M, Schneider DS. 2007. A specific primed immune response in drosophila is dependent on phagocytes. PLoS Pathog 3(3): e26.

Ratner S, Vinson B. 1983. Phagocytosis and Encapsulation: Cellular Immune Responses in Arthropoda. Amer. Zool. 23: 185-194.

Tabunoki H, Dittmer NT, Gorman MJ, Kanost MR. 2019. Development of a new method for collecting hemolymph and measuring phenoloxidase activity in *Tribolium castaneum*. BMC Research Notes. 12:7.

Wingate Water Bears: A Survey of Tardigrades on University Campus

Tyler Castle

Faculty Mentors: Dr. Shem D. Unger, Assistant Professor of Biology and Mark Rollins,

Instructor of Biology

ABSTRACT

Tardigrades, also known as water bears or moss piglets, are microscopic invertebrates that are found in a variety of aquatic and terrestrial habitats and are capable of surviving the vacuum of space, high radiation, or completely drying out. These ubiquitous microscopic animals are reportedly common in areas in and around Union County, but research on their presence has yet to be undertaken. Due to their small size, water bears are often overlooked when surveying for biodiversity. Moreover, a method of sampling that provides a reliable detection rate has yet to be developed for sampling both tardigrades and other microscopic invertebrates locally. Tardigrades may form a "tun" state when inactive if the environment is dry for a sustained period of time. Therefore, to that end, I sampled various locations around the campus of Wingate University in North Carolina to determine if I could detect tardigrades both following precipitation events and without, and by either soaking them in water for different time periods. These habitats included moss near the aquatic outflow of Stegall Pond, and on moss and lichen from trees on campus. Moss and lichen were collected, submerged in water for either 3 or 24 hours, then surveyed under the microscope for the presence of tardigrades. I successfully detected Eutardigrada or "unarmored" tardigrades from moss in trees, moss near the creek, and from lichen in trees alongside other invertebrates identified using iNaturalist. iNaturalist is a social network where images of wildlife findings are uploaded for the sake of identification and mapping. Non-target microorganisms present on campus in these same moss and lichen microhabitats included springtail insects, snout mites, rotifers, false scorpions, centipedes, and nematode worms. This research provides evidence for microorganism diversity across Wingate's campus and will be used in future organismal biology laboratories.

INTRODUCTION

Tardigrades, also known as water bears or moss piglets, are ubiquitous, eight-legged invertebrates that are often overlooked due to their microscopic size (less than 1mm) and clear bodies, even though they are found almost everywhere in the world (Miller 2011, Bordenstein 2016). This is possibly due to them being difficult to detect without magnification. Water bears

are capable of surviving extreme environments such as drying out, radiation (Orellana et al. 2018), and even the vacuum of space (Jonsson et al. 2008). Therefore, an understanding of local biodiversity should incorporate methods that include surveying for this elusive microscopic animal so that they are represented in biological assessments. Tardigrades have received some renewed attention with recent surveys conducted in the southeastern US in Tennessee (Nelson et al. 2020) and in some urban locations in New Jersey (Shaw and Miller 2013). Moreover, few surveys targeting tardigrades have been conducted in North Carolina, outside of a few studies from the Great Smoky Mountains National Park (Nelson & Bartels 2013, Bertolani et al. 2014) and in the Pisgah National Forest (Bartels et al 2016), with little attention paid to other areas of the piedmont of North Carolina or even semi-rural areas around Wingate's campus.

While tardigrades are ubiquitous and found across many habitats, their presence is likely linked to precipitation, as they are capable of living under low water conditions forming a dried out, "tun" state, and awaiting favorable wet conditions (Glime 2017). Presently, it is unclear if sampling for tardigrades locally requires rain events or further submersion in water following field collection. Therefore, developing a technique for detecting tardigrades is important if sampling for tardigrade diversity is to be incorporated into any undergraduate biology curriculum as a laboratory activity. Previous studies have sampled both moss and lichen or leaf litter for tardigrades (Hinton et al. 2016, Stec et al. 2020), but no surveys have been conducted locally on Wingate University's campus. In addition, developing a laboratory activity that can be used by introductory biology students can serve to engage students in discovering the local biodiversity of the microcosm world which surrounds them when walking around campus, but is largely overlooked.

Herein, I describe an efficient, reliable method for collecting and detecting water bears on Wingate University's Campus. Moreover, I report on other microorganisms encountered while surveying samples for tardigrades. This study contributes to our general body of knowledge on both field and laboratory techniques utilized for the detection of water bears. Information from this study will be incorporated into future organismal biology laboratory activities for the Wingate Biology Department.

METHODS

Field Collection

We sampled both moss and lichen habitats on Wingate's campus (Figure 1). Two separate moss species were sampled in order to identify tardigrades across habitats to increase the potential to detect tardigrades where they are likely to occur. Lichen and moss were keyed out using iNaturalist. The two species of moss were common tree-skirt moss, *Pseudanomodon attenuates,* and Sphagnum or peat moss, *sphagnum sp*. moss (genus). For sphagnum moss three quarter -sized portions of moss were sampled after precipitation and then soaked in spring water for 3 hours and 24 hours. *Pseudanomodon attenuatus* was more difficult to portion, so one full Petri dish of moss was sampled. *Sphagnum sp.* moss was also soaked in spring water for 3hrs and 24 hrs. The same sampling methods were repeated for both mosses, but with no previous precipitation. To augment our sampling, we also collected samples of Powdery Axil-bristle lichen, *Myelochroa aurulenta*, and processed the lichen similar to our methodology for moss samples.

Figure 1. Type of Moss (common tree skirt and sphagnum, *left* and *right*) and lichen (center) were sampled across habitats on Campus. Note sphagnum was near Stegall stream or Stegall pond outflow, while other trees sampled for moss and lichen were in the Wingate campus quad area.

Identifying Microorgansms

Once the moss had soaked in a Petri dish for the appropriate time, all the water from the moss or lichen was then squeezed onto another Petri for examination under a dissecting scope (Figure 2). We then scanned the Petri dish under the dissecting microscope on either a dark or light background (Figure 3), to search for living micro-organisms. Once a prospective organism was identified it was transferred to a slide using a disposable micropipette with ~50 microliters of water. Images were taken using a combination of cell-phones and either a Leica EZ4HD dissecting microscope or a Leica ICC50W compound microscope. We used iNaturalist to identify both moss and lichen and other organisms found within samples.

Figure 2. Laboratory methods used for sampling water to detect tardigrades.
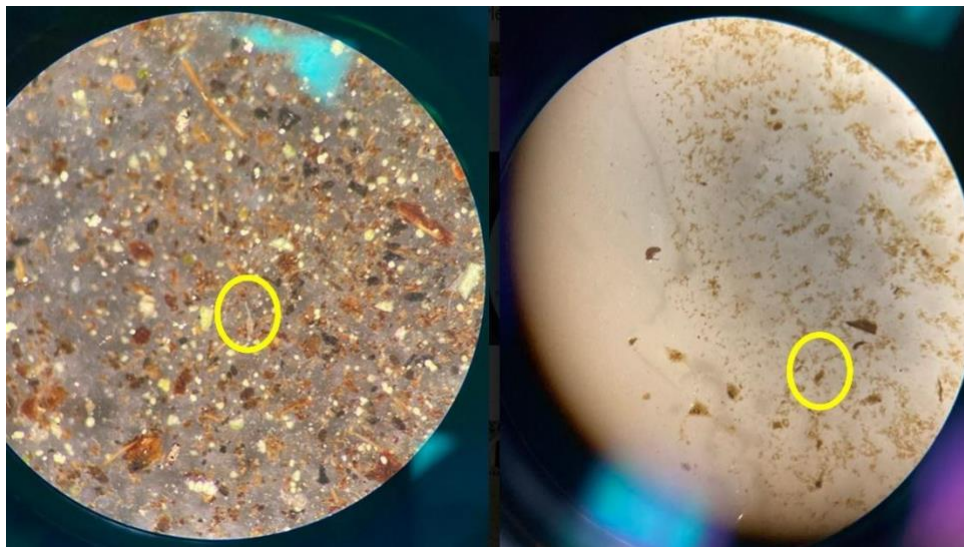
Figure 3. View under dissecting microscope showing debris in the water of Petri dishes under dark background (*left*) and light background (*right*). Highlighted area shows rotifer (*left*) and tardigrade (*right*).

RESULTS

I successfully found tardigrades and other microorganisms across several habitats on campus (Figure 4), depending largely on whether samples were soaked in spring water for either 3 or 24 hours or if they were collected following precipitation. The most effective method for consistently finding tardigrades present on Wingate University's campus habitats includes precipitation and moss (Table 1), as this combination took the least amount of time to discover tardigrades in squeezed water from moss following rain. Time spent searching and successfully detecting tardigrades was variable, but was approximately 5 minutes if they were present in lichen, and approximately 10 minutes in moss.

Figure 4. Representative tardigrade images, highlighting anatomy including gut, claws, pharynx, and stylet (upper left and right), found in this study on Wingate's campus.
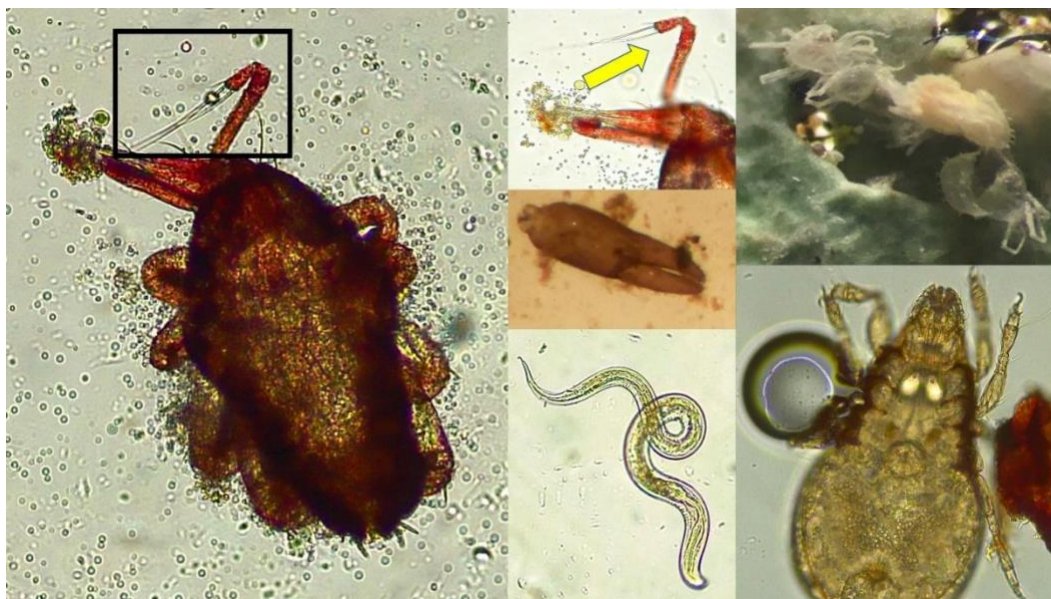
Figure 6. Representative non-targeted organisms found in lichen including Bdellidae snout mite (*left*) with the elbowed palps shown in detail (*upper center*), centipede (*upper right*), false scorpion pedipalps (*middle center*), nematode (*lower center*), and mite (*lower right*).

While we detected water bears, tardigrades were unable to be keyed out to species, due to the difficulty in observing structures limited by both microscope and availability of dichotomous keys. However, I detected several water bears in the biological class Eutardigrada or "unarmored" water bears found in both moss and lichen. Other non-target organisms I identified from moss include aquatic mites (*Hydrachnidia*), *Euchlanis* rotifer, *Brachionus* rotifer, nematode worms, and *Isotomurus* springtail insects (Figure 5). Organisms identified from the lichen sample include snout mites from the Family Bdellidae, Order Trombidiformes, nematode worms, and rotifers. Moreover, I also observed sheds and fragments of a false scorpion, Order Psuedoscorpiones, and also a centipede, Class Chilopoda (Figure 6) from the 24-hour lichen sample.

Figure 5. Representative non-targeted organisms found in moss include *Isotomurus* springtail (*top left*), *Euchlanis* rotifers (*bottom left*), Nematodes (*upper middle and right*), and water mites (*lower right*).

When sampling 3 hours soaked in water lichen samples, I found it was more difficult to extract the water from the lichen, regardless of precipitation. I also noted when collecting lichen following precipitation that the water in the Petri dish was discolored and took on a brown-green appearance. Moreover, when surveying the 24-hour lichen sample, I found over 10 nematode worms, as they were readily observed moving in the water surrounding the lichen.

Time and Precipitation	*Myelochora aurulenta* **Lichen**	*Sphagnum sp.* **moss**	*Pseudanomodon attenuatus* **moss**
3 HR + Precipitation	*ND*	*P*	*P*

24 HR + Precipitation	*P*	*P*	*P*
3 HR – Precipitation	*ND*	*ND*	*ND*
24 HR – Precipitation	*P*	*P*	*P*

Table 1. Treatment for sampling tardigrades and relative success of detection method for lichen, *Sphagnum* moss, and *Pseudanomodon* moss. ND = Tardigrades Not detected, P = Tardigrades Present. Treatments include time of water saturation with precipitation (+) and without precipitation (-).

DISCUSSION

I detected the presence of tardigrades and other microscopic invertebrates successfully during this study using novel methodologies for surveying water from moss and lichen, which will be incorporated into new undergraduate biology laboratories. Clearly tardigrades and other organisms I detected during this study represent a unique diversity and window into the microcosms of Wingate University's campus. I found several tardigrades across moss species, both near water and on trees. Lichen contained many of the same organisms with additional organisms observed, indicating that it might be important to sample both lichen and moss not only for tardigrades but for the presence of additional organisms, as they likely represent unique habitats on campus. It is likely that the amount of moisture available to both tardigrades and other organisms increased the likelihood of detecting moving microorganisms in the samples collected. Therefore, I recommend sampling for these microorganisms following precipitation events and soaking in spring water to increase the chances of finding both water bears and associated microorganisms like rotifers, worms, and other tiny arthropods.

Interestingly, I observed several other organisms, including nematodes, which have been found to be the prey of tardigrades (Tumova et al., 2022). However, many tardigrades commonly found in lichen and moss often consume moss protonema and green algae within lichen (Momeni et al. 2022). Moreover, where tardigrades are found in habitats with both nematodes and rotifers, as we observed, typically either tardigrades or rotifers are more dominant (Zawierucha et al. 2021). Subsequently, mites can also be found in patchy environments in forested or urban areas within moss, lichen, and even the bark of trees (Aoki 1967). Therefore, my observation of finding tardigrades within these habitats on campus can be explained by the availability of food sources, however, further research is needed to elucidate the ecological connections between tardigrades and other organisms I found in this study.

I qualitatively noted that when sampling with lichen it was easier to notice organisms moving relative to sampling the moss, likely due to some of the soil being retained when straining water to sample. Quantitatively, I observed tardigrades with more ease in water drained from lichen compared to moss, as it is likely with moss samples, some portion of soil may have been present, and in general water from moss had slightly more sediment present. Based on my observations for finding organisms in both lichen and moss, I would recommend soaking samples for 24 hours when compared to only soaking them for 3 hours, as I readily found more microorganisms when surveying 24-hour samples. Alternatively, it is possible that when sampling, samples collected following rain events increase the likelihood of finding active microorganisms moving in the water, as they are easier to detect when some movement in the debris was observed. When searching for both tardigrades and other microorganisms for both lichen and moss, it is important to properly focus the dissecting scope magnification to view both

the top and lower surface of the water, as organisms may be found in different layers of the Petri dish.

Future studies could sample more aquatic habitats, such as within stream and pond areas, as well as in other terrestrial areas. In addition, further replication could strengthen the detectability of tardigrades. Moreover, using increased magnification identification of tardigrade species might help us understand more about the taxonomic diversity of tardigrades. I did find a number of other interesting organisms inhabiting the moss samples, and it is likely some of these may have been encountered while removing water from prepared samples, as sampling included portions of the soil, where nematodes are common alongside other soil invertebrates. It is also worth noting that the sphagnum moss was much more difficult to observe. The extra sediment made the water murky and made identifying organisms much more difficult. The *P. attenuatus* moss did not contain the extra sediment which made the identification much easier.

While there are limited studies on environmental factors influencing the presence and the relative abundance of tardigrades in North America, the level of disturbance and presence of sulfur dioxide deposition appear to negatively impact moss and lichen communities, and thus tardigrades (Nelson et al. 2020). Therefore, I recommend further sampling across unique Wingate University habitats, which can aid in the identifying environmental impacts of the presence of tardigrades.

In conclusion, this research study demonstrates that tardigrades require some time searching to detect successfully in suspended water when collected from moss or lichen. Tardigrades, alongside other microorganisms, are an integral part of many different types of habitats on university campuses and further study could investigate potential predator-prey densities of both water bears and other smaller invertebrate arthropods we discovered living in

45

moss and lichen. Microscopic creatures are often overlooked, yet provide a window into the

microscopic ecosystems, an important, yet overlooked area of biological study. These research

findings will be incorporated into a future laboratory in organismal biology, which will allow

undergraduate biology majors to experience first-hand the importance of micro-diversity in and

around Wingate University's campus.

REFERENCES

Aoki, J. 1967. Microhabitats of orbatid mites on a forest floor. Bulletin of the Natural Science Museum of Tokyo 10: 133-140.

Bartels, P. J., C. J. Mormino, and D. R. Nelson. 2016. Aquatic tardigrades of North Carolina with a new North American record of a lotic tardigrade, *Dactylobiotus haplonyx maucci*, 1980 (Eutardigrada: Macrobiotidae). Journal of the North Carolina Academy of Science 132: 26-30.

Bertolani, R., P.J. Bartels, R. Guidetti, M. Cesari, and D. R. Niseon. 2014. Aquatic tardigrades in the Great Smoky Mountains National Park, North Carolina and Tennessee, U.S.A., with the description of a new species of *Thulinius* (Tardigrada, Isohypsibiidae. Zootaxa 3764: 524-536.

Bordenstein, S. 2016. Tardigrades (water bears). http://serc.carleton.edu/microbelife/topics/tardigrade/index.html (2016-09-01)

Glime, J.M. 2017. Tardigrade Survival. Chapter 5-1 in Glime, J.M. Bryophyte Ecology, Volume 2, Bryological Interaction. Ebook available at http:digitalcommons.mtu.edu/bryophyte-ecology2/

Goldstein, B., and M. Blaxter. 2002. Tardigrades Current Biology

Hinton, J. G., H. A. Meyer, and B. Peet. 2016. A contribution to the tardigrade fauna of Georgia. Georgia Journal of Science 74: 1-8.

Jonsson, K., E. Rabbow, R. Schill, M. Ringdhal, and P.. Rettberg. 2008. Tardigrades survive exposure to space in low earth orbit. Current Biology 18: R729-R731.

Miller, W. 2011. Tardigrades. American Scientist. http://www.americanscientist.org/issues/pub/2011/5/tardigrades/1 American

Momeni, S., J. Fuentes-Gonzalez, and J. Pienaar. 2022. How to culture limnoterrestrial heterotardigrades. Invertebrate Biology 141: e12360.

Nelson, D. R, and P. J. Barlets. 2013. Species richness of soil and leaf litter tardigrades in the Great Smoky Mountains National Park (North Carolina/Tennessee). Journal of Limnology 72: 144-151.

Nelson, D. R., R. A. Fletcher, R. Guidetti, M. Roszkowska, D. Grobys, and L. Kaczmarek. 2020. Two new species of Tardigrada from moss cushions (*Grimmia* sp.) in a xerothermic habitat in northeast Tennessee (USA, North America), with the first identification of males in the genus *Viridiscus*. PeerJ 8:e10251 http://doi.org/10.7717/peerj.10251.

Nelson, D. R., P. J. Bartels, and S. R. Fegley. 2020. Environmental correlates of tardigrade community structure in mosses and lichens in the Great Smoky Mountains National park

(Tennessee and North Carolina, USA). Zoological Journal of the Linnean Society 188: 913-924.

Orellana, R., Macaya, C., Bravo, G., Dorochesi, F., A. Cumsille, R. Valencia, C. Rojas, and M. Seeger. 2018. Living at the Frontiers of Life: Extremophiles in Chile and their potential for bioremediation. Frontiers in Microbiology: 9: 1-25.

Shaw, M. W., and W. R. Miller. 2013. Tardigrades of North America: a preliminary survey of New Jersey, U.S.A. Newsletter of the New York Microscopial Society, 7: 36-56.

Stec, D., D. V., Tumanov, and R. M. Kristensen. 2020. Integrative taxonomy identifies two new tardigrade species (Eutardigrada: Macrobiotidae) from Greenland. European Journal of Taxonomy 614: 1-40.

Tumova, M., D. Stec, L. Michalczyk, and M. Devetter. 2022. Buccal tube dimensions and prey preferences in predatory tardigrades. Applied Soil Ecology, 170: 104303.

Zawierucha, K., D. L. Porazinska, G. F. Ficetola, R. Ambrosini, G. Baccolo, J. Buda, J. L. Ceballos, M. Devetter, R. Dial, A. Cranzetti, U. Fuglewicz, L. Gielly, et al. 2020. A hole in the nematosphere: tardigrades and rotifers dominate the cryoconite hole environment, whereas nematodes are missing. Journal of Zoology, 313, 18-36.

Waterborne Livestock Pathogens Detection in Water Bodies Around the Wingate

University Campus

Harley Linton

Faculty Mentor: Dr. Debra A Davis, Associate Professor of Biology

ABSTRACT

High numbers of waterborne pathogens may result in outbreaks in local water sources, quickly affecting animals and humans in nearby farms and communities. Bacterial livestock pathogens *Mannheimia haemolytica*, *Bacillus anthracis*, *Mycobacterium bovis*, *Salmonella enterica*, *Escherichia coli*, and *Shigella dysenteriae* cause enteric and pulmonary diseases and are often found in watersheds near farms where they may enter community water sources via agricultural runoff. These pathogens were hypothesized to be present in water bodies around Wingate University (WU) in North Carolina, a rural campus surrounded by livestock farms. WU Campus Lake, Smith Street Creek, and the unnamed creek that runs through the Frisbee Golf Course were sampled. The serial dilution agar plate technique and specialized media were used to target the pathogens. Bacterial isolates categorized into morphotypes using observed morphological and biochemical characteristics of the pathogens were identified using phylogenetic analysis of the 16S rRNA and *rpoB* gene sequences. Multiple bacterial morphotypes were found to be resistant to human and veterinarian (specifically Tilmicosin) antibiotics. Only the *Bacillus anthracis* morphotype was phylogenetically related to its expected species; all other morphotypes were closely related within the same Phylum or family, but not the expected genus. However, the genera within which some of the morphotypes were phylogenetically classified are natural waterborne pathogens (such as *Aeromonas*) and bear further study. In conclusion, we must reject the hypothesis that all listed pathogens are present in the WU water bodies and acknowledge that there are other pathogens present that were not initially considered.

INTRODUCTION

Waterborne livestock microbial pathogens are harmful to many populations and can cause outbreaks if water bodies are not appropriately routinely observed and maintained. Microbial pathogens that may result in local epidemics through water contamination include viruses, fungi, protozoa, and bacteria (McAllister and Topp, 2012; Wingender and Flemming, 2011). These pathogens may originate from the waste of livestock (farm animals) populations housed near water sources, agricultural watersheds, and runoff from rural areas (Mawdsley *et al.*,

49

1995; McAllister and Topp, 2012). Direct leakage into water supplies, faulty drainage systems, feces directly deposited by animals into permeable soils, and surface or subsurface runoff due to heavy rainfall are all possible routes of microbial contaminants to nearby water sources. Manmade water sources used for recreational purposes are routinely monitored for contamination, however natural fresh and marine water bodies also used by humans may contain aquatic microbes that are naturally pathogenic (Fewtrell and Kay, 2015; McAllister and Topp, 2012). This study aims to discover whether specific livestock pathogens (listed below) are in the recreational water bodies around the Wingate University campus in Wingate, North Carolina. This campus is embedded in a rural community, surrounded on either side by farms of various sizes. The major recreational water body is WU Campus Lake, used by students and community members for canoeing, fishing, and sometimes swimming; at the north end of this location is a livestock farm. Any animal waste picked up within this watershed could easily contaminate this recreational location resulting in infectious disease and potentially a campus-wide and/or community epidemic.

For this study, the bacterial pathogens of interest are *Bacillus anthracis*, *Escherichia coli*, *Mannheimia haemolytica*, *Mycobacterium bovis*, *Salmonella enterica*, and *Shigella dysenteriae*. *Bacillus anthracis* is the only bacterium naturally found in the environment (Beesley *et al.*, 2010) while the others listed are most commonly the causative agents of waterborne epidemics and natural microbial inhabitants of animal intestines (Tomley and Shirley, 2009). All of these organisms can persist in water for ≥16 days at moderate temperatures of 25°C, and at least 6 weeks at temperatures as low as 6°C (McAllister and Topp, 2012). The regional location of the university is in a hot and humid environment for the majority of the year, where average high/low temperatures are 22°C/8°C (WorldClimate, 2022). Freshwater bodies in this region are

50

prime locations for these bacteria to maintain viability for prolonged periods increasing their likelihood to result in public health emergencies as each has the potential to cause a variety of human illnesses. *Mannheimia haemolytica* and *Mycobacterium bovis* can cause bovine respiratory disease in livestock and humans (Kumar *et al.* 2015; de Azevedo *et al.* 2017); *M. bovis* is a slow-growing organism that results in tuberculosis lung infections. *Bacillus anthracis* is a soil bacterium that can also be found in the respiratory and enteric tract of humans and animals, and causes all forms of anthrax, cutaneous, inhalation, and gastrointestinal (Kolton *et al.* 2017). *Escherichia coli* and *Shigella dysenteriae* may cause diarrhea in varying severities and if prolonged may result in shock and death (Zhu *et al.* 2016), however, some strains are symbiotic partners in the natural microbiome of humans, while *Salmonella enterica* can cause typhoid fever (Andino and Hanning 2015).

Classic microbiological techniques were used to isolate bacteria from water collected at 3 locations in Wingate, NC that were within or relatively close to the WU campus. WU Campus Lake was chosen because of the farm on the north end side of its property that may contaminate the lake through agricultural runoff. The creek on the Frisbee Golf Course flows directly from the lake which allows for the livestock waste from the farm to flow into surrounding creeks. Smith Street creek flows through a residential area and is a part of Meadow Branch, a stream that flows southward through Eastern North Carolina with multiple livestock farms along its route in Union County where the university is also located (TopoZone, 2022). All of these locations are in close proximity to residential areas and the college. They are used for fishing and other recreational purposes, particularly WU Campus Lake, therefore they are highly probable to serve as the source of a community-wide epidemic if contaminated. All of the waterborne isolates that morphologically (by cellular and colonial growths on plates, and metabolic enzyme production)

matched the above listed pathogens were phylogenetically analyzed using DNA sequences of biomarker genes 16S rRNA (present in all bacteria) and *rpo*B (gene that codes for a transcription enzyme present in all bacteria, with conserved sequences in different taxa of bacteria). Analysis of the morphological and phylogenetic data provided credible presumed identities of the isolated bacteria.

PROCEDURE

On September 24th, 2021, water samples were collected in sterile 50mL conical tubes from the following locations: WU Campus Lake, Smith Street creek, and the unnamed creek along the Frisbee Golf Course on campus. Water temperature (°C) was measured using a mercury thermometer and the pH of the water samples (collected in sterile 15mL conical tubes) was measured using universal strips (LabRat Supplies, LLC, North Carolina, USA). The water samples were serially diluted to 10^{-3} in sterile dH$_2$O, and 100μL of the stock sample, and each dilution was aseptically spread onto the following agar plates: Egg Yolk (EYA) to isolate *Mycobacterium bovis*, Salmonella Shigella (SSA) for *Salmonella enterica* and *Shigella dysenteriae*, 5% Sheep's blood (SBA) for *Bacillus anthracis* and *Mannheimia haemolytica*, and Eosin Methylene Blue (EMB) for *Escherichia coli*. Spread plates were incubated at 30°C for 24 hours (parameters also used for all subsequent incubations) and the observed morphology (shape, colour, elevation, edges etc. as they appeared on the plates) of colonial growths were recorded. Bacterial colonies (5-10) that exhibited the morphological characteristics (morphotypes) for the expected pathogens were chosen from each agar type and patched in a grid formation onto sterile agar. They were later purified using the four-way streak method and purity was confirmed using the Gram staining method which provided Gram reaction and cellular shape and arrangement.

Four-way streaks were subcultured each week for isolate maintenance on Tryptic Soy Agar (TSA) and EYA (*M. bovis* and *B. anthracis* morphotypes only).

To further confirm the isolates' presumed identities based on morphology, additional pathogen-specific biochemical tests were performed. Presumed *B. anthracis* colonies were inoculated on SBA plates to determine their hemolytic properties. MacConkey agar (MCA), SBA, and TSA plates were inoculated with presumed *M. haemolytica* isolates to confirm if they fit the expected characteristics. Metabolic substrate preferences were determined via carbohydrate fermentation broths (1% each of monosaccharides, glucose and maltose, and the disaccharide, mannose; Reiner, 2012), triple sugar iron (TSI) agar slant (0.1% glucose, and 1% of each disaccharide, lactose and sucrose; Lehman, 2005), and sulfur-indole-motility (SIM) agar deep (containing the amino acids cysteine and tryptophan; MacWilliams, 2009) inoculations. Tryptophanase production was confirmed with the addition of Kovac's reagent (Carolina Biological Supply Company, North Carolina, USA) to the culture in the SIM deep. To confirm the production of respiratory enzymes cytochrome oxidase and catalase, the bacterial cultures were respectively treated with N'-tetramethyl-p-phenylenediamine dihydrochloride (Carolina Biological) and 3% hydrogen peroxide. Potassium nitrate broths were inoculated to determine the isolates' ability to respire anaerobically (without oxygen) reducing the alternative terminal electron acceptor, nitrate. Post-incubation cultures were treated with sulfanilic acid and alpha-naphthylamine reagents, and possibly zinc to confirm the reduction of nitrate. These tests helped to narrow down the isolates to only those that (mostly) matched expected biochemical and morphological characteristics of the named pathogens and 13 morphotypes were further studied.

The morphotypes' susceptibility to commercial antibiotics manufactured for human infections was determined using the Kirby-Bauer method. Erythromycin (concentration 15μg),

neomycin (30µg), novobiocin (30µg), chloramphenicol (30U), penicillin (10U), streptomycin (10µg), tetracycline (30µg), and kanamycin (30µg) discs were stamped onto previously inoculated agar plates using a sensi-disk dispenser and incubated for 24hrs at 30°C. After incubation, bacterial susceptibility was determined from diameters (mm) of zones of inhibition compared to a standardized chart. Susceptibility to the antibiotic tilmicosin, administered to treat livestock respiratory infections, particularly those caused by *M. haemolytica* (McClary *et al.*, 2011), was determined by inoculating the morphotypes on agar containing the antibiotic (final concentration 1.6µg/mL) and observing the amount of growth after incubation.

To obtain DNA for phylogenetic analysis, genomes were extracted from all morphotypes using the Zymo Research © Quick-DNA Fungal/Bacterial microprep kit (California, USA). The genomic quality was confirmed by electrophoresing the DNA in a 0.8% Sodium Borate agarose gel, stained with GelRed nucleic acid stain (Biotium Corp., California, USA), and comparing the DNA bands with the fragments in the 1kb DNA ladder (New England BioLabs [NEB], Massachusetts, USA) to estimate band sizes. Amplification of the 16S rRNA gene was accomplished using Polymerase Chain Reaction (PCR) conducted in a T100 Thermocycler (BioRad Laboratories Inc., California USA). Amplicons (copies of the gene fragment) were created using universal primers 27FHT [AGRGTTTGATYMTGGCTCAG] and 1492RHT [GGYTACCTTGTTACGACTT] (10µM each; Integrated DNA Technologies Inc. [IDT], Iowa, USA) which targeted the 16S rRNA gene in the genome, and One*Taq*® master mix (final concentration 1X; NEB) which contained the additional components to synthesize DNA copies. The PCR cycle parameters were initial denaturation at 95°C for 15 mins, followed by 30 cycles of denaturation at 94°C for 30 secs, annealing at 58°C for 30 secs, extension at 72°C for 1 min and 45secs, and a final extension after the cycles at 72°C for 7 minutes, finally held at 12°C until

removed from the thermocycler. Amplification success was determined using agarose gel electrophoresis as previously described except the 100bp DNA ladder (NEB) was used to confirm the amplicons were of the expected 1500 base pair (bp) size.

For more stringent phylogenetic analysis of the morphotypes, amplification of areas in the 16S rRNA gene that were conserved in the genera (taxonomic specific groups based on DNA similarity) *Escherichia*, *Mycobacterium*, and *Salmonella* were amplified using primers ECA75F/ECR619R (Sabat *et al*., 2000), AFB (acid-fast bacillus primers; Lee *et al*., 2011), and *pan*B-F/R (Sukhnanand *et al*., 2005), respectively. Primers that target the housekeeping gene, *rpo*B, which codes for the B subunit of RNA polymerase (transcription enzyme), in the genera *Bacillus* and *Mannheimia* were also used (Christensen *et al*., 2004). PCR reaction mixes were as described previously with each primer in the set at a 10μM concentration and parameters for the PCR cycles used are listed in Table 1. As described previously amplification success was determined via agarose gel electrophoresis, however for these primer sets the Low Molecular Weight ladder (NEB) was used to confirm amplicon size (Table 1).

Successfully amplified genes were mailed to Eton Bioscience Inc. (North Carolina, USA) where they were sequenced on an ABI 3730xl DNA Sequencer. Abi chromatograms of each sequence were observed in Chromas (Technelysium Pty Ltd, Brisbane, Australia), and further edited to remove low-quality areas of the sequences. Fasta format of the sequences were created in Chromas, and forward and reverse versions (created from opposite ends of the DNA fragment) of each sequence were aligned and further edited in BioEdit software (Hall, 1999). Edited sequences were uploaded in BLASTn (Basic Local Alignment Search Tool, nucleotide; NCBI, 2022) and compared to submitted sequences in the GenBank database (NCBI, 2022a) to acquire preliminary identities based on sequence similarity. Neighbor-joining phylogenetic trees (Saitou

and Nei, 1987) were created in MEGA 11 (Tamura *et al.*, 2021) using isolate gene sequences and reference sequences acquired from GenBank. Phylogenetic distances were calculated using the Jukes-Cantor Method (Jukes and Cantor, 1996), with all ambiguous positions removed for each sequence pair compared, and the resulting trees were tested with 1000 bootstraps to ensure accuracy of the representation of evolutionary relationships.

Primers [expected amplicon size]	Initial Denaturation	Number of cycles	Denaturation/ Extension step in each cycle	Annealing temperature	Final extension
ECA75F/ ECR619R [544 bases]	94°C for 4 mins	40	94°C for 45secs/ 72°C for 1min 45secs	60°C for 20secs	72°C for 2 mins
AFB [593 bases]	92°C for 2 mins	35	95°C/72°C for 20secs	55°C for 20secs	72°C for 5mins
*pan*B F/R [997 bases]	95°C for 9 mins	40	95°C for 30secs/ 72°C for 60secs	Touchdown PCR, 30secs [65°C – 55°C]	72°C for 10mins
*rpo*B (*Bacillus*) [318 bases]	95°C for 5 mins	30	95°C for 30secs/ 72°C for 60secs	45°C to 55°C for 30secs	72°C for 5mins
*rpo*B (*Mannheimia*) [518 bases]	95°C for 5 mins	35	94°C/ 72°C for 30secs	54°C for 30secs	72°C for 1 min

Table 1: PCR parameters for genus specific primers.

Results

The pH at all three sampling locations was neutral, however, the temperatures differed; it was 28°C at Campus Lake, 25°C at Smith Street Creek, and 21°C at Frisbee Golf Course Creek on the day of collection. The water at all locations was very murky as it had rained the day

before sample collection (Figure 1). This may have added more nutrients and possibly microorganisms to the water from surrounding areas. Serial dilution plates exhibited a variety of colonial morphologies (Figure 2) and bacterial isolates that matched the expected characteristics of the targeted genera *Bacillus* (BA isolates; EYA and SBA plates), *Escherichia* (EC isolates; EMB plates), *Shigella* and *Salmonella* (Sg and S isolates, respectively; SSA plates), *Mycobacterium* (MB isolates; EYA plates), and *Mannheimia* (MH isolates; SBA) were chosen. For each bacterial type, between 25 and 27 isolates were picked and patched onto new plates. Expected colonial and cellular morphologies and Gram reactions for each pathogen are outlined in Tables 2 and 3 (also see Figures 2 and 3). Included in the tables are the isolates that matched which were maintained and further characterized biochemically. BA1 from EYA plates was inoculated onto SBA media and produced a hemolytic reaction that did not match the expected description (Figure 4). The isolate was further explored as there was the possibility that as this unknown bacterium was isolated from the environment it could have additional or different characteristics that did not perfectly match with the expected pathogen. In addition, all MH isolates produced the expected complete hemolysis on SBA and were also inoculated on MCA to confirm lactose fermentation. All morphotypes were inhibited on the MCA, which was unexpected, however, the general colonial characteristics on TSA and hemolysis, indicated the classification was correct (Figure 5).

The biochemical tests allowed for further insight into the bacteria's respiratory and metabolic preferences. All isolates except for MH1 did not produce cytochrome oxidase (no colour change with added reagent), while BA1, EC1, EC2 were the only isolates that lacked catalase enzyme (produced no O_2 bubbles with the addition of 3% H_2O_2) and this was an unexpected result. Inoculations and subsequent test results in SIM deeps indicated that only Sg1

57

produced indole (cherry red color with the addition of Kovac's) and all MB, S, Sg, and EC2 morphotypes exhibited motility (Figure 6). However, only EC2 produced cysteine desulfurase and blackened the SIM medium with H_2S production.

For nitrate reduction, and carbohydrate fermentation tests most observations matched the expected results. All morphotypes except for EC1 (unexpected result) produced nitrate reductase and anaerobically respired nitrate. TSI test results varied (Figure 7); most morphotypes fermented glucose only, if not all sugars, and some had additional gas production. EC1 did not ferment any sugars (unexpected), however, S2 and EC2 produced H_2S byproducts as exhibited by blackening of the agar slant in addition to fermenting sugars. All morphotypes produced acid upon fermenting the carbohydrates glucose, mannose, and sucrose, however, the BA1 and the MB morphotypes did not have any gas production in the inner Durham tubes as expected.

All bacterial morphotypes were resistant to at least one of the commercial antibiotics screened, however, all but MH1 and MH2 were resistant to penicillin. MB1, MB3, EC1, and EC2 were resistant to 5 or more of the commercial antibiotics but not the livestock antibiotic, tilmicosin (Table 4). All the MH and S morphotypes exhibited growth on plates containing tilmicosin, which is used to treat pneumonia and *M. haemolytica* infections, indicating their resistance.

The DNA was successfully extracted, and 16S rRNA genes were amplified and sequenced for each morphotype, confirming that all isolates were indeed prokaryotic and bacterial (Figure 8). Using the genus-specific primer sets AFB for *Mycobacterium* and *rpo*B for *Mannheimia* for PCR resulted in amplicons that were the incorrect size and suggested that the morphotypes were not actually of the expected genera (Figure 9). PCR using the *pan*B primers for *Salmonella* failed outright (twice), however, *rpo*B was successfully amplified for BA1 and

the *Escherichia* specific 16S rRNA primers (ECA75F/ECR619R) produced the correct size amplicon in all EC morphotypes (Figure 9). Phylogenetic analysis of the 16S rRNA (Figure 10, MH3's sequence was of low quality and is seen as an outlier) and genus-specific sequences (Figures 11 – 14) provided confirmation of the PCR results. In the neighbor-joining phylogenetic trees, the morphotypes did not cluster (group) closely with any of the expected genera which indicates that their DNA/gene sequences are not similar to the pathogens of interest. BA1, however, is indeed a *Bacillus* species and is quite possibly closely related to *Bacillus anthracis* (Figure 14). EC morphotypes are all members of the phylum Pseudomonadota though not of the genus *Escherichia* (Figure 11), however, EC3 clustered tightly with the *Siccibacter colletis* reference sequence. MB morphotypes clustered tightly with reference sequences of the genus *Bacillus,* except for MB2 which is not related to either the *Mycobacterium* or *Bacillus* genera (Figure 12). Reference sequences of the genus *Aeromonas*, are closely related to the MH morphotypes except for MH2 which clustered with neither *Mannheimia* or *Aeromonas* reference sequences (Figure 13).

Figure 1: Sample collection from the stream along the frisbee golf course around Wingate University Campus Lake (D. Davis photo).
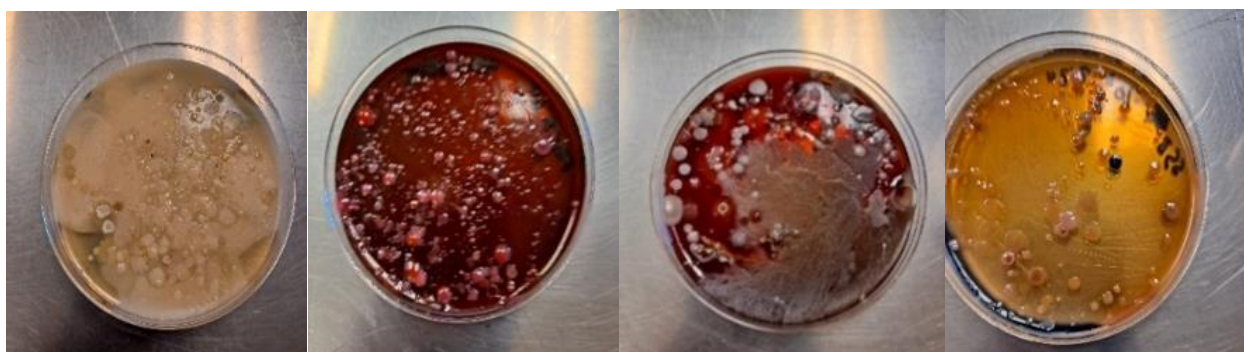
Figure 2: Spread plates on specialized media (left to right, Egg Yolk [EYA], 5% Sheep's Blood [SBA], Eosin Methylene Blue [EMB], and Salmonella-Shigella [SSA] agars) for the various samples collected (Harley Linton [HL] photos).

Figure 3: Pick and patch plates with the bacterial morphotypes of presumed pathogens on various media (left to right, EYA, SBA, EMB, SSA agars; HL photos).

Bacteria	Expected Colonial Morphology	Isolates that Matched
Mannheimia haemolytica	Circular, smooth/glistening, convex, white, and entire edge	MH1, MH2, and MH3
Mycobacterium bovis	Irregular, rough, undulate, flat, and milky white	MB1, MB2, and MB3
Bacillus anthracis	Circular, smooth, convex, entire edge, and white/yellow	BA1

Salmonella enterica	Circular, smooth, entire edge, white, and raised	S2
Shigella dysenteriea	Yellow, circular, undulate, smooth, and flat	Sg1
Escherichia coli	Cream, circular, entire edge, smooth, and flat	EC1, EC2, EC3, and EC4

Table 2: Expected colonial morphology of each pathogen and the isolates that matched.

Bacteria	Expected Cellular Morphology	Isolates that Matched
Mannheimia haemolytica	Gram-negative, rod-shaped coccobacillus bacterium, and complete hemolysis	MH1, MH2, and MH3
Bacillus anthracis	Gram positive, rectangular, rod-shaped bacterium with square ends, and chain formation is common	BA1
Mycobacterium bovis	Gram positive, aerobic bacterium, acid-fast, and rod-shaped	MB1, MB2, and MB3
Escherichia coli	Gram-negative, envelope has three layers: cytoplasmic membrane, peptidoglycan, and rod-shaped	EC1, EC2, EC3, and EC4
Salmonella enterica	Gram-negative, rod-shaped enterobacterium, and peritrichous flagella	S2
Shigella dysenteriae	Gram-negative, rod-shaped, and non- spore-forming	Sg1

Table 3: Gram status, expected cellular morphology and the isolates that matched.

Figure 4: SBA with BA1; this organism produced complete destruction of red blood cells (β hemolysis) reaction which is not common for *B. anthracis* (HL photo).

Figure 5: SBA, TSA, and MCA media plates streaked with MH morphotypes. SBA shows β hemolysis and TSA shows the colonial morphology, whereas as MCA agar has no growth (HL photos).
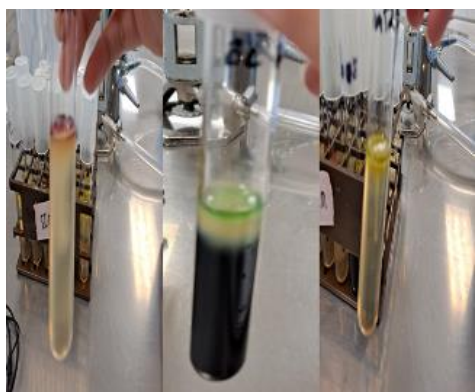

Figure 6: Various SIM test results. From left to right: indole production (red ring on top), cysteine desulfurase production (blackening of medium), neither indole nor cysteine desulfurase production (HL photos).
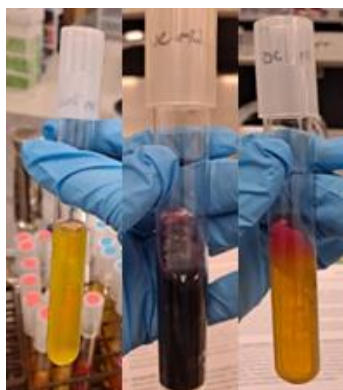
Figure 7: TSI results of various morphotypes. From left to right: MB1 shows fermentation of all three sugars (yellow medium), S2 shows sulfur production (blackening of the medium), MH2 anaerobic fermentation of glucose only (only the bottom half of the medium is yellow; HL photos).
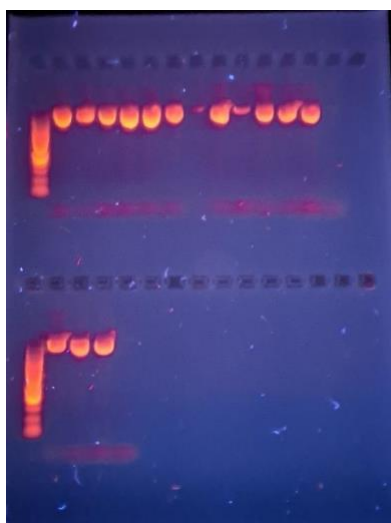

Figure 8: Agarose gel of 16S rRNA PCR amplicons showing all morphotypes contain the targeted gene. First row (L to R): 100bp DNA Ladder, MH1, EC1, Sg1, MB1, MB2, S2, EC2, BA1, MB2, MH2, EC3, positive control (*E. coli*), negative control (dH₂O). Second row (L to R): 100bp DNA ladder, EC4, MB3, positive control, negative control (HL photos).
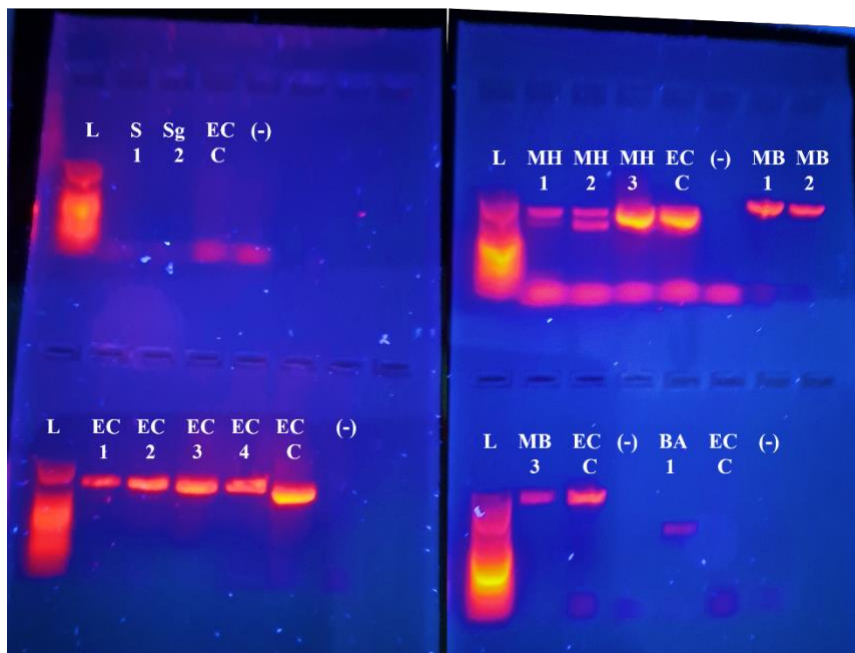
Figure 9: Genus specific PCR results. The first picture is of the *pan*B primer PCR (top row) and the ECA75F/ ECR619R primer PCR (bottom row). The second picture includes the AFB and *rpo*B (*Mannheimia*) primer PCR (top row) and *rpo*B (*Bacillus*) primer PCR (bottom row). (HL photos)
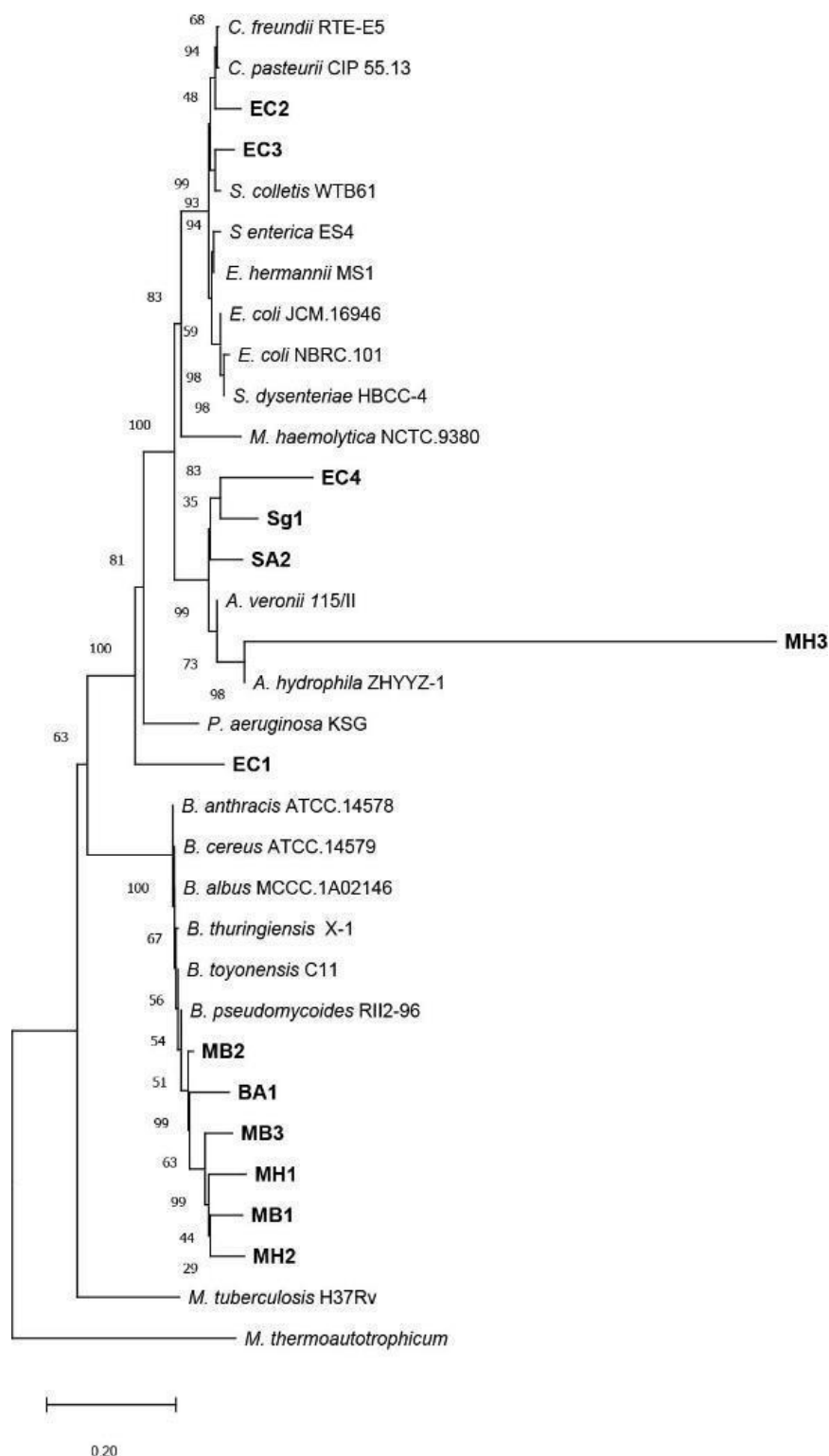
Figure 10: Neighbor-joining phylogenetic tree of 16SrRNA sequences of morphotypes and reference sequences of closely related organisms. Evolutionary distances were calculated by Jukes-Cantor method and all positions containing gaps and missing data were eliminated. None of the samples were phylogenetically related to the pathogens of interest.

Figure 11: Neighbor-joining phylogenetic tree of 16SrRNA sequences (using ECA75F/ ECR619R primers) of *Escherichia* morphotypes and reference sequences.

Figure 12: Neighbor-joining phylogenetic tree of 16SrRNA sequences (using AFB primers) of *Mycobacterium* morphotypes and reference sequences.

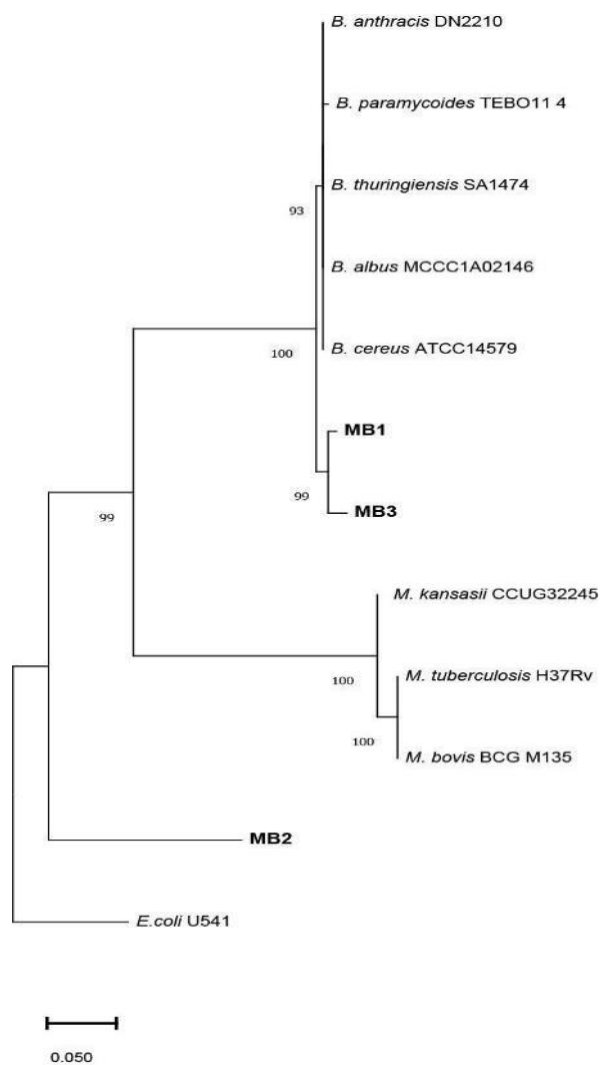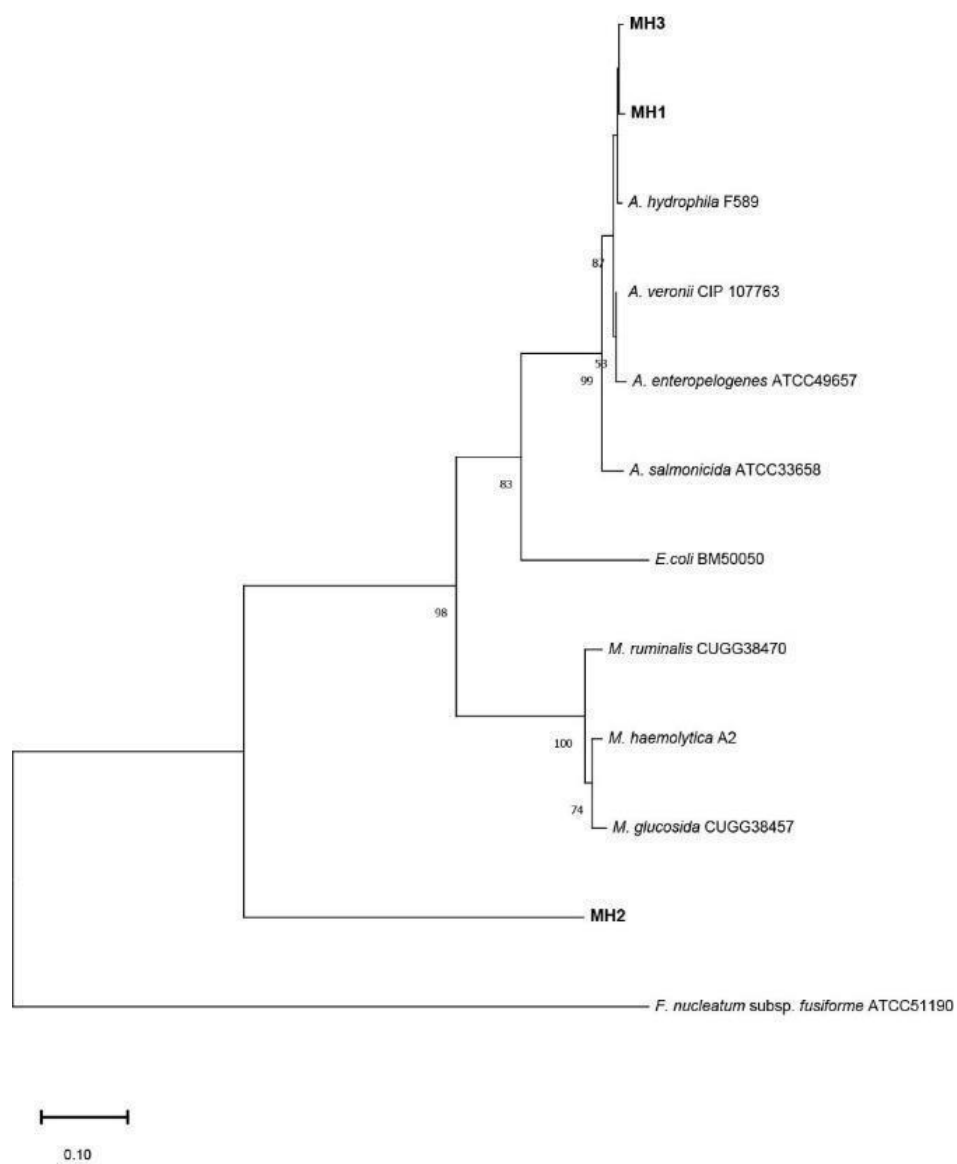Figure 13: Neighbor-joining phylogenetic tree of *rpo*B sequences of *Mannheimia* morphotypes and reference sequences.
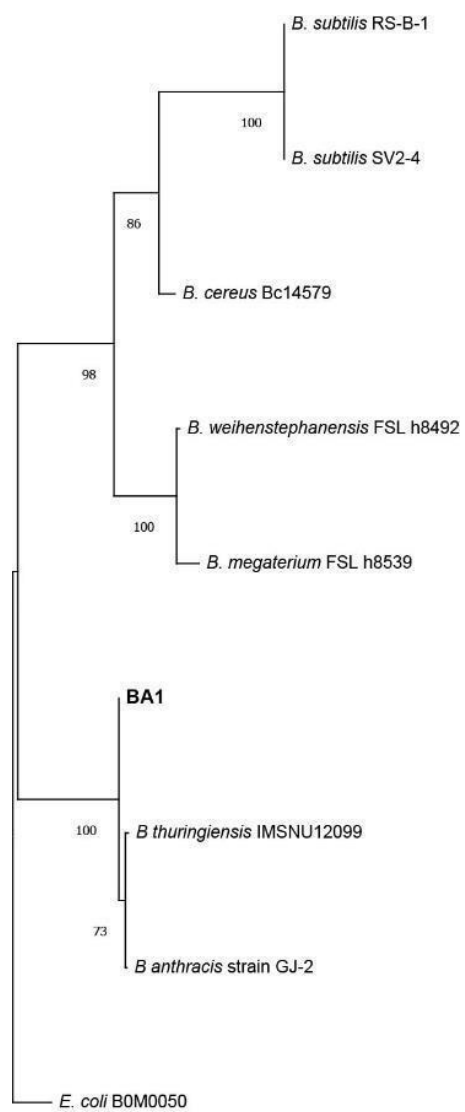
Figure 14: Neighbor-joining phylogenetic tree of *rpo*B sequences of *Bacillus* morphotypes and reference sequences.

Bacteria	Susceptible	Intermediate	Resistant
MH1	7	1	1
MH2	8	0	1
MH3	4	3	2
MB1	3	0	6
MB2	3	4	2
MB3	3	1	5
BA1	5	1	3
S2	2	3	4
Sg1	2	4	3
EC1	0	0	9
EC2	0	2	7
EC3	2	4	3
EC4	3	3	3

Table 4: Susceptibility of bacterial morphotypes to commercial (8) and livestock (tilmicosin) antibiotics. All MH and S morphotypes were resistant to tilmicosin. Highlighted in red font are the samples that were resistant to 5 or more of the commercial antibiotics.

Discussion/Conclusions

The results of this study indicate from the first colonial growth observed on serial dilution plates that the water bodies surrounding WU's campus have the potential for harboring pathogenic microorganisms. This is not surprising as there are a number of aquatic microbial genera that are capable of pathogenicity towards humans and animals, for example, *Aeromonas*

and *Vibrio* (Vally *et al*., 2004). While the pathogens that were targeted (*Bacillus anthracis*, *Escherichia coli*, *Mannheimia haemolytica*, *Mycobacterium bovis*, *Salmonella enterica,* and *Shigella dysenteriae*) were not successfully isolated in this study, other potentially harmful pathogens were and that is a cause for concern considering the water bodies explored are frequented by humans, livestock and wildlife. Successfully isolated organisms include *Bacillus, Citrobacter, Enterobacter, Siccibacter,* and *Aeromonas* which are all genera that can be waterborne and contain species that are etiological agents of respiratory and intestinal diseases in both human and livestock (Vally *et al*., 2004; Sanders Jr. and Sanders, 1997; Aminharati *et al*., 2019; Svobodova *et al*., 2017; Bottone, 2010). In addition, a number of these isolates were also antibiotic-resistant (Table 4) which indicates their ability to evade treatment and could create a public health emergency should there be an epidemic. These genera have similar morphological characteristics to those that were of interest, which is why they were initially presumed to be the target pathogens.

The culture-dependent procedures provided data that indicated the isolates were mostly similar to those that were expected for described type strains of the pathogens of interest, however among some morphotypes there were variations in test results which were initially attributed to environmental strains that may have adapted new characteristics to survive its surroundings (Mahoney *et al*. 2010). The biochemical test results varied for some of the samples and were questionable, particularly for the potential *S. enterica* and *E. coli* morphotypes. This could be seen in EC1, where the TSI test results exhibited no preference for glucose or lactose which is uncommon for *E. coli.* While type strains are usually described based on clinical isolates, there are substrate preference variations in environmental strains due to the availability of particular nutrients in the environment (Mahoney *et al*. 2010).

Phylogenetic analysis provided irrefutable evidence that while these isolated

morphotypes may have appeared to be morphologically similar to the targeted pathogens, they

were not identical. BLAST results and neighbor-joining trees provided definitive evidence that

the evolutionary relationships between the targeted pathogens and the isolated morphotypes were

strained at best. Morphotypes did not group with the expected pathogen reference sequences in

phylogenetic clusters in the main 16S rRNA or genus-specific trees, except for BA1, which

clustered with *B. anthracis* and *B. thuringiensis* yet not with *B. cereus*. It is important to note,

that this placement of BA1 with *B. anthracis*/*B. thuringiensis* in the *rpo*B phylogenetic tree

(Figure 14) is indicative of a more stringent genetic relationship with these species. *B. anthracis*

is taxonomically classified as a member of the *B. cereus* group and is usually quite difficult to

distinguish genetically and morphologically (Pilo and Frey 2011), therefore its position in the

*rpo*B tree is likely accurate. Regardless, based on the reference organisms that the morphotypes

are phylogenetically closely related to, these isolates are potentially harmful human and animal

pathogens. The tilmicosin-resistant MH1 and MH3 are likely species of *Aeromonas*, and the

species *A. hydrophilia* is found in warm humid climates where it is a waterborne pathogen of

warm and cold-blooded animals that causes multiple types of infections (Vally *et al*., 2004). The

multi-drug resistant MB1 and MB3 morphotypes were closely related to *Bacillus cereus*, which

is a species that is associated with food poisoning in humans (Bottone, 2010). The evolutionary

ancestry of the MB2 isolate could not be discerned with the primer sets that were used as in

neither the 16S rRNA (Figure 10) or AFB (Figure 12) trees were there any clear similarities with

any of the reference sequences, therefore its identity cannot be confirmed and requires further

study. The least harmful to animals and humans is the EC3 isolate which is closely related to the

genus *Siccibacter* as seen in both 16S rRNA trees (Figures 10 and 11) and species in this genus include plant pathogens that generally inhabit freshwater bodies (Svobodová *et al.*, 2017).

In conclusion, the hypothesis that waterborne livestock pathogens *Bacillus anthracis*, *Escherichia coli*, *Mannheimia haemolytica*, *Mycobaterium bovis*, *Salmonella enterica,* and *Shigella dysenteriae* were present in water bodies of Wingate University campus is rejected. While these organisms were not successfully isolated from the recreational water samples of this study, other animal pathogens, some multi-drug resistant, that may cause respiratory and intestinal diseases in the humans and animals of the community are present. Future studies of these water bodies should consider taking multiple water samples, collected over time including when livestock are active and grazing, and utilizing more extensive molecular techniques for accurate detection of pathogens such as DNA microarrays and nucleic acid targeting. For instance, a DNA-based system optimized for bacterial pathogens could be created based on the SureSelect target enrichment system which was developed to detect viruses that infect livestock (Oba *et al.*, 2018). SureSelect targets nucleic acids in samples based on sequence similarity with the nucleic acids of pathogens of interest. Considering phylogenetic analysis provided our most credible results, this would provide more accurate initial results and could provide quicker results and pinpoint which water samples to investigate for the presence of harmful microorganisms.

REFERENCES

Andino A, Hanning I. 2015. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. *ScientificWorldJournal.*, 2015: 520179. [doi.org/10.1155/2015/520179](doi.org/10.1155/2015/520179).

Aminharati, F, Ehrampoush, MH, Soltan Dallal, MM, Yaseri, M, Dehghani Tafti, AA, Rajabi, Z. 2019. *Citrobacter freundii* Foodborne Disease Outbreaks Related to Environmental Conditions in Yazd Province, Iran. *Iran J Public Health 48*(6), 1099–1105.

Beesley CA, Vanner CL, Helsel LO, Gee JE, Hoffmaster AR. 2010. Identification and characterization of clinical *Bacillus* spp. isolates phenotypically similar to *Bacillus anthracis*. *FEMS Microbiol Lett. 313*(1): 47–53. doi:10.1111/j.1574-6968.2010.02120.x.

Bottone EJ. 2010. *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev*, *23*(2), 382–398. https://doi.org/10.1128/CMR.00073-09

Christensen H, Kuhnert P, Olsen JE, Bisgaard M. 2004. Comparative phylogenies of the housekeeping genes atpD, infB and rpoB and the 16S rRNA gene within the Pasteurellaceae. *Int J Syst Evol Microbiol. 54*(Pt 5): 1601-1609. doi: 10.1099/ijs.0.03018-0. PMID: 15388716.

de Azevedo Issa M, Martins Soares Filho P, Fonseca Júnior AA, Arrais Hodon M, Cristian Dos Santos L, Karlisson Pimenta Dos Reis J, Cerqueira Leite R. 2017. Comparative study of *Mycobacterium bovis* primary isolation methods. *Braz J Microbiol, 48*(1), 139–144. doi.org/10.1016/j.bjm.2016.07.026

Fewtrell L, Kay D. 2015. Recreational Water and Infection: A Review of Recent Findings. *Curr Envir Health Rpt* 2**,** 85–94. [doi.org/10.1007/s40572-014-0036-6](doi.org/10.1007/s40572-014-0036-6)

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser 41*:95-98.

Jukes TH, Cantor CR. 1969. Evolution of protein molecules in mammalian protein metabolism. *Academic Press, New York,* pp. 21-132.

Kolton CB, Podnecky NL, Shadomy SV, Gee JE, Hoffmaster AR. 2017. *Bacillus anthracis* gamma phage lysis among soil bacteria: an update on test specificity. *BMC Res Notes*. *10*(1). doi:10.1186/s13104-017-2919-8.

Kumar J, Dixit SK, Kumar R. 2015. Rapid detection of *Mannheimia haemolytica* in lung tissues of sheep and from bacterial culture. *Vet World*. *8*(9):1073–1077. doi:10.14202/vetworld.2015.1073-1077.

Lee HS, Park KU, Park JO, Chang HE, Song J, Choe G. 2011. Rapid, sensitive, and specific detection of *Mycobacterium tuberculosis* complex by real-time PCR on paraffin-embedded human tissues. *J Mol Diagn*, *13*(4), 390–394. doi.org/10.1016/j.jmoldx.2011.02.004

Lehman D. 2005. Triple Sugar Iron Agar Protocols. *American Society for Microbiology*. [accessed May 31, 2022] Available from https://asm.org/ASM/media/Protocol-Images/Triple-Sugar-Iron-Agar-Protocols.pdf?ext=.pdf

MacWilliams MP. 2009. Indole Test Protocol. *American Society for Microbiology*. [accessed
May 31, 2022] Available from https://asm.org/getattachment/200d3f34-c75e-4072-a7e6-
df912c792f62/indole-test-protocol-3202.pdf

Mahoney JC, Gerding MJ, Jones SH, Whistler CA. 2010. Comparison of the pathogenic
potentials of environmental and clinical *Vibrio parahaemolyticus* strains indicates a role
for temperature regulation in virulence. *Appl Environ Microbiol*, *76*(22), 7459–7465.
doi.org/10.1128/AEM.01450-10

Mawdsley JL, Bardgett RD, Merry RJ, Pain BF, Theodorou MK. 1995. Pathogens in livestock
waste, their potential for movement through soil and environmental
pollution. *Appl Soil Ecol*, *2*(1), 1–15. doi.org/10.1016/0929-1393(94)00039-A

McAllister TA, Topp E. 2012. Role of livestock in microbiological contamination of water:
Commonly the blame, but not always the source, *Front Anim Sci*, *2*(2), 17–
27. doi.org/10.2527/af.2012-0039

McClary DG, Loneragan GH, Shryock TR, Carter BL, Guthrie CA, Corbin MJ, Mechor GD.
2011. Relationship of in vitro minimum inhibitory concentrations of tilmicosin against
Mannheimia haemolytica and *Pasteurella multocida* and in vivo tilmicosin treatment
outcome among calves with signs of bovine respiratory disease. *J Am Vet Med Assoc,
239*(1): 129-135. doi.org/10.2460/javma.239.1.129

NCBI. 2022. Basic Local Alignment Search Tool. National Center for Biotechnology and
Information (NCBI), National Institutes of Health, Rockville, Maryland [accessed April
18th, 2022]. Available from https://blast.ncbi.nlm.nih.gov/Blast.cgi

NCBI. 2022a. GenBank. National Center for Biotechnology and Information, National Institutes of Health, Rockville, Maryland [accessed April 18th, 2022]. Available from https://www.ncbi.nlm.nih.gov/genbank/.

Oba M, Tsuchiaka S, Omatsu T, Katayama Y, Otomaru K, Hirata T, Aoki H, Murata Y, Makino S, Nagai M, Mizutani T. 2018. A new comprehensive method for detection of livestock-related pathogenic viruses using a target enrichment system. *Biochem Biophys Res Commun*, *495*(2), 1871–1877. doi.org/10.1016/j.bbrc.2017.12.017

Pilo P, Frey J. 2011. *Bacillus anthracis*: molecular taxonomy, population genetics, phylogeny and patho-evolution. *Infect Genet Evol*. *11*(6):1218-24. doi: 10.1016/j.meegid.2011.05.013.

Reiner, K. 2012. Carbohydrate fermentation protocol. *American Society for Microbiology*. [accessed May 31, 2022] Available from https://asm.org/ASM/media/Protocol-Images/Carbohydrate-Fermentation-Protocol.pdf?ext=.pdf

Sabat G, Rose P, Hickey WJ, Harkin JM. 2000. Selective and sensitive method for PCR amplification of Escherichia coli 16S rRNA genes in soil. *Appl Environ Microbiol*. *66*(2): 844-9. doi: 10.1128/AEM.66.2.844-849.2000.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406-425

Sanders WE, Jr, Sanders CC. 1997. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. *Clin Microbiol Rev*, *10*(2), 220–241. doi.org/10.1128/CMR.10.2.220

Sukhnanand S, Alcaine S, Warnick LD, Su WL, Hof J, Craver MP, McDonough P, Boor KJ, Wiedmann M. 2005. DNA sequence-based subtyping and evolutionary analysis of selected *Salmonella enterica* serotypes. *J Clin Microbiol. 43*(8):3688-98. doi: 10.1128/JCM.43.8.3688-3698.2005.

Svobodová B, Vlach J, Junková P, Karamonová L, Blažková M, Fukal L. 2017. Novel method for reliable identification of *Siccibacter* and *Franconibacter* strains: from "Pseudo-*Cronobacter*" to new Enterobacteriaceae genera. *Appl Environ Microbiol, 83*(13), e00234-17. doi.org/10.1128/AEM.00234-17

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol 38* (7): 3022-3027

Tomley FM, Shirley MW. 2009. Livestock infectious diseases and zoonoses. *Philos Trans R Soc Lond B Biol Sci., 364*(1530), 2637–2642. doi.org/10.1098/rstb.2009.0133

Topozone. 2022. Watson TOPO Map in Union County, NC. Locality LLC. [accessed June 4th, 2022] Available from https://www.topozone.com/north-carolina/union-nc/city/watson-21/

Vally H, Whittle A, Cameron S, Dowse GK, Watson T. 2004. Outbreak of *Aeromonas hydrophila* wound infections associated with mud football. *Clin Infect Dis. 38* (8): 1084–1089. doi.org/10.1086/382876

Wingender J, Flemming HC. 2016. Biofilms in drinking water and their role as reservoir for pathogens, *Int J Hyg Environ Health, 214* (6), 417-423. doi.org/10.1016/j.ijheh.2011.05.009.

WorldClimate. 2022. North Carolina average weather, climate, and temperature information. [accessed May 31, 2022] Available from http://www.worldclimate.com/climate/us/north-carolina

Zhu Z, Wang W, Cao M, Zhu Q, Ma T, Zhang Y, Liu G, Zhou X, Li B, Shi Y, Zhang J. 2021. Virulence factors and molecular characteristics of *Shigella flexneri* isolated from calves with diarrhea. *BMC Microbiol. 21*(1). doi:10.1186/s12866-021-02277-0.

Anatomical and Physiological Sex Differences:

How They Impact Exercise Response and Injury Rates

Isabella Lluhi

Faculty Mentor: Dr. Acchia Albury, Associate Professor of Biology Department

ABSTRACT

In humans, anatomical and physiological sex differences exist in the musculoskeletal and endocrine systems. During puberty and afterward, women experience hormone fluctuations during their menstrual cycle. These concentrations and changes of these hormones significantly impact the development of females and their exercise performance, injury prevalence, recovery following injury, and disease risk following menopause. Throughout development, estrogen affects the rate of bone growth and the shape and size of the female pelves, contributing to the larger and stronger bones and smaller Q angles demonstrated by males.

Anatomically, the sexes differ in body composition, lean muscle mass, bone strength and density, muscle fiber type, heart size, blood volume amount, lung geometry, and airway size. Due to these anatomical, physiological, and hormonal differences, women exhibit a greater oxidative capacity, greater use of fat as an energy substrate, and greater fatigue resistance. These differences contribute to differing responses to exercise and varied prevalences of injuries and diseases. Specifically, women are at an increased risk for bone stress injuries, ACL injuries, and concussions. Post-menopause, women are also increasingly susceptible to CVD, osteoporosis, and osteoarthritis. The varying concentrations of the two main sex hormones are a major contributing factor for many anatomical and physiological differences present between the sexes. As a result, the sex hormones indirectly contribute to differences seen in exercise response and injury prevalence. This research shows the importance of identifying these sex differences and understanding how they can impact an individual's health throughout their lifetime.

INTRODUCTION

In most humans, anatomical and physiological sex differences exist in the musculoskeletal and endocrine systems. Anatomically, females and males differ in body fat percentages and distribution, skeletal muscle size, bone strength, and density, and muscle fiber

types. Naturally, men demonstrate larger amounts of skeletal muscle, larger and stronger bones, and smaller quadriceps (Q) angles. In addition to having less skeletal muscle mass, females also have slower and smaller muscle fibers which contribute to their lower contractile force and increased resistance to fatigue.

Hormonally, significant differences between the sexes occur during maturation and are seen following this transition. Specifically, the greater concentration of estrogen in females during puberty pushes their bones to fuse sooner than their male counterparts. Women also experience hormone fluctuations during their menstrual cycle and begin to lose estrogen at menopause. These estrogen fluctuations throughout a woman's life can contribute to decreased tendon and ligament strength during different phases of their cycle and a loss of bone tissue post-menopause. This paper will examine the anatomical and physiological differences between the sexes due to the varying concentrations of the sex hormones and how these differences contribute to exercise response and the prevalence of injuries.

SEX HORMONES AND PUBERTY

Most sex differences observed between males and females develop due to the secretion of sex hormones and through genes located on the X and Y chromosomes (Marrocco & McEwen, 2016). Throughout a child's life, growth, development, and maturation occur until they reach adulthood (Kenney et al., 2020). During maturation, the adult form is obtained, which allows the individual to become fully functional. At this time, the child enters adolescence, which begins with the onset of puberty and ends once growth and developmental processes have been completed. On average, adolescence ranges from 8-19 years for females and 10-22 years for males. At puberty, secondary sexual characteristics begin to develop, allowing for the potential for sexual reproduction (Kenney et al., 2020).

Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus and helps to regulate hormone concentrations in both sexes by increasing the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Production of LH and FSH, produced by the pituitary gland, increases at puberty. As the production of LH and FSH increases, the sex hormones are stimulated to be released. The two main sex hormones, androgens and estrogens, are responsible for regulating development, puberty, and reproduction (Tortora & Derrickson, 2017). These sex hormones also impact bone growth and health, body weight and composition, mood and brain function, metabolism, and cardiovascular health.

Estrogens are predominantly produced by the ovaries and androgens by the adrenal gland and testes. However, the androgens produced by the adrenal glands are able to convert to estrogens. Estrogens are present in three primary forms, which include estradiol, estrone, and estriol. Females contain much higher levels of estrogens and low levels of androgens. In contrast, males have high levels of androgens, particularly testosterone produced by the testes, and low levels of estrogens. During puberty, testosterone helps bring about the developmental changes experienced by males in their sex organs and secondary sexual characteristics (Tortora & Derrickson, 2017).

At the beginning of puberty, most women begin their reproductive cycle, which occurs once a month from their first menstruation to menopause, with the average cycle ranging from 24-36 days (Tortora & Derrickson, 2017). This reproductive cycle is controlled by GnRH and consists of four main phases: menstruation, the preovulatory or follicular phase, ovulation, and the postovulatory or luteal phase. Menstruation lasts for the first five days of the cycle, typically. At this time, blood, tissue fluid, mucus, and epithelial cells are shed from the uterus due to declining levels of progesterone and estrogens.

After menstruation and before ovulation is the preovulatory phase which typically lasts for six to thirteen days. A dominant follicle within the ovary begins to enlarge and mature to become ready for ovulation during this phase. Here, estrogen concentration is at its highest concentration during the cycle. Ovulation usually starts around day 14 of a cycle with the high concentration of estrogens stimulating the release of the hormones GnRH, LH, and ultimately FSH. In response, the now mature follicle ruptures to release the secondary oocyte or female gamete. This egg then travels through the fallopian tubes towards the uterus, where it will either encounter a sperm cell or die.

Following ovulation, the postovulatory phase begins and lasts from days 15-28 of the cycle. Throughout this phase, the ruptured follicle within the ovary starts to transform into the corpus luteum. The corpus luteum, once formed, begins to release progesterone and small amounts of estrogen. At this time, these hormones are helping to maintain the thickened lining of the uterus while waiting for the implantation of a fertilized egg. If a fertilized egg does not come, the corpus luteum dies after two weeks, and as a result, progesterone and estrogens decrease. Between the ages of 45 and 55, menopause occurs, which is known as the permanent cessation of menstruation (Tortora & Derrickson, 2017). At this point, there are few remaining ovarian follicles. As a result, the ovaries become less responsive to hormonal stimulation, and the production of estrogens declines, contributing to increased sympathetic activity.

SEX DIFFERENCES WITHIN THE BODY SYSTEMS

Musculoskeletal System

Sex differences in the musculoskeletal system exist regarding overall body composition, the density, size, and shape of bones, muscle size, muscle range of motion (ROM), and muscle

fiber type composition. The amount of fat and its distribution varies between males and females, with subcutaneous fat being roughly 8% thicker in women than in men. There is typically additional fat found on women at the hips, thighs, breasts, and back of arms. This distribution is also known as a pear-shaped body fat distribution (Karastergiou et al., 2012). In contrast, men are more prone to gaining visceral fat, which is located within the abdominal cavity. Women also present higher body mass indexes and roughly 10% more body fat when compared to men (Karastergiou et al., 2012).

Sex differences in the skeletal system exist primarily due to estrogen's role in human bone growth (Dunsworth, 2020). This effect is seen in the differing statures and pelvic shapes. On average, adult males reach a final height of 70 inches and females, 64 inches. Estrogen is responsible for accelerating the "loss of progenitor cells in the resting zone of the long bone" epiphyseal plate (Dunsworth, 2020). Therefore, due to the presence of estrogen, the epiphyseal plate will be pushed to closure much faster than if estrogen was not present. The closure of the epiphyseal plate signifies that growth in length has stopped. As males go through puberty, they experience high levels of testosterone and low levels of estrogen. In contrast, females encounter higher estrogen levels during puberty.

Low doses of estrogen enhance bone growth by increasing the production of growth hormone and insulin-like growth factor 1 (IGF-1), whereas, at higher concentrations, estrogen inhibits IGF-1 (Dunsworth, 2020). As a result of low estrogen levels, males' epiphyseal plate stays open longer, and growth is enhanced, which contributes to their greater stature. While men continue to grow, females stop growing earlier as their epiphyseal plates close due to the increased estrogen levels. Since women's menstrual cycle is dependent on estrogen, as their growth slows to a stop, their monthly cycle begins. In addition to greater stature, men also have

larger, denser, and stronger bones than women, which is partly explained by estrogen's role in bone growth (Baker et al., 2020).

Women's pelves fuse earlier but expand more than those of males (Dunsworth, 2020). Female pelves fuse earlier than males due to the role of estrogen in bone development previously mentioned. As women go through puberty, between the ages of 8 and 18, their pelves will expand slightly to reach these larger dimensions. The larger size is thought to be caused by the space taken up by the reproductive organs and the presence of estrogen and relaxin in the tissues of the pelvic region.

Specifically, the pelvic floor muscles and other surrounding muscles and ligaments contain estrogen receptors (ER), which are absent in typical skeletal muscles. Due to the presence of these receptors, it is hypothesized that these muscles are under special hormonal control, which allows them to influence the bones to which they are adjacent or anchored (Dunsworth, 2020). In addition to this, the volume of the internal female reproductive organs may also contribute to the expanded female pelvis. These organs include the vagina, uterus, fallopian tubes, cervix, and ovaries. In contrast, the male reproductive system is located primarily outside the body, with the main internal structure being the prostate. Due to the different dimensions of pelves between sexes, females exhibit greater Q angles than males. The Q angle is the angle between the quadriceps muscle and the patellar tendon. The larger Q angles exhibited by women contribute to greater knee valgus, increasing their risk for injuries mentioned later in this paper.

Several muscular differences exist between the sexes, including size, fiber type composition, and range of motion. On average, males contain more lean muscle tissue and faster and larger muscle fibers. Several studies reported larger muscle fiber cross-sectional areas in

males regardless of the muscle fiber type (Haizlip et al., 2015; Horwath et al., 2021; Jeon et al., 2019; Trevino et al., 2019). This is likely due to the relatively greater skeletal muscle mass of males. The maximal isometric force has also been reported to be significantly lower in female individuals than males, with a -23% difference in type I fibers and -20% in type II (Jeon et al., 2019). When examining the vastus lateralis muscle specifically, men contained greater amounts of contractile tissue than women, which contributed to greater motor unit action potentials (Trevino et al., 2019).

On average, men also contain a greater proportion of type II fibers than women (Horwath et al., 2021). The type I and type IIA muscle fibers in women also tend to be slower than those in males, contributing to lower contractile velocity and a higher oxidative capacity. However, the higher oxidative capacity in these fibers allows for increased endurance, fatigue resistance, and recovery (Haizlip et al., 2015). These variations could be caused by estrogen, which has been shown to impact fiber size, contractility, muscle weight, and regeneration and minimally affect fiber-type distribution.

Satellite cells are muscle stem cells that are located between the sarcolemma and basement membrane of muscle fibers. These cells are involved in muscle tissue growth, repair, and regeneration following injury or disease (Horwath et al., 2021). In type II fibers, women contained fewer satellite cells compared to men. The lower concentrations of these cells in type II fibers specifically could influence how women respond to and recover from certain types of exercise.

Women exhibit greater ROM than men, with ROM in women being related to tolerance to muscle stretch rather than passive muscle stiffness as seen in men (Miyamoto et al., 2018). In particular, women demonstrated greater ROM in ankle dorsiflexion, which may predispose them

to muscular injuries at the ankle and calf. When examined, it appears that muscle stiffness, muscle stretch, and muscle slack angle are not influenced by the fluctuations of hormones throughout the menstrual cycle. However, the hormones estrogen and relaxin have been reported to decrease tendon and ligament strength (Hewett et al., 2006). Due to this effect, women experience increased passive joint laxity depending on their hormone cycle.

Respiratory, & Cardiovascular Systems

Regarding the cardiovascular system, females, on average, have smaller hearts and a smaller blood volume due to their smaller body size. As a result, women exhibit higher resting and exercise heart rates, and lower stroke volumes but similar cardiac output values when compared to males (Kenney et al., 2020).

Similarly, women have smaller lungs, smaller airways, and different lung geometry than males in the respiratory system, even when matched for height (Ansdell et al., 2020). Women also have smaller cross-sectional areas of the trachea and longer, narrower thoraxes than males, particularly at the bases (Dominelli et al., 2019). Regarding lung geometry, women often have a "prismatic" shape that consists of smaller bases and apices of the lungs. In contrast, men have a "pyramidal" shape which includes larger lung bases than the apices. However, the diaphragm in females appears to be more resistant to fatigue than in males (Ansdell et al., 2020).

Due to these structural differences, women breathe more frequently and experience reduced ventilatory capacity, mechanical ventilatory constraints, greater ventilatory work, and therefore less efficient ventilation (Sheel et al., 2016). The greater mechanical work and low efficiency are demonstrated by higher oxygen uptake of the respiratory muscles (VO_2RM) during exercise (Dominelli et al., 2019). In addition to this, women also show lower VO_2 max values

than their male counterparts. At maximal exercise intensities, women dedicated ~14% of whole-body VO_2, whereas men only devoted ~9% (Ansdell et al., 2020). Research has indicated that the more significant work of breathing in women results from differences in physical size, breathing patterns, and lung function (Sheel et al., 2016).

SEX DIFFERENCES DURING AND IN RESPONSE TO EXERCISE

Males and females also vary in response to and during exercise, particularly in their muscle metabolism, fatigue resistance, and muscle hypertrophy due to anatomical, physiological, and hormonal differences. Due to the fluctuating sex hormone concentrations throughout the menstrual cycle, women experience performance changes during different phases, particularly their energy substrate metabolism, muscular strength, thermoregulation, and aerobic performance.

Regarding metabolism, males and females differ in their oxidative capacity and energy substrate metabolism. In general, research has demonstrated that women utilize more fat and fewer carbohydrates for energy than men at the same exercise intensity (Hunter, 2014). Both estrogen and progesterone are reported to play a role in substrate metabolism, with estrogen being primarily responsible for greater glycogen storage and promoting insulin sensitivity. In contrast, progesterone may promote insulin resistance (Oosthuyse & Bosch, 2010). Estrogen has also been shown to increase lipolysis, intramuscular fat stores, and cellular free fatty acid oxidation, contributing to increased fat utilization during exercise. Specifically, when estrogen concentrations are highest during the follicular phase, muscle glycogen stores are spared, and fat oxidation increases, contributing to a lower respiratory exchange ratio (RER) (Oosthuyse & Bosch, 2010).

Estrogen is also thought to affect muscular strength, power, stiffness, and tendon stiffness. This influence is demonstrated by increased strength and power during the late follicular and ovulatory phase and performance reductions in the luteal phase when progesterone levels are elevated (Carmichael et al., 2021). Reductions in muscle and tendon stiffness were noted in phases with high estrogen concentrations. It is thought that estrogen reduces this stiffness by decreasing collagen synthesis, which contributes to a reduction in collagen density in muscles and connective tissues (Carmichael et al., 2021).

Progesterone is thought to contribute to the increased pre-and post-exercise internal body temperatures during the luteal phase compared to the follicular phase. During this phase, decreased blood flow to the skin and a "delayed sweating threshold during exercise" are also noted (Giersch et al., 2020). Estrogen could also be partly responsible for this increase in temperature during this phase as it has vasodilatory effects and therefore may impact blood flow to the skin. As a result of these impacts, research suggests that women may experience more significant strength gains and muscle growth during the follicular phase and may require more extended recovery periods during the luteal phase (Roberts et al., 2020).

When examining varying hormone concentrations between males and females, females have lower levels of "testosterone, free-testosterone, and insulin-like growth factor-binding protein 1" (Roberts et al., 2020). Testosterone is thought to aid in skeletal muscle hypertrophy by promoting the replication and activation of satellite cells (Sinha-Hikim et al., 2006). This effect is demonstrated following resistance training programs where males exhibited greater hypertrophy and strength gains than females.

Regarding their oxidative capacity, men demonstrate greater glycolytic capacity, whereas females tend to have greater muscle oxidative capacity (Ansdell et al., 2020). This is shown by

the greater mitochondrial respiratory rates and greater proportional area of type I muscle fibers, indicating that the skeletal muscles in females are more suited to creating ATP via oxidative phosphorylation during work. Women also have slower Ca^{2+}ATPase activity than men, contributing to greater fatigue resistance during exercise (Hunter, 2014).

Along with a greater oxidative capacity and diminished Ca^{2+}ATPase activity, reduced muscular fatigue in women can also be attributed to their slower contraction rates and vasodilatory responses during exercise. Women's muscles are metabolically slower due to Ca^{2+} ATPase activity and slower contractile properties, contributing to more fatigue-resistant muscle fibers (Hunter, 2014). Women also demonstrate more significant vasodilatory responses to their skeletal muscles during work than males. This enhanced response helps to increase blood flow to the muscles, decrease the accumulation of metabolites, and is thought to offset muscle fatigue. In contrast, males' blood flow is often more restricted because they are often stronger and "exert more intramuscular pressure onto the feed arteries" (Hunter, 2014).

SEX DIFFERENCES IN THE PREVALENCE OF INJURIES AND DISEASE

As a result of anatomical, neuromuscular, and hormonal differences, the sexes vary in their prevalence of various injuries. In particular, women are at an increased risk for bone stress injuries, anterior cruciate ligament (ACL) injuries, and concussions. In contrast, males are at an increased risk of upper extremity, hip/groin, thigh, Achilles tendon, and foot injuries. Following menopause, women are also increasingly vulnerable to diseases like cardiovascular disease (CVD), osteoporosis, arthritis, and metabolic disease, to name a few (Lobo et al., 2014).

Multiple studies have determined that females appear to be at higher risk for bone stress injuries (Lin et al., 2018). This type of injury is a common running injury related to overuse and

is caused by "cumulative microtrauma to the bone" (Hollander et al., 2021). One possible explanation for the increased prevalence of these injuries in females is the female athlete triad. The female athlete triad usually occurs due to disordered eating and is characterized by low energy availability, menstrual disturbance, and low bone mineral density. As a result of this, the bones of female athletes may be more susceptible to these injuries (Hollander et al., 2021).

Women are also at an increased risk for ACL injuries due to several anatomical and neuromuscular differences. Anatomical factors include increased Q angle, decreased femoral notch width, general joint laxity, and increased flexibility. Although females' Q angle or pelvis width could increase injury rates, it does not appear to affect an individual's risk of injuring their ACL. However, the smaller femoral notch has been shown to predispose females to ACL injuries due to it contributing to a smaller and weaker ACL (Hewett et al., 2006). General joint laxity has also been shown to increase an individual's risk of ACL injury significantly. Due to joint laxity affecting both hyperextension and valgus knee motion, it can increase the strain put on the ACL. Women also tend to be more flexible than their male counterparts, particularly in their hamstrings. The increased hamstring flexibility is another factor that could potentially be responsible for the "decreased dynamic control of the knee" (Hewett et al., 2006).

The four main neuromuscular factors that predispose female athletes to ACL injuries are ligament dominance, quadriceps dominance, leg dominance, and core instability. Ligament dominance has been demonstrated by the "inability to control lower extremity frontal plane motion during landing and cutting" (Myer et al., 2011). This imbalance is exhibited by higher knee valgus angles caused by increased hip adduction and internal rotation (Bien, 2011).

Quadricep dominance has also been found to be a significant contributor to ACL injuries in females. Females have been found to have "increased activation of their quadriceps relative to

91

their hamstrings" and slower hamstring activation (Hewett et al., 2006). This imbalance can contribute to the inability to control dynamic valgus and anterior tibial translation and rotation (Hewett et al., 2006). Both the quadriceps and hamstrings are vital for stabilizing the knee. Under normal conditions, the quadriceps and hamstring co-contract and compress the joint to protect the ligaments and decrease the valgus and varus laxity of the knee. With a reduced ability to balance muscular recruitment between these muscles, the risk of ACL injury is significantly increased. Leg dominance refers to the differences in strength, flexibility, coordination, and control between limbs where the dominant side typically accepts larger forces (Bien, 2011). It has been found, however, that the asymmetry can vary depending on the sports movement.

Core instability is demonstrated in female athletes as the weakness of core stabilizers. This instability is responsible for increased trunk motion and lateral trunk flexion, which have been shown to possibly increase knee valgus loading (Bien, 2011). In other words, there is an imbalance between the "inertial demands of the trunk and control and coordination to resist it" (Myer et al., 2011) which results in excessive trunk motion. These four neuromuscular deficits could be "indicative of decreased dynamic knee joint control" (Myer et al., 2011), which may underlie significant ACL injury risk.

In addition to bone stress and ACL injuries, females are more susceptible to concussions and have more prolonged symptoms following a concussion (McGroarty et al., 2020). Women may be more predisposed to concussions due to biomechanical and hormonal differences, including shorter and more narrow necks, less head mass, and, therefore, less neck strength (Lin et al., 2018; McGroarty et al., 2020). In addition to this, research also suggests that women have a lower tolerance or lower biomechanical threshold for concussions. Hormonally, some research

has indicated that the menstrual cycle phase and estrogen and progesterone concentrations at the time of injury may predict concussion recovery outcomes among women.

The cessation of menstruation, also referred to as menopause, occurs around the ages of 40 and 50 in women and reduces estrogen production, contributing to increased sympathetic activity. As a result of menopause, women become increasingly vulnerable to diseases that affect hormone-responsive tissues such as the brain, the cardiovascular system, and bones (Lobo et al., 2014). The risk of CVD increases significantly in women following menopause due to the altered distribution of fat and the protective effect of estrogen. Although menopause does not primarily influence weight gain, women begin accumulating more abdominal fat, which aids in the development of insulin resistance, the incidence of diabetes, and an increase in low-density lipoprotein cholesterol (Lobo et al., 2014). Due to these factors, the increase in abdominal fat increases women's risk for CVD following menopause.

Blood pressure regulation also changes due to the increased sympathetic activity associated with estrogen loss from menopause. Until reaching their forties and fifties, women experience slow rises in blood pressure and have a higher prevalence of hypotensive disorders than males (Joyner et al., 2016). After this, their blood pressure begins rising much quicker, and by the time they are seventy years old, more women are hypertensive than men. With continued aging, the rates of hypertension continue to increase in women. This rapid increase in hypertension rates is partly caused by the loss of estrogen during menopause. Without estrogen, sympathetic activity increases, which increases vasoconstriction sensitivity and responsiveness, therefore increasing blood pressure (Joyner et al., 2016).

The loss of estrogen following menopause also significantly impacts women's bones and connective tissue, contributing to the increased incidence of osteoporosis and osteoarthritis. Bone

93

remodeling is a natural process that occurs throughout our lives and involves bone resorption and formation. Bone resorption involves removing old bone, whereas bone formation replaces this removed tissue with new bone (Lobo et al., 2014). As previously mentioned, estrogen plays a role in developing the skeletal system and also contributes to bone remodeling processes. Due to the decreased estrogen production following menopause, additional bone resorption occurs, resulting in further bone loss. This bone loss dramatically increases the risk of osteoporotic fracture in women as the bones become more brittle and fragile. Along with this, there is also a loss of dermal connective tissue, more specifically in the "medial arterial layers and intervertebral disc as well as articular cartilage" (Lobo et al., 2014).

Men are also at an increased risk for several injuries, including upper extremity, hip/groin, thigh, Achilles tendon, and foot injuries. Although males have a higher incidence of total injuries, research has indicated that the type of sport dramatically influences the rate of these injuries (Zech et al., 2021). For example, males have demonstrated higher overall injury rates in sports like soccer and handball, whereas females have a higher incidence of injuries in basketball. For male runners specifically, there is an increased incidence of Achilles tendinopathies. Several intrinsic and extrinsic factors could explain this increased prevalence. For starters, the Achilles tendon is prone to overuse injuries. Males also tend to have a higher lifetime cumulated load, increasing their risk of overuse injuries in running. The higher training and match load and more contact situations in male sports are also thought to be contributing factors to the higher prevalence of overall injuries in sports (Hägglund et al., 2009). Hormonally, estrogen influences collagen synthesis, which could aid in the Achilles tendon's healing capacity, which may decrease the risk of these injuries in females (Hollander et al., 2021).

CONCLUSION

Many anatomical and physiological differences exist between the sexes regarding the endocrine and musculoskeletal systems. During puberty and afterward, women experience hormone fluctuations during their menstrual cycle. These concentrations and changes of these hormones significantly impact the development of females and their exercise performance, injury prevalence, recovery following injury, and disease risk following menopause. Throughout development, estrogen affects the rate of bone growth and the shape and size of the female pelves, contributing to the larger and stronger bones and smaller Q angles demonstrated by males.

Anatomically, the sexes differ in body composition, lean muscle mass, bone strength and density, muscle fiber type, heart size, blood volume amount, lung geometry, and airway size. Due to these anatomical, physiological, and hormonal differences, women exhibit a greater oxidative capacity, greater use of fat as an energy substrate, and greater fatigue resistance. These differences contribute to differing responses to exercise and varied prevalences of injuries and diseases. Specifically, women are at an increased risk for bone stress injuries, ACL injuries, and concussions. Post-menopause, women are also increasingly susceptible to CVD, osteoporosis, and osteoarthritis. In contrast, men are at an increased risk of Achilles tendinopathies. Note that the prevalence of male injuries is greatly influenced by the type of sport the individual plays.

The varying concentrations of the two main sex hormones are a major contributing factor to many anatomical and physiological differences present between the sexes. As a result, the sex hormones indirectly contribute to differences seen in exercise response and injury prevalence. This research poses questions regarding the possibility of additional roles of estrogen and testosterone within the body systems of men, particularly regarding bone health throughout a male's lifetime. For example, seeing as the loss of estrogen post-menopause increases women's

95

risk for many diseases, why do men not also experience osteoporosis, osteoarthritis, etc., throughout their lives if estrogen concentration is always low in males? With this in consideration, could testosterone help with the regulation of bone remodeling later in life? Additionally, would transgender males to females who have undergone feminizing hormone therapy experience an altered prevalence of injuries due to the increased concentration of estrogen within their bodies? This research shows the importance of identifying these sex differences and understanding how they can impact an individual's health throughout their lifetime.

REFERENCES

Ansdell, P., Thomas, K., Hicks, K. M., Hunter, S. K., Howatson, G., & Goodall, S. (2020). Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Experimental Physiology*, *105*(12), 2007–2021. https://doi.org/10.1113/EP088548

Baker, B. S., Chen, Z., Larson, R. D., Bemben, M. G., & Bemben, D. A. (2020). Sex differences in bone density, geometry, and bone strength of competitive soccer players. *Journal of Musculoskeletal & Neuronal Interactions*, *20*(1), 62–76.

Bien, D. P. (2011). Rationale and implementation of anterior cruciate ligament injury prevention warm-up programs in female athletes. *Journal of Strength and Conditioning Research*, *25*(1), 271–285. doi.org/10.1519/jsc.0b013e3181fb4a5a

Carmichael, M. A., Thomson, R. L., Moran, L. J., & Wycherley, T. P. (2021). The Impact of Menstrual Cycle Phase on Athletes' Performance: A Narrative Review. *International Journal of Environmental Research and Public Health*, *18*(4), 1667. https://doi.org/10.3390/ijerph18041667

Dominelli, P. B., Molgat-Seon, Y., & Sheel, A. W. (2019). Sex Differences in the Pulmonary System Influence the Integrative Response to Exercise. *Exercise and Sport Sciences Reviews*, *47*(3), 142–150. https://doi.org/10.1249/JES.0000000000000188

Dunsworth, H. M. (2020). Expanding the evolutionary explanations for sex differences in the human skeleton. *Evolutionary Anthropology: Issues, News, and Reviews*, *29*(3), 108–116. https://doi.org/10.1002/evan.21834

Giersch, G. E. W., Morrissey, M. C., Katch, R. K., Colburn, A. T., Sims, S. T., Stachenfeld, N.

    S., & Casa, D. J. (2020). Menstrual cycle and thermoregulation during exercise in the

    heat: A systematic review and meta-analysis. *Journal of Science and Medicine in Sport*,

    23(12), 1134–1140. https://doi.org/10.1016/j.jsams.2020.05.014

Hägglund, M., Waldén, M., & Ekstrand, J. (2009). Injuries among male and female elite football

    players. *Scandinavian Journal of Medicine & Science in Sports*, *19*(6), 819–827.

    https://doi.org/10.1111/j.1600-0838.2008.00861.x

Haizlip, K. M., Harrison, B. C., & Leinwand, L. A. (2015). Sex-Based Differences in Skeletal

    Muscle Kinetics and Fiber-Type Composition. *Physiology*, *30*(1), 30–39.

    https://doi.org/10.1152/physiol.00024.2014

Hewett, T. E., Myer, G. D., & Ford, K. R. (2006). Anterior Cruciate Ligament Injuries in Female

    Athletes Part 1, Mechanisms and Risk Factors. *The American Journal of Sports*

    Medicine, *34*(2), 299–311. doi.org/10.1177/0363546505284183

Hollander, K., Rahlf, A. L., Wilke, J., Edler, C., Steib, S., Junge, A., & Zech, A. (2021). Sex-

    Specific Differences in Running Injuries: A Systematic Review with Meta-Analysis and

    Meta-Regression. *Sports Medicine*, *51*(5), 1011–1039. https://doi.org/10.1007/s40279-

    020-01412-7

Horwath, O., Moberg, M., Larsen, F. J., Philp, A., Apró, W., & Ekblom, B. (2021). Influence of

    sex and fiber type on the satellite cell pool in human skeletal muscle. *Scandinavian*

    Journal of Medicine & Science in Sports, *31*(2), 303–312.

    https://doi.org/10.1111/sms.13848

Hunter, S. K. (2014). Sex differences in human fatigability: mechanisms and insight to

    physiological responses. *Acta Physiologica*, *210*(4), 768–789.

    https://doi.org/10.1111/apha.12234

Jeon, Y., Choi, J., Kim, H. J., Lee, H., Lim, J.-Y., & Choi, S.-J. (2019). Sex- and fiber-type-

    related contractile properties in human single muscle fiber. *Journal of Exercise*

    Rehabilitation, *15*(4), 537–545. https://doi.org/10.12965/jer.1938336.168

Joyner, M. J., Wallin, B. G., & Charkoudian, N. (2016). Sex differences and blood pressure

    regulation in humans: Sex differences and human blood pressure. *Experimental*

    Physiology, *101*(3), 349–355. https://doi.org/10.1113/EP085146

Karastergiou, K., Smith, S. R., Greenberg, A. S., & Fried, S. K. (2012). Sex differences in

    human adipose tissues – the biology of pear shape. *Biology of Sex Differences*, *3*(1), 13.

    https://doi.org/10.1186/2042-6410-3-13

Kenney, W. L., Wilmore, J. H., & Costill, D. L. (2020). *Physiology of sport and exercise*

    (Seventh edition). Human Kinetics.

Lin, C. Y., Casey, E., Herman, D. C., Katz, N., & Tenforde, A. S. (2018). Sex Differences in

    Common Sports Injuries. *PM&R*, *10*(10), 1073–1082.

    https://doi.org/10.1016/j.pmrj.2018.03.008

Lobo, R. A., Davis, S. R., De Villiers, T. J., Gompel, A., Henderson, V. W., Hodis, H. N.,

    Lumsden, M. A., Mack, W. J., Shapiro, S., & Baber, R. J. (2014). Prevention of diseases

    after menopause. *Climacteric*, *17*(5), 540–556.

    https://doi.org/10.3109/13697137.2014.933411

Marrocco, J., & McEwen, B. (2016). Sex in the brain: hormones and sex differences. *Dialogues in Clinical Neuroscience*, *18*(4), 373–383. https://doi.org/10.31887/DCNS.2016.18.4/jmarrocco

McGroarty, N. K., Brown, S. M., & Mulcahey, M. K. (2020). Sport-Related Concussion in Female Athletes: A Systematic Review. *Orthopaedic Journal of Sports Medicine*, *8*(7), 232596712093230. https://doi.org/10.1177/2325967120932306

Miyamoto, N., Hirata, K., Miyamoto-Mikami, E., Yasuda, O., & Kanehisa, H. (2018). Associations of passive muscle stiffness, muscle stretch tolerance, and muscle slack angle with range of motion: individual and sex differences. *Scientific Reports*, *8*(1), 8274. https://doi.org/10.1038/s41598-018-26574-3

Myer, G. D., Brent, J. L., Ford, K. R., & Hewett, T. E. (2011). Real-time assessment and neuromuscular training feedback techniques to prevent anterior cruciate ligament injury in female athletes. *Strength & Conditioning Journal*, *33*(3), 21–35. doi.org/10.1519/ssc.0b013e318213afa8

Oosthuyse, T., & Bosch, A. N. (2010). The Effect of the Menstrual Cycle on Exercise Metabolism: Implications for Exercise Performance in Eumenorrhoeic Women. *Sports Medicine*, *40*(3), 207–227. https://doi.org/10.2165/11317090-000000000-00000

Roberts, B. M., Nuckols, G., & Krieger, J. W. (2020). Sex Differences in Resistance Training: A Systematic Review and Meta-Analysis. *Journal of Strength and Conditioning Research*, *34*(5), 1448–1460. https://doi.org/10.1519/JSC.0000000000003521

Schedler, S., Kiss, R., & Muehlbauer, T. (2019). Age and sex differences in human balance

    performance from 6-18 years of age: A systematic review and meta-analysis. *PLOS ONE*,

    14(4), e0214434. https://doi.org/10.1371/journal.pone.0214434

Sheel, A. W., Dominelli, P. B., & Molgat-Seon, Y. (2016). Revisiting dysanapsis: sex-based

    differences in airways and the mechanics of breathing during exercise: Dysanapsis.

    Experimental Physiology, *101*(2), 213–218. https://doi.org/10.1113/EP085366

Sinha-Hikim, I., Cornford, M., Gaytan, H., Lee, M. L., & Bhasin, S. (2006). Effects of

    Testosterone Supplementation on Skeletal Muscle Fiber Hypertrophy and Satellite Cells

    in Community-Dwelling Older Men. *The Journal of Clinical Endocrinology &*

    Metabolism, *91*(8), 3024–3033. https://doi.org/10.1210/jc.2006-0357

Tortora, G. J., & Derrickson, B. H. (2017). *Principles of anatomy & physiology* (Fifteenth

    edition).

Trevino, M. A., Sterczala, A. J., Miller, J. D., Wray, M. E., Dimmick, H. L., Ciccone, A. B.,

    Weir, J. P., Gallagher, P. M., Fry, A. C., & Herda, T. J. (2019). Sex-related differences in

    muscle size explained by amplitudes of higher-threshold motor unit action potentials and

    muscle fibre typing. *Acta Physiologica*, *225*(4), e13151.

    https://doi.org/10.1111/apha.13151

Zech, A., Hollander, K., Junge, A., Steib, S., Groll, A., Heiner, J., Nowak, F., Pfeiffer, D., &

    Rahlf, A. L. (2021). Sex differences in injury rates in team-sport athletes: A systematic

    review and meta-regression analysis. *Journal of Sport and Health Science*,

    S2095254621000545. https://doi.org/10.1016/j.jshs.2021.04.003

How College Seniors Are Using Social Media Platforms to Brand Themselves for

Prospective Careers

Sydney Dinkins

Faculty Mentor: Dr. Kristin Stowe, Professor of Economics and Finance

ABSTRACT

In this day and age, almost everyone has the opportunity to create a professional image that makes them appear to be the perfect candidate for the job they desire. The purpose of this study is to uncover how college seniors are using social media platforms to brand themselves for their prospective careers. Previous research suggests a need for personal branding to be taught in higher education. It also suggests that college students perceive personal branding as important and proposes a positive correlation between personal branding and career success. In this study, a survey was presented to college seniors across several campuses through a few different outlets. The collected data was then analyzed between two groups, (1) business majors and (2) non-business majors. The results indicate that many college seniors understand the importance of personal branding and engage in different methods to create a personal brand; however, a student's major has little impact on the topics investigated.

INTRODUCTION

In this paper I argue that in this day and age, with social media, one can present themself as a perfect candidate for the job they want. Everyone has the opportunity to craft their professional image to get the career they desire. The purpose of this study is to uncover how college seniors are using social media platforms to brand themselves for their prospective careers. This study was conducted with a specific focus on the following platforms: LinkedIn, Instagram, Facebook, and Twitter.

Two hypotheses are included in this research: (1) college seniors designate certain profiles for setting up their professional identity, meaning a career-oriented profile versus a life-oriented profile geared towards friends and family, and (2) college seniors create profiles that highlight their skills and abilities. One purpose of this research is to investigate how college

seniors are using social media to present themselves in a way that attracts the employers they want to attract. It should also reveal how college seniors view the importance of personal branding in their career-seeking efforts.

PERSONAL BRANDING IN HIGHER EDUCATION

Personal branding has become increasingly important in the realm of higher education. Girard, Pinar, and Lysiak (2020) conducted a study on student perceptions of personal branding in higher education. In a survey of college students from two universities, the authors examined the student perception of 28 attributes relevant to student branding and student performance self-evaluation, both measured using a 5-point scale. These attributes include self-motivation, critical thinking skills, leadership, etc. The results indicated that the student performance self-evaluation is lower than the perceived importance of the personal branding attributes. The authors call for universities to fill this gap by providing better education on these attributes.

In another study related to higher education, Parrot (2019) used the unique method of rich pictures to gain insight into the student perspective of 'brand-me.' According to Parrot (2019), "Rich pictures is a soft systems methodology and used to capture the thinking process of individuals using icons, graphics, symbols, underlining, and directional arrows to visually communicate feelings and affect" (p. 172). As part of this methodology, students in the study were asked to privately draw pictures as answers to questions about their personal brand and were then asked to explain their drawings. The results offer more understanding as to different student perceptions of personal branding to be used by teachers in higher education. For example, two of the student drawings in this study indicate that the participants perceive shaping outward appearance as the best way to brand oneself. The study also offers encouragement for

this method to be used in more research projects as it is beneficial for not only readers but for the self-evaluation of the study participants as well.

When it comes to personal branding in the 21st century, one cannot forget the importance of social media. There is research that highlights this aspect and offers educators in higher education tools to teach personal branding through social media. Hutchins (2016) and Wetsch (2012) both use research to provide educators with personal branding assignments involving social media. Hutchins focuses on LinkedIn, while Wetsch covers many platforms including LinkedIn, Twitter, Blogging, HootSuite, Facebook, Google+, and YouTube. Both authors use past research to explain the importance of social media in personal branding and to develop relevant assignments to assist educators. For example, Hutchins (2016) includes an assignment on content creation, in which students get to practice creating useful content with LinkedIn's publishing feature (p. 143).

PERSONAL BRANDING THROUGH SOCIAL MEDIA

There is much more research exploring the use of social media for branding oneself. Viţelar (2019) focuses on the use of social media for personal branding, specifically by the "tech-savvy" Generation Z. From a questionnaire given to 100 Romanian Gen Z'ers, Gen Z being one of the generation groups currently attending college, Viţelar discovered that all 100 respondents had an online presence, with Instagram being the most popular platform. 85.7% of respondents perceived building a personal brand as something everyone should do. This study illustrates the Gen Z desire to differentiate and network through social media.

Girard & Pinar (2021) explore student perceptions of using social media to build their personal brand, as well as the various social media platforms used in this effort. Through an online survey conducted at two universities, research supports that students comprehend the

importance of a personal brand and work hard to build one. It also indicates that there are differences in the perception of social media platforms pertaining to relevance (meaning how relevant a platform is to the process of establishing a personal brand).

In another article about the importance of online branding, Watson (2019) uses past research to support the use of professional online portfolios ("POP"). After describing the challenges graduating college students face, Watson encourages the use of "POP" to create a personal brand and build confidence. This is useful for teachers and students as it provides another way, outside of typical social media, to create a personal brand. Watson lays out instructions and advice for building POP and includes advice for issues that may arise. For example, he instructs students to create "an engaging, narrative about me page" (p.159) which would provide students with the opportunity to tell their stories and stand out to potential employers.

Kleppinger & Cain (2015) offer yet more encouragement and urgency to the personal branding phenomenon. Using past research, these authors emphasize the importance of building a professional online presence through the use of social media. They highlight the advantages and disadvantages of online personal branding, how social media is used in the process of building a personal brand, and mistakes that educators have made in teaching this concept. For example, they emphasize the ability to "speak on behalf of [oneself] through positive and thought-provoking social media posts" (p. 3). This study is part of an effort to increase awareness about the importance of personal branding and why it is essential that it be taught correctly.

PERSONAL BRANDING AND CAREER SUCCESS

There is also research that highlights how personal branding has impacted career success. Amoako & Adjaison (2012) use past research to support the relationship between personal branding and career success. Their research suggests that "there is a positive relationship between personal branding and personal performance," given that personal branding improves the perception of an individual's value in society. The authors state that "personal branding influences performance through good working environment, proactive cultures and the achievement of one's positive self-esteem" (p.125). They also highlight a difference between personal branding and employee branding, in which personal branding is more focused on the individuals' natural characteristics and employee branding is focused on the employee adapting to the brand of the organization.

In a study involving 54 in-depth interviews, Khedher (2019) discovered six dimensions to personal branding that add value to the employability of college graduates. These include "cultural capital, social capital, verbal self-presentation, mediated self-presentation, authenticity, and appearance." The purpose of Khedler's research is to communicate how personal branding can result in perceived employability. The conclusion encourages the idea of graduates being thought of as a brand.

METHODOLOGY

In this study, a survey created through Qualtrics was presented to college students across ten campuses in North Carolina, South Carolina, and Delaware through social media, email, and other forms of direct messaging. From these methods of distribution, 47 usable anonymous responses were recorded. Participants were asked several questions related to personal branding. These questions fall into four categories: (1) social media usage, (2) social media platform relevancy, (3) personal branding methods, and (4) self-evaluation. The desired outcome was to

determine how college seniors are branding themselves for future employment. The first set of questions was meant to uncover seniors' initial perception of the importance of social media and how often they engage with social media accounts. The purpose of the questions following was to detect which platforms were discerned as most relevant to personal and professional branding. This was followed by an attempt to unveil what methods participants use in branding themselves. Lastly, participants were given seven statements meant to evaluate effort-level regarding branding through social media by choosing a level of agreement from a 5-point scale.

It was deemed most useful to analyze the data between two groups: business majors and non-business majors. The reason for this is that business majors are, more often than not, required to take courses where personal branding may be discussed and emphasized.

RESULTS

Social Media Usage

To gauge how often participants are active on social media, they were asked to choose an option from a 3-point scale ranging from (1) very often to (2) not often. Figure 1 shows a similar amount of activity between business and non-business majors. More than half of the respondents use social media very often.

Figure 2 indicates the differences in how business versus non-business majors perceive the importance of personal branding. Respondents seem to perceive personal branding as having some level of importance. A chi-squared test for a contingency table was used to determine whether perceived importance is independent of major. The resulting p-value (p=0.242) demonstrates that perceived importance is independent of major, meaning a college senior's major does not significantly affect how they view the importance of personal branding.

Social Media Platform Relevancy

Participants in this survey were asked to indicate which apps they consider most relevant for personal branding and were given the ability to select as many of the options as they deemed correct. They were also given an "other" option as part of this question. The results are shown in Figure 3.

A chi-squared test for a contingency table was used to uncover whether platform relevancy is independent of major. With a p-value of 0.460, it can be concluded that relevancy is independent of major, meaning major does not affect college seniors' perspective on which platforms are most relevant for personal branding.

Personal Branding Methods

There are many aspects to personal branding, and many ways one can engage in this process. To get a glimpse into exactly how college seniors are choosing to brand themselves, they were asked to choose from a few different factors. They were, again, given the ability to select all that apply to them. The results are displayed in Figure 4. A chi-squared test for a contingency table revealed a p-value of 0.827, which indicates that method choice is independent of major. This means that a student's major has no effect on the methods college seniors choose to utilize in the process of personal branding.

Self-evaluation

As the survey came to an end, participants were given several statements not only meant to evaluate effort-level regarding branding through social media but also to allow students to self-evaluate their efforts. Some questions were also intended to assist universities in their efforts to create a personal branding mindset among college seniors. Data from two statements were chosen for analysis. Tables 1 and 2 display the results for the selected statements.

Table 1		
Self-evaluation: I have worked hard to develop a strong personal brand		
	Some Level of Agreement	**Neither Agree nor Disagree**	**Some Level of Disagreement**
Business Majors	29%	33%	38%
Non-business Majors	18%	41%	41%

Table 2		
School Evaluation: I feel that my university is helping me to develop a strong personal brand		
	Some Level of Agreement	**Neither Agree nor Disagree**	**Some Level of Disagreement**
Business Majors	38%	43%	19%
Non-business Majors	23%	41%	36%

A chi-squared test for a contingency table was used to evaluate if agreeance level is independent of major when self-evaluating. The resulting p-value ($p= 0.709$) indicates that it is independent, which means a student's major does not affect whether they agree that they have worked hard to develop a strong personal brand. The same test was used to analyze the results in Table 2. With a p-value of 0.367, it can be concluded that a student's agreeance level regarding their university helping them develop a strong personal brand is independent of the student's major, meaning major has no effect.

LIMITATIONS

There are limitations to this data. The number of responses was considerably low, and over half of the participants are Wingate University students. I hypothesize that a larger number of participants from a wider range of schools would increase the quality of this study. A more in-depth statistical analysis could also provide even more interesting and useful information about this topic. It may also be beneficial to do the same study with all college students rather

than narrowing the scope to only include seniors. Future research should include a wider scope for participants and investigate how universities are teaching personal branding skills.

CONCLUSION

Today's digital age provides many opportunities for college seniors to brand themselves for their prospective careers. This study sought to uncover how college seniors discern the significance of personal branding as well as how they engage in the personal branding process through social media platforms. The results show that college seniors perceive and understand the importance of personal branding. The data analysis was based on comparing business majors versus non-business majors. This analysis revealed that how students choose to brand themselves and how they feel about how universities are helping is not dependent on whether they are a business major, or not.

Two hypotheses were investigated in this research: (1) college seniors designate certain profiles for setting up their professional identity, meaning a career-oriented profile versus a life-oriented profile geared towards friends and family, and (2) college seniors create profiles that highlight their skills and abilities. Results seem to indicate that seniors recognize Instagram and LinkedIn as the most relevant platforms for setting up a personal brand. The study also shows that highlighting skills and abilities is a key factor that seniors consider when developing their personal brand, along with building a network and keeping a consistent image.

Survey on Personal Branding through Social Media for College Students

This study is meant to uncover ways in which college students are using social media to brand themselves. The survey should take approximately 5 minutes to complete. Please answer all questions truthfully. Thank you for your participation.

- Please fill out this survey on personal branding through social media. If you would like to continue, click "I agree to participate."
    - I agree to participate
    - I do not agree to participate
- What year are you in college?
    - Freshman
    - Sophomore
    - Junior
    - Senior
- What career field are you planning to enter? (ex. Medical, Business, etc.)
    - Type Box
- How many personal social media accounts do you have?
    - 1
    - 2
    - 3
    - 4
    - 5 or more
    - None
- How often are you active on these accounts?
    - Very often
    - Somewhat often
    - Not often
- How often do you post on social media?
    - Very often
    - Somewhat often
    - Not often
- How do you view personal branding in regard to your future success?
    - Very Important
    - Somewhat Important
    - Not Important

If you answered very important or somewhat important, please answer these follow up questions.

- How confident are you in your ability to create a personal brand?
    - Very Confident
    - Somewhat Confident

- - Not Confident
- What social media apps do you consider most relevant for personal branding?
  - Instagram
  - Facebook
  - LinkedIn
  - Twitter
  - Other
- How many of your social media accounts are public?
  - 1
  - 2
  - 3
  - 4
  - 5 or more
  - None of them
- How many of your social media accounts do you use as a professional branding tool for future employment?
  - 1
  - 2
  - 3
  - 4
  - 5 or more
  - None of them
- What apps do you use for professional branding?
  - Instagram
  - Facebook
  - LinkedIn
  - Twitter
  - None
  - Other
- When engaging in personal branding through social media, what, if any, of the following factors do you consider?
  - Building a network
  - Keeping a consistent image
  - Regular Engagement/Posting
  - Sharing Diverse Content about personal attributes
  - Highlighting personal skills and characteristics
  - None of the above

Please indicate how much you agree or disagree with the following statements:

(This section of the survey came from an existing source. Cited: Girard, T., & Pinar, M. (2021). Examining the use of Social Media in Building a Student Personal Brand and the Impact of Demographics. *Journal of Marketing Development and Competitiveness*, 15(3), 7-18.)

- I have used social media presence to elevate my own personal branding
  - Strongly Agree
  - Agree
  - Somewhat Agree
  - Do not agree nor disagree
  - Somewhat disagree
  - Disagree
  - Strongly Disagree
- I actively manage my personal branding on social media
  - Strongly Agree
  - Agree
  - Somewhat Agree
  - Do not agree nor disagree
  - Somewhat disagree
  - Disagree
  - Strongly Disagree
- I make sure that my personal brand on social media reflects a consistent image of me
  - Strongly Agree
  - Agree
  - Somewhat Agree
  - Do not agree nor disagree
  - Somewhat disagree
  - Disagree
  - Strongly Disagree
- I have taken advantage of the networking opportunities on campus or online
  - Strongly Agree
  - Agree
  - Somewhat Agree
  - Do not agree nor disagree
  - Somewhat disagree
  - Disagree
  - Strongly Disagree
- Most social media platforms are not good places to create a personal brand
  - Strongly Agree
  - Agree
  - Somewhat Agree
  - Do not agree nor disagree
  - Somewhat disagree
  - Disagree
  - Strongly Disagree
- I have worked hard to develop a strong personal brand
  - Strongly Agree
  - Agree

- o   Somewhat Agree
- o   Do not agree nor disagree
- o   Somewhat disagree
- o   Disagree
- o   Strongly Disagree
● I feel that my university is helping me to develop a strong personal brand
  - o   Strongly Agree
  - o   Agree
  - o   Somewhat Agree
  - o   Do not agree nor disagree
  - o   Somewhat disagree
  - o   Disagree
  - o   Strongly Disagree

End of survey

Figure 1:
Social Media Activity

Figure 2:
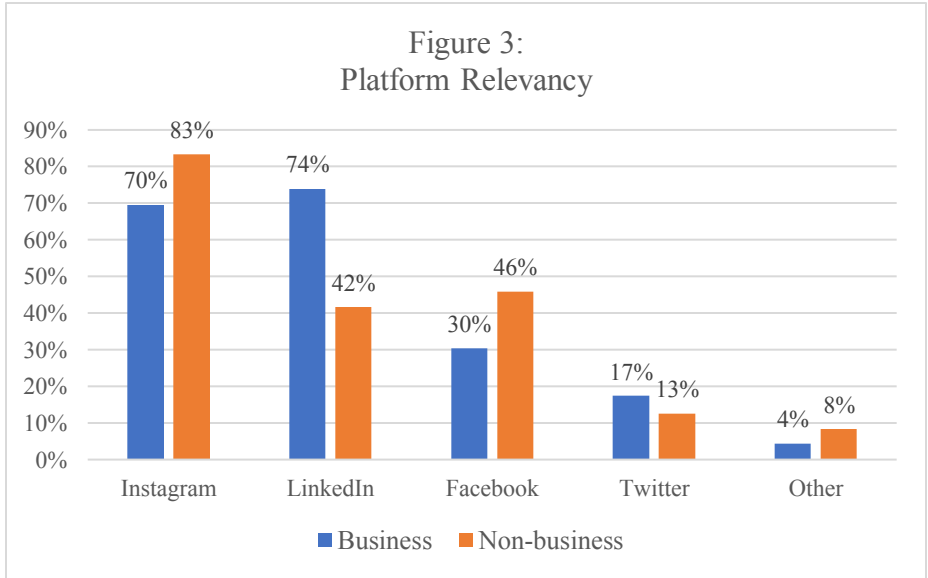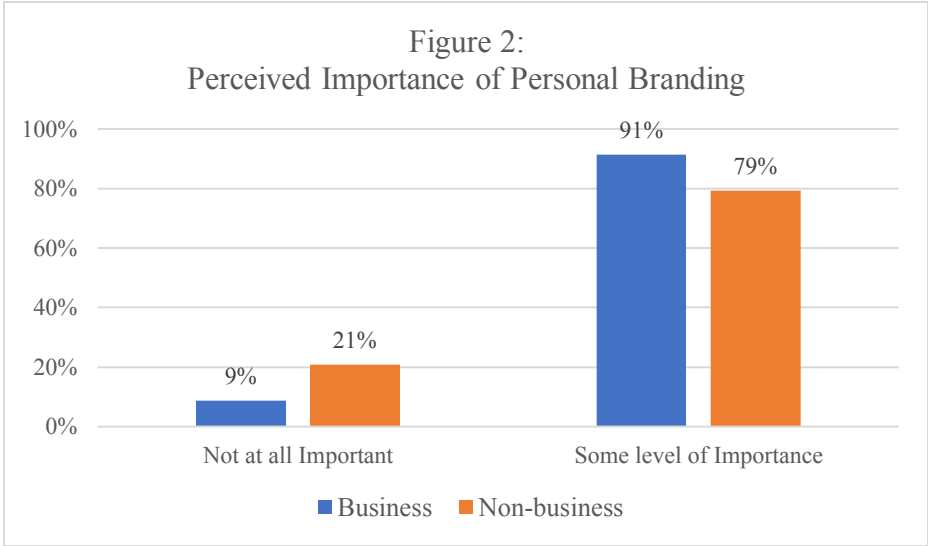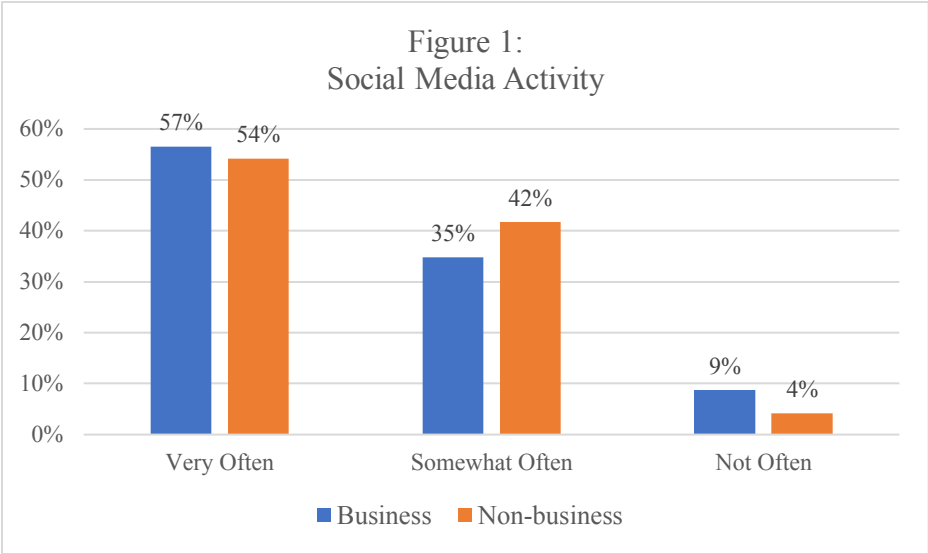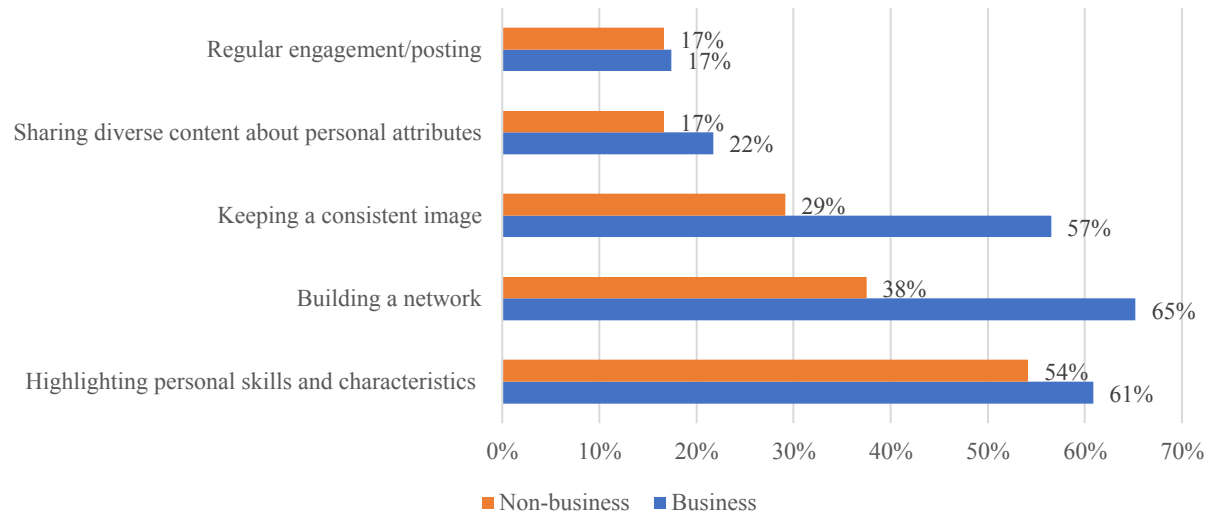Perceived Importance of Personal Branding

Figure 3:
Platform Relevancy

Figure 4:
Personal Branding Methods

REFERENCES

Amoako, G. K., & Adjaison, G. K. (2012). Non-empirical Analysis of the Relationship Between Personal Branding and Individual Performance. *Journal of Marketing and Operations Management Research*, 2(3), 117-128.

Girard, T., & Pinar, M. (2021). Examining the use of social media in building a student personal brand and the impact of demographics. *Journal of Marketing Development and Competitiveness*, 15(3), 7-18.

Girard, T., Pinar, M., & Lysiak, L. (2020). Understanding student perceptions of their personal branding in higher education. *Journal of Higher Education Theory and Practice*, 20(13), 136-146.

Hutchins, A. L. (2016). Beyond resumes: LinkedIn for marketing educators: An international journal. *Journal of Research in Interactive Marketing*, 10(2), 137-147.

Khedher, M. (2019). Conceptualizing and researching personal branding effects on the employability. *Journal of Brand Management*, 26(2), 99-109.

Kleppinger, C. A., & Cain, J. (2015). Personal Digital Branding as a Professional Asset in the Digital Age. *American journal of pharmaceutical education*, 79(6), 79.

Parrott, P. (2019). Rich pictures in qualitative research in higher education: The student as consumer and producer in personal branding. *International Journal of Work - Integrated Learning*, 20(2), 171-187.

Vițelar, A. (2019). Like me: Generation Z and the use of social media for personal branding. *Management Dynamics in the Knowledge Economy*, 7(2), 257-268.

Watson, M. (2019). Using Professional Online Portfolios to Enhance Student Transition Into the Poststudent World. *Business & Professional Communication Quarterly*, 82(2), 153–168.

Wetsch, L. R. (2012). A personal branding assignment using social media. *Journal of Advertising Education*, 16(1), 30-36,4.

Comparison of Academic Preparedness of Latino Students for College

Jocelyn Hernandez

Faculty Mentor: Kristin Stowe, Professor of Economics and Finance

ABSTRACT

The Latino population is growing at a rapid rate, becoming one of the largest ethnic groups in the United States. However, they remain one of the least likely groups to apply to college and pursue higher education. Studies imply that immigration, language barriers, and the transition into college are all things Latino students struggle with when pursuing higher education. Through a self-designed survey, undergraduate students from a variety of ethnicities were asked about three distinct stages of college: prior perception about college, transitioning into college, and their college performance. In the results, students' answers reflected that there are certain perceptions that are more significant than others, such as asking professors for help and how much impact family had in their decision making. The students were asked how they felt about their preparedness and their own point of view.

BACKGROUND

As the fastest-growing minority group, Latinos are a big part of the new education realm. In 2020, Latino's made up 25% of the K-12 population; The enrollment rate for Latinos in higher education is 21% compared to 24% for Whites (Excelencia in Education). Only 24% of Latinos obtained an associate degree or higher compared to 46% of White students. North Carolina has the 11th largest Latino population in the United States. Latinos make up 16% of the K-12 population, and 22% of the Latino population have an associate degree or higher in North Carolina. The 4-year institution with the highest Latino enrollment rate is the University of North Carolina at Charlotte at only 9%. Wingate only has 2.96% of Latino students (Data USA, 2019). The data collected will demonstrate these perceptions on the scale of a small private university.

LITERATURE REVIEW

In many cases, Latino students are faced with challenges that prevent them from having the same skills and academic preparedness as groups. These perceptions are caused by cultural differences as there is a clear difference in the experience of immigrant students, non-native speakers, and those who are new to the college realm. Crucial perceptions such as family influences and self-identity can play a key role in whether students enroll in higher education or not (Jabber, 2019).

IMMIGRATION

Immigration is one of the key causes of the disparities involved in the performance. Most of the research conducted about the cultural and academic challenges for college readiness was conducted on Mexican-origin families since they make up the highest percentage of Latin and Hispanic immigrants and 27.6% of the foreign-born population (Yasuike, 2019). However, many of the challenges found are also the same experienced by other Latin American and Hispanic groups such as language barriers and cultural differences.

Undeniably the biggest challenge for immigrants is the language barriers that come with living in a new country. The students themselves find it difficult to make progressive growth in their courses when they do not know the language used by instructors. Nearly one-third of students in a survey conducted by Murrillo and Schall enrolled in elementary school speaking only Spanish. From an early age, programs created to help English learners such as Accelerated Reader only create frustration for Spanish-speaking students which often makes them fall behind (Murillo & Schall, 2016). Additionally, this often leads to the sentiment of internalized oppression in which people are aware of their disadvantage but are not aware of the systemic institutions behind it. The language barrier often causes parents to feel ostracized from any

involvement in their student's academic career. Although they are supportive through motivation and challenging work (Yasuike, 2019), they are often purposely shut out by the school's system (Monzo, 2016).

The other major drawback for achieving the highest academic potential is the lack of knowledge immigrants have about their opportunities. Sometimes the lack of knowledge about scholarships creates missed opportunities (Yasuike, 2019). Other times, they do not have the resources to prepare for college. Often, immigrant students are denied access to courses that could help them succeed in preparing for college because they are deemed not yet prepared - putting Latino students further behind their peers (Wagner, K., et al, 2017). The cultural differences between the United States and their home country can lead to mistrust from parents of students in the academic system, but since it is the only opportunity to obtain scholarships and monetary support, it creates an imbalance of power in Latin American family dynamics. (Yasuike, 2019; Monzo 2016). There is also a great factor of fear when it comes to reaching out for help if students or their parents are undocumented. This power imbalance thus creates a rocky relationship between immigrants and the education system.

LANGUAGE BARRIERS

An important thing to note is that immigrant students are not the only ones who face challenges in language barriers. Some first-generation students also face the challenges of being ESL students (English as a Second Language). Although the idea of teaching ESL classes may seem beneficial at first, the cons can often outweigh the pros depending on the type of curriculum. Often, students are placed in Transitional Bilingual programs that try to transition Latino students into only speaking English (Garza-Reyna, 2019). The lack of accountability for these effects is what leads students to fall behind (Melguizo, 2021). Most of the students who are

put into ESL courses also live in poverty (Giraldo-García, et al., 2016). This creates double segregation in the disadvantage of college readiness for these students. All three of the previous authors agree that the best way to combat the drawbacks of ESL courses is to have better staffing or a better curriculum (Giraldo-Garcia, 2016) that integrates both languages (Garza-Reyna, 2016) as it would lead students to be more prepared for college. Finding ways to combat disadvantages is crucial in breaking down whatever harmful patterns have been placed previously, as they are not working in favor of bilingual students. Creating mentoring programs and allowing students to feel empowered and motivated is key when adjusting to new languages (Giraldo-Garcia, 2016).

TRANSITION INTO COLLEGE

As Latin American students enter the higher education system, they have a harder time adapting to a system for which they have not been prepared. One of the biggest problems for Latinos in their academic careers is the retention rate (Aguilar and Kim, 2019). Latino students have the highest dropout rate out of all ethnicities. Their percentage is 12 percent, in comparison to White students who face a 5 percent dropout rate, and African Americans who face a 7 percent dropout rate (Giraldo-Garcia, 2016). In the transition to college, many key parts come into play, of which one of the most important for Latinos is family. Before even beginning the choice of pursuing an academic career, students often prioritize their family's influence in their decision-making (Jabbar, H, et. al, 2019), which can lead to benefits and drawbacks. Sometimes students are influenced by the thought that the investment that they put into a college degree will not benefit them overall and is not worth pursuing (Aguilar, 2019). If neither parents nor students are educated about the benefits of a career, they will close these doors and limit their achievements. Students may feel pressured to satisfy their family's needs instead of their own. They may also

121

feel the cultural pressure of being the "first in the family" and take on this identity which can help students create a sense of community with other students (Jabbar 2017; Clayton 2019).

Due to the increase in first-generation Latino students, programs have been developed recently to aid students not only financially but to transition culturally into the college environment. For example, Advancement Via Determination (AVID) and Gaining Early Awareness and Readiness for Undergraduate Programs (GEAR UP) are both high school programs that promote rigorous preparedness for college. Since White peers are more likely to have taken more Advanced Placement (AP) courses than Latino students (Gurantz, O et. al, 2017), programs such as the National Hispanic Recognition Program help aid high schoolers in scoring better on college readiness exams. These programs can help with key perceptions leading to successful enrollment for Latino students applying for college. For example, students who have higher self-esteem and believe in themselves have a 115% higher chance of enrolling in college (Aguilar and Kim, 2019). To make meaningful change, contribution to these programs is important so that more students can experience the worth of higher education.

Latino students face unique challenges when it comes to their academic careers. Many of the obstacles begin at an early age when they are first introduced to the English Language and remain when they are ready to initiate a college career journey. Legal status also hinders Latinos from gaining equal opportunities compared to their counterparts of the same ethnicity. Family is one of the key influences that can motivate the student, but sometimes there is not enough access to knowledge about where to find useful academic or career information, or the oppression experienced within a family affects all members. Regardless, there are innovative programs that can better prepare students such as the National Hispanic Recognition Program, Hispanic Scholarship Fund, and Latin American Women Association; these programs offer access to the

various kinds of help that are available. Academia is difficult to navigate as a minority, but Latin Americans have more obstacles than most can see.

METHODOLOGY

The goal of the study is to determine if there are significant perceptions depending on the ethnicity of students on their own academic preparedness for higher education. Information was collected through a survey which was sent out to undergraduate students at Wingate University via an anonymous link through email from clubs and the School of Business. The survey consisted of twenty multiple-choice questions and three short answer questions. There were questions regarding transition into college, feelings about college, and college performance. The questions varied on scales of 1 (Strongly agree) to 5 (Strongly Disagree). There were also questions regarding the GPA or first-generation status of the respondents. The survey had 103 respondents from varied ethnicities: 40 Hispanics, 23 Asians, 6 Black/African American, 28 White, and 6 who identified themselves as "Other."

HYPOTHESES

*H1*: There will be a significant difference in the preparation of students depending on their ethnicities.

H2: There will be a significant difference in the college experience for students depending on their ethnicities

.H3: There will be a significant difference in the performance of students in college depending on their ethnicity

RESULTS

Transition into College

The research investigates if the ethnicity of the respondents impacts their self-assessment on the following criteria: high school performance, high school preparedness, support coming into college, and family influence in college decisions. High school performance was determined based on how the student rated themselves as an A-student, a B-student, etc. Similarly, there were questions regarding whether students felt their school had prepared them for college, whether they had a dedicated support system before going to college, and whether their family had a strong influence on their decision making. These questions were based on a four-point scale ranging from 1 (Strongly Agree) to 4 (Strongly Disagree).
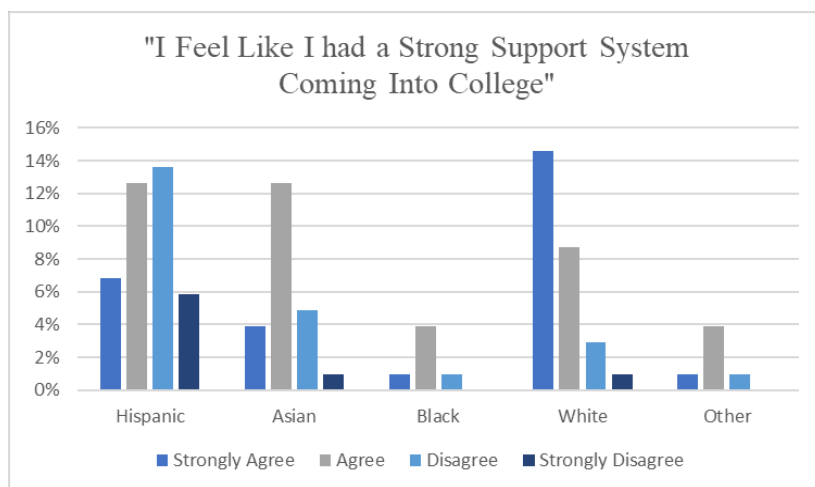
In Table 1, the p-values of all the previous questions demonstrate that the only factor that had a significant impact based on the ethnicity of the respondent is whether family had a strong influence on college decision making. Table 1 also shows the averages in the student's responses, to show the distribution of the answers. In the "High School Performance" row, Asian

students ranked their high school grades higher than other groups. The lowest ranking group was White students with an average of 1.29. The "High School Preparedness" row illustrates whether the students felt like they were prepared well for college. Black and Asian students on average felt less prepared than White students. The "Support" question asked students whether they felt they had a dedicated support system going into college. Both Figure 1 and Table 1 show the distribution for this question. Hispanic students tended to skew more toward the "Disagree" side than the rest of the groups, while White students tended to skew more toward the "Agree" side.

Table 1: Comparing Student's Feelings About Going to College

Comparing Students Feelings about the College Experience										
	Hispanic		Asian		Black		White		Other		P-Value
	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	
Stressed about classes	3.13	3.00	3.30	3.00	3.00	3.00	3.04	3.00	3.50	3.50	0.75
Left Out or Lonely	2.28	2.00	2.22	2.00	1.83	2.00	2.14	2.00	2.67	2.50	0.69
Positive Experience	2.05	2.00	2.09	2.00	2.00	2.00	2.11	2.00	2.00	2.00	0.28
Approaching Professors	2.15	2.00	2.13	2.00	1.50	1.00	1.64	2.00	1.50	1.00	.04*
Benfit from Organiations	2.20	2.00	2.35	2.00	2.17	2.50	2.04	2.00	2.00	2.00	0.17

Based on a scale of 1-4 *significance value of .10

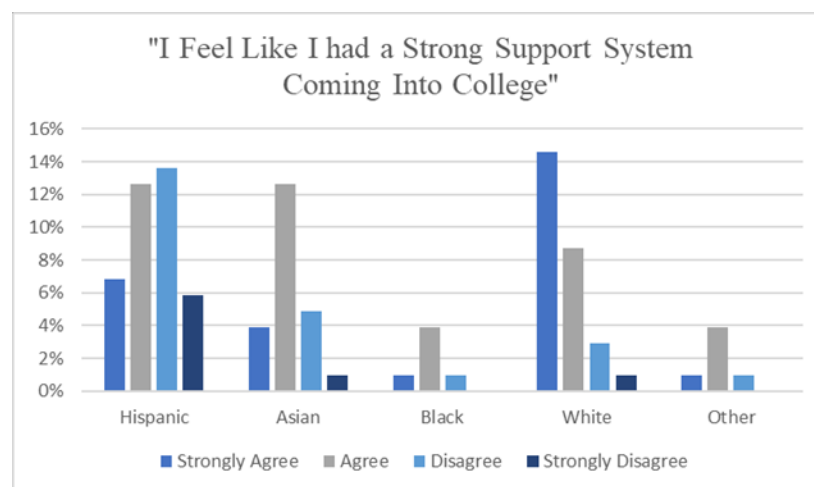"I Feel Like I had a Strong Support System Coming Into College"

Figure 1: "I Feel Like I Had Strong Support System Coming into College"

Feelings About College

Several questions were based on stress levels in college classes, whether students feel left out, overall positive experience, approaching professors for help, and benefits from college academic organizations. All the questions were based on a scale of 1 (Strongly Agree) and 4 (Strongly Disagree).

Table 2 shows the average in responses based on the mean. The stress level, feeling of being left out, positive experience, and benefit from academic organization results all proved to be independent of student ethnicities. The "Stress About Classes" question demonstrates how students feel about their college classes. All students scored similarly in this section, with a small range of .50. In the row "Feeling Left Out or Lonely," students answered regarding how they felt on campus. Black students expressed that they have felt as though they were more likely to find an organization to fit into, compared to the other students. Most students agreed that they have a positive experience regardless of ethnicity. However, the ability to approach professors for help was significant among Hispanic and Asian students. Finally, the "Benefiting from College Organization" row shows that most students agree that they benefit from organizations, with students in the "other" category agreeing the most.

Table 2: Comparing Student's Feelings About the College Experience

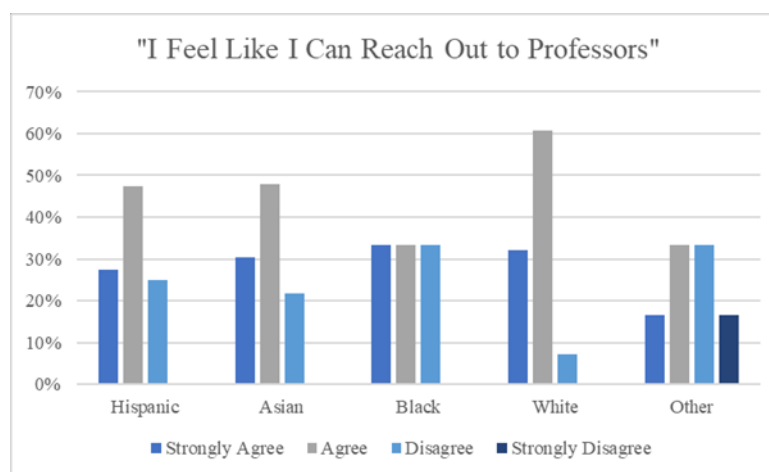| Comparing Students Feelings about Going College										
	Hispanic		Asian		Black		White		Other		P-Value
	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	
HS Performance	1.55	1.00	1.61	1.00	1.50	1.50	1.29	1.00	1.50	1.00	0.47
HS Prepardness	2.40	2.00	2.61	3.00	2.67	3.00	1.86	2.00	2.00	2.00	0.35
Support	2.48	2.50	2.13	2.00	2.00	2.00	1.64	1.00	2.00	2.00	0.25
Decision	1.65	1.50	1.43	1.00	1.17	1.00	1.79	1.50	1.50	1.50	0.08*

Based on a scale of 1-4 *significance value of .10

Figure 2: Comparing Student's Feelings About the College Experience

Performance in College

The last set of questions is based on the student's performance in college and their involvement in clubs and organizations. The self-reported GPA range is from a scale of 1 (Less than 2.5) to 4 (3.5 or Higher). For these questions, none of the responses were dependent on ethnicity.

Table 3 also shows the mean values to show the average responses to each of the questions in which the variety of responses is not dependent on ethnicity. In the row regarding "Reflection of Grades," students were asked whether their grades reflected their academic goals.

Asian students' answers leaned towards the "Disagree" category while White students responded toward the "Strongly Agree" side. Figure 3 and Table 3 both demonstrate the way that students evaluated their grades in connection to their academic goals. Hispanic and White students were most likely to rate themselves A-students, while Black and Asian students rated themselves B-students. The responses were not dependent on ethnicity and ranged differently amongst the groups, but they do hold a change based on their high school performance. All ethnicities tended to rate themselves as better students in college compared to high school. The GPA row gave the students ranges to select their college GPA from. Asian students tended to lean towards the 3.0 or lower range while the other students tended to lean towards the 3.5 and higher range. For the "Number of Organizations" students are involved in, the most involved were Hispanic students and the least involved were Black students.

Table 3: Comparing Student's Performance in College

Comparing Students' Performance in College						
	Hispanic	Asian	Black	White	Other	P-Value
	Mean	Mean	Mean	Mean	Mean	
Reflection of Grades	2.00	2.52	2.17	1.43	1.67	0.48
College Performance	1.65	2.00	2.00	1.25	1.50	0.92
GPA	3.25	2.87	3.00	3.54	3.67	0.29
Number of Org	2.55	2.09	1.83	2.39	2.33	0.39

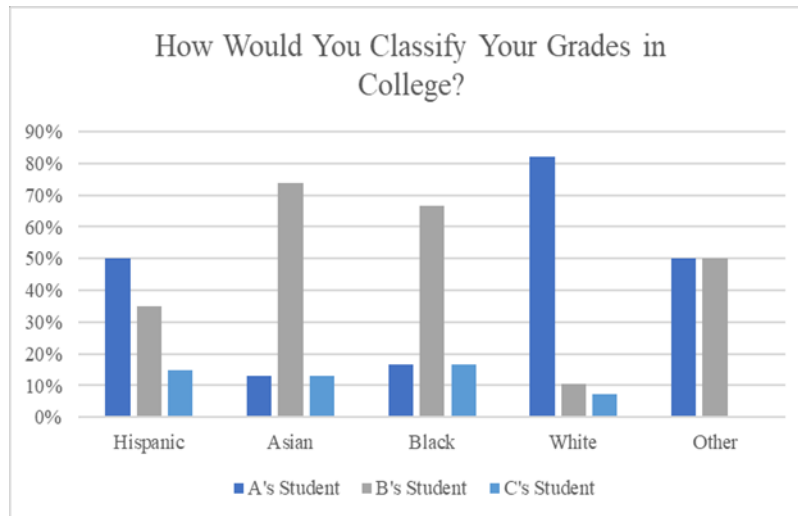Based on a scale of 1-4 *significance value of .10

Figure 2: Comparing Student's Feelings About the College Experience

LIMITATIONS

The survey took place at Wingate University, which is a small school. The number of respondents is limited since the survey was only distributed through a limited number of organizations and groups. Although the goal was to distribute the survey to as many students as possible, more groups could have participated. Some participants did not answer all the questions; therefore, they were not able to be part of the analysis. More respondents would have led to more accurate results. It is important to note that the results were based on the student's reported perceptions of themselves, and not experimental data.

CONCLUSION

The Latino population remains behind their White counterparts both in enrollment and graduation rates. Three of the main causes of this lag are immigration, language barriers, and the transition into college. From the research conducted, some perceptions play a significant role in the way Latino students feel about going to and being in college. The respondents showed that family plays a significant role in their decisions in going to college. In another question, the

respondents showed that they have a harder time reaching out to professors in comparison to other groups. Overall, most perceptions did not have a significant difference regarding the student's ethnicity statistically. However, the study did have limitations as the sample size for each category could have been bigger, and there were some incomplete responses.

REFERENCES

Aguilar, S. J., & Kim, A. Y. (2019). Hispanic students' sense of control in relation to post-secondary enrollment outcomes. *Social Sciences, 8*(2), 2-10.

Clayton, A. B., Medina, M. C., & Wiseman, A. M. (2019). Culture and community: Perspectives from first year, first-generation-in-college Latino students. *Journal of Latinos and Education, 18*(2), 134-150.

Garza-Reyna, G. L. (2019). The academic preparedness of Latino students in dual language and transitional bilingual education programs. *Journal of Latinos and Education, 18*(4), 340-348

Giraldo-García, R. J., Galletta, A., & Bagaka's, J. G. (2019). The intersection of culture and institutional support for Latino students' academic success: Remediation or empowerment? *Journal of Latinos & Education*, *18*(1), 68–80

Gurantz, O., Hurwitz, M. and Smith, J. (2017), College Enrollment and Completion Among Nationally Recognized High-Achieving Hispanic Students. *Journal of Policy Analysis and Management, 36*: 126-153

Jabbar, H., Serrata, C., Epstein, E., & Sánchez, J. (2019). "Échale Ganas": Family support of Latino/a community college students transfer to four-year universities. *Journal of Latinos & Education*, *18*(3), 258–276.

Latino College Completion: United States. Excelencia in Education. (n.d.). 2022, from https://www.edexcelencia.org/research/latino-college-completion

Melguizo, T., Flores, S., Velásquez, D., & Carroll, T. (2021). Lost in the transition: The cost of college-readiness English standards misalignment for students initially classified as English learners. *The Journal of Higher Education (Columbus), 92*(5), 815-846.

Monzó, L. D. (2016). "They don't know anything!": Latinx immigrant students appropriating the oppressor's voice. *Anthropology & Education Quarterly, 47*(2), 148-166

Murillo, Luz A., Schall, Janine M. (2016). "They Didn't Teach Us Well": Mexican-Origin Students Speak Out About Their Readiness for College Literacy. *Journal of Adolescent & Adult Literacy*, 60(3), 315– 323.

Wagner, K., Dymes, L., & Wiggan, G. (2017). Tracking students through life: A critical structural analysis of academic tracking of Mexican immigrant students in the United States and Korean immigrant students in Japan. *The Urban Review, 49*(5), 875–894.

Yasuike, Akiko (2019) The academic success of undocumented Latino students: School programs, non-profit organizations, and social capital, *Journal of Latinos and Education,* 18(1), 42-5.

Wingate University. Data USA. (2019).

An Economic Impact Analysis of Union County Public Schools

By Jonas Soerensen

Faculty Member: Dr. Kristen Stowe, Professor of Economics and Finance

ABSTRACT

This paper examines the economic impact of the Union County Public Schools on Union County using the software program IMPLAN to estimate the economic impact of construction projects and ongoing operations. This paper concludes that the five previous years of construction expenditures added approximately $76 million to the GDP and supported 936 jobs. The ongoing operations added approximately a total of $20 million and supported approximately 5,235 jobs. Furthermore, 600 property values located near four schools have been sampled to examine the nearby school quality's impact on the property value using regression analysis. The quality of a nearby public school is concluded to have an impact on property values.

LITERATURE REVIEW

The methods behind conducting an economic impact analysis can vary from study to study; however, a common practice is to use the input/output IMPLAN software. The software utilized by IMPLAN is built on the input/output model developed by Wassily Leontief, the Nobel Prize recipient in Economics. The economic input/output model is a quantitative model that recognizes the interdependence of different sectors within an economy. Bae & Dall'erba (2016) concluded in their research on the construction of solar plants that when choosing the software, it is vital that the software has the appropriate sector where the construction is taking place as this will result in more accurate estimations. Furthermore, Mulligan, Jackson, and Krugh (2013) concluded that there was a remarkable similarity in results from the IMPLAN software compared to the ACDS (Arizona Community Data Set) model. This finding of similarity adds validity to the IMPLAN

software. Schuyler (1997) examined 19 past economic impact analyses of community colleges and concluded that it is important to understand that an economic impact analysis provides a narrow picture of the actual impact and recommended that qualitative data should be collected to give a broader representation of the impact of the institution. Furthermore, economic impact analysis can be a valuable tool for policymakers because it provides them with an estimate of what will happen to the economy if a certain policy is changed (Schuyler, 1997).

Other studies have examined the usefulness of an economic impact analysis conducted by IMPLAN. Crompton (2020) concluded that computer general equilibrium (CGE) models are more accurate than the IMPLAN, however, this model is more expensive and complex. It is important to note these limitations when designing an economic impact analysis using the software IMPLAN.

ECONOMIC IMPACT ANALYSES OF HIGHER EDUCATION INSTITUTIONS

Research conducted in the past has concluded that higher education institutions have varying economic impacts on the surrounding economy. An analysis conducted of Bowling Green State University in Ohio concluded that every dollar the university received in state funding created an economic output of eight dollars in the state of Ohio (Carol & Smith, 2006). A more recent economic impact analysis of the University of Tennessee Health Science Center College of Pharmacy concluded that the University's Pharmacy School added a total of $94.1 million to the economy of Tennessee and supported 763 jobs excluding those directly employed by the University's Pharmacy school. (Chisholm-Burns, Chang, & Cooper, 2020). Khalaf, Jolley, and Clouse (2021) identified a guide to best practices when doing an economic impact analysis using the IMPLAN software. Interest payments and depreciation should be excluded when looking at the institution's output as this results in a more conservative estimate. Also, student spending

should be estimated using a consumer expenditure survey and spending categories should be adjusted to avoid overlap between ongoing operations. The study also emphasizes the importance of treating construction projects as one-time purchases. Furthermore, the survey discusses how to handle visitor spending; however, visitor spending is not applicable for county public schools as it is for colleges.

ECONOMIC IMPACT ANALYSES OF K-12 SCHOOLS

The previously discussed studies have mainly been examining the economic impact of ongoing operations by the institutions. However, new construction projects by institutions are also an important factor. The Business Economic and Community Outreach Network at Salisbury University examined the economic impact of The Wicomico County Public Schools in Maryland for the fiscal year (ending in 2018) and concluded that every dollar in the operational budget generated local spending of $1.66. Furthermore, each job supported directly by the school indirectly supported 0.3 jobs in the local economy. (Business Economic and Community Outreach Network). The Neshoba County School District in Mississippi economic impact analysis showed that the nine construction projects done in 2018-2022 resulted in 4.2 million dollars in direct, indirect, and induced effects from the 3.3 million dollars that were invested in these construction projects. The projects supported 39 jobs through the construction period. (Cambong, Brown, & Miller, 2021). Furthermore, it is important to note that the economic impact of K-12 schools differs from the impact of higher education institutions as factors such as visitor and student spending are not relevant.

SCHOOL QUALITY'S IMPACT ON RESIDENTIAL HOUSING VALUES

Past research has concluded that the quality of a K-12 school district has an impact on the housing prices in that area. However, past research has defined school quality in several different ways. Jud and Watts (1981) examined housing prices in Mecklenburg County based on the racial composition of the students and the average performance on the state test for reading skills for third graders. Chiodo, Hernández-Murillo, and Owyang (2010) also used test scores as a measure of school quality; however, this study used test scores for the entire school rather than the third-grade level. Courtney, Murray, and Osman (2013) conducted a similar study by looking at the Williamsburg-James City County Public Schools in Virginia used Virginia's Standards of Learning (SOL), Scholastic Aptitude Test (SAT), and American College Test (ACT) scores as a measure of the quality of the school. Seo and Simons (2009) estimated school quality's impact on housing prices based on a more complex measure of school quality. They included five factors: input factors, output factors, value-added of year-to-year progress in output; efficiency of output to tax rates; and parent and peer characteristics. These five factors were chosen because they are all publicly available and can therefore be a part of the decision-making process for parents when moving, thereby having an influence on housing prices.

School quality measures differ from study to study; however, the models that are used to estimate school quality's effect on housing prices are similar. Jud & Watts (1981) used a nonlinear model that included the following vectors of variables: describing the public schools, indicating the zoning classification of the property, describing the land-use characteristics of the neighborhood, describing the socioeconomic characteristics of the neighborhood, measuring the quality of structure, describing the size of the structure, and lot size. Seo and Simons (2009) also used a framework where the housing price is a function of similar vectors of variables; however,

136

this framework also contains an error term that acknowledges that the model results are estimates. It seems that the framework of estimating school quality's effect on housing prices has remained almost the same through time, but that the variables included in the models have become more complex.

Jud and Watts (1981) concluded that an increase of one grade level in the third graders' reading test is associated with a 5.2 percent to 6.2 percent increase in the value of an average house in the county. Chiodo, Hernández-Murillo, and Owyang (2010) concluded that one standard deviation increase or decrease from the mean in school-level test scores resulted in a roughly 10 percent change in the average housing price. Courtney, Murray J., & Osman (2013) concluded that the Williamsburg-James City County Public Schools in Virginia contributed to a rise in property values in the county of $54.4 million due to high school graduate's additional spending and a rise in test scores Lastly, Seo and Simons (2009), which included the most complex measure of school quality, concluded that one standard deviation change in performance index, efficiency, teacher salary, or fourth-grade math pass rate would result in a 3-5 percent change in the average housing price.

RESEARCH QUESTIONS

Research Question 1: Does the quality of a Union County public high school have an impact on real estate valuations in the area close to the school?

Research Question 2: Do the ongoing operations of all Union County Public Schools have an economic impact on Union County?

Research Question 3: What is the economic impact of all Union County Public Schools?

IMPLAN

Methodology

IMPLAN is used to estimate the economic impact of Union County Public Schools'

construction and ongoing operations. The construction expenses are based on the reported capital

expenditures for the last five years. Union County Public schools spent a total of over $112

million on construction projects for the period 2016-2020. (Union County Board of Education,

2020). In IMPLAN sector 53 (Construction of new educational and vocational structures) is used

for the capital expenditure function labeled "Building and Site Improvements" and capital

expenditures on Furniture and Equipment have been assigned to sector 369 (Institutional

furniture manufacturing). IMPLAN adjusts the dollar amounts from previous years for inflation,

so the reported amounts reflect the value of the 2022 US dollar.

The dollar amount used in IMPLAN for ongoing operations is based on the Statement of

Expenses for the Fiscal year that ended June 30th, 2020. Furthermore, the capital expenditures on

vehicles are included in the ongoing operation section because it is assumed that maintaining a

fleet of school buses is an ongoing operation for the Union County Public Schools. The

expenditures of over $1.5 million on vehicles have been assigned to sector 343 (Motor vehicle

body manufacturing). The expenses for the school minus deprecation and payroll which

amounted to over $7.6 million were assigned to sector 480 (Elementary and secondary schools).

The payroll expenses of $10.6 million have been assigned to sector 542 (Employment and

payroll of local government (education)). Depreciation has been ignored as this is not relevant

for economic impact purposes. Furthermore, IMPLAN's advanced settings were modified to

incorporate the approximate 5,000 employees on the payroll of Union County Public Schools.

IMPLAN Terminology

IMPLAN uses certain terminology which is explained in Graph 1. Direct effects are impacts where the change can be measured directly such as changes in wages or production. Indirect effects are purchases of goods and services from business to business because of the direct impact. This results in supply chain effects, business tax impacts, and changes in wages paid. The changes in wages paid lead to changes in household spending. The change in household spending leads to changes in local and import purchases, wages paid, and household tax. These are induced effects. Value added is simply a measure of gross domestic product.

Graph 1: IMPLAN Terminology

Source: https://blog.implan.com/what-is-implan
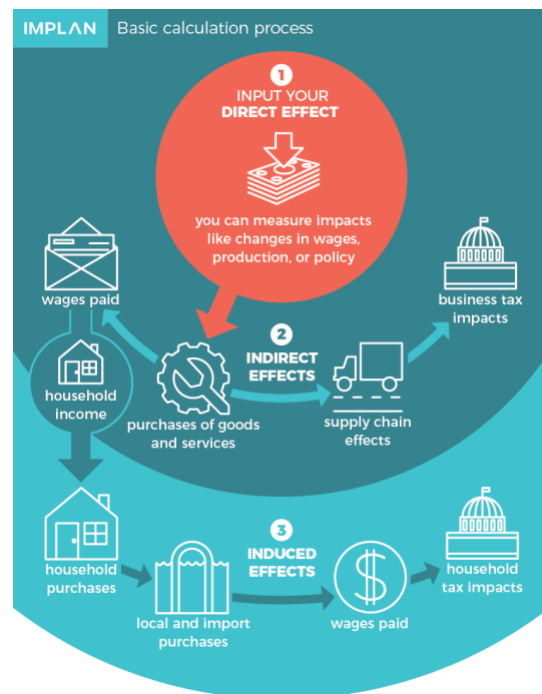
RESULTS

The past five years of construction work for Union County Public Schools have resulted in approximately 76 million dollars in added value or GDP. (Graph 2). The sector that benefited the

most over this period is the construction sector. The owner-occupied dwellings sector added just over 3 million dollars due to induced impacts which is the result of household income because of the added income from the construction projects.

Graph 2: Value Added as Result of Construction Expenditures 2016-2020
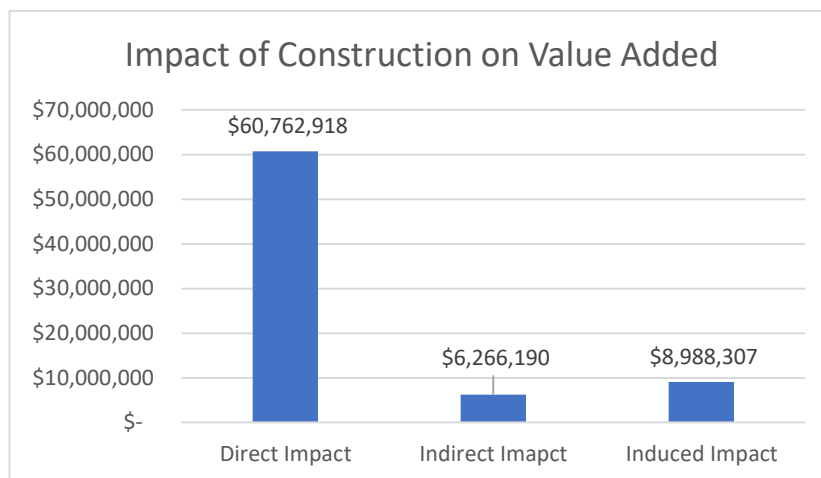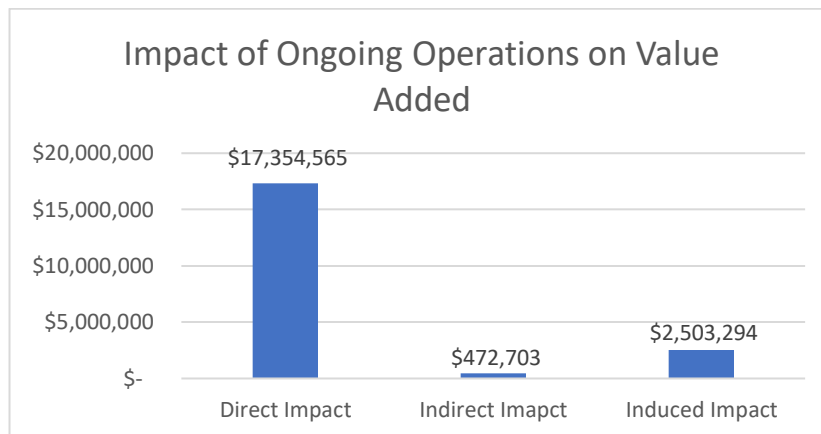

In addition to the added GDP, these construction projects also support a total of approximately 936 jobs in Union County. Approximately 741 are a direct result of the construction projects and include the construction workers hired to perform the construction (Graph 3). The construction projects have supported approximately 74 jobs due to indirect effects, which are the jobs supported because of business-to-business purchases. The construction projects resulted in approximately 121 supported jobs due to induced effects, which are the jobs supported because of the additional household spending that the construction projects generate.

**Graph 3: Supported Jobs as a Result of Construction Expenditures 2016-2020**

Impact of Construction on Employment

- Direct Impact: 741
- Indirect Imapct: 74
- Induced Impact: 121
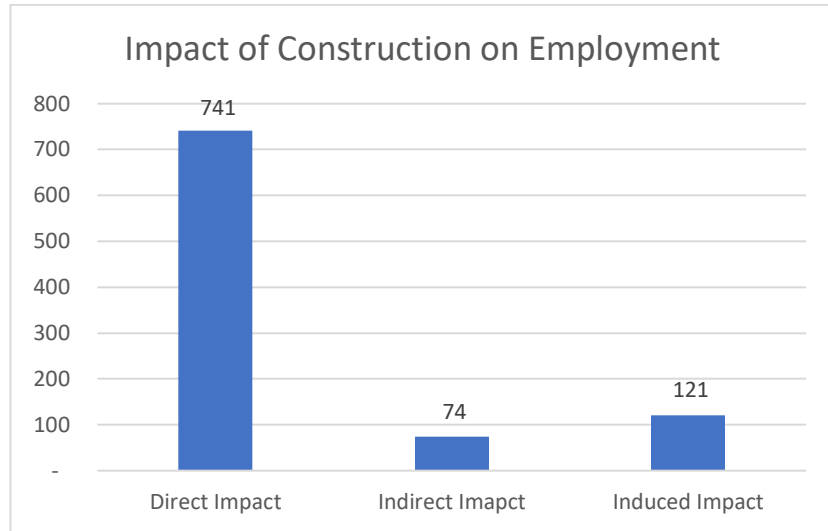
The ongoing operations of the schools have economic impacts of just under $20 million of additional GDP in Union County (Graph 4). The sector contributing the most to the added GDP is the employment and payroll of local government (education) sector which is the salaries and benefits provided to the employees of the UCPS. This sector adds approximately $10 million to the GDP of Union County.

Graph 4: Value Added as Result of Ongoing Operations 2020

Impact of Ongoing Operations on Value Added

- Direct Impact: $17,354,565
- Indirect Imapct: $472,703
- Induced Impact: $2,503,294

The ongoing operations support approximately 5,235 jobs in Union County (Graph 5). Around 5,000 of these jobs are on the payroll of the local government in Union County. The ongoing operations support approximately 8 jobs through indirect effects, which are business-to-business purchases, and approximately 34 jobs because of increased household spending, which are the induced effects.

**Graph 5: Jobs Supported as a Result of Ongoing Operations 2020**
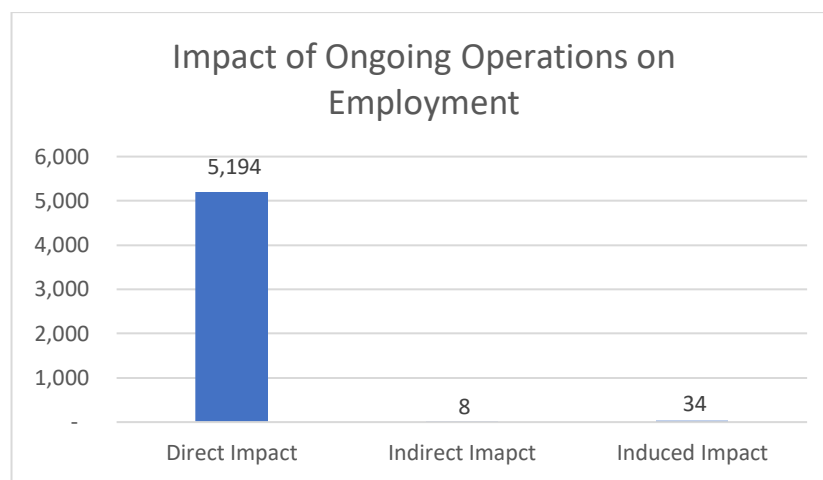
SCHOOL QUALITY'S IMPACT ON PROPERTY VALUES

Methodology

To estimate the impact of Union County Public Schools' quality on housing prices, four high schools will be examined. This is done by comparing 150 randomly selected property values in an area close to each school to different measures of the quality of the school. The 150 property values for each school were selected by extracting properties in neighborhoods close by the schools from the county's tax scroll. To achieve four evenly sized samples, 150 property values were randomly selected. Forest Hills High School, Porter Ridge High School, Parkwood High

School, and Marvin Ridge High School are the four high schools selected as they are placed in the eastern, northern, southern, and western parts of Union County.

Map 1: Union County Public Schools
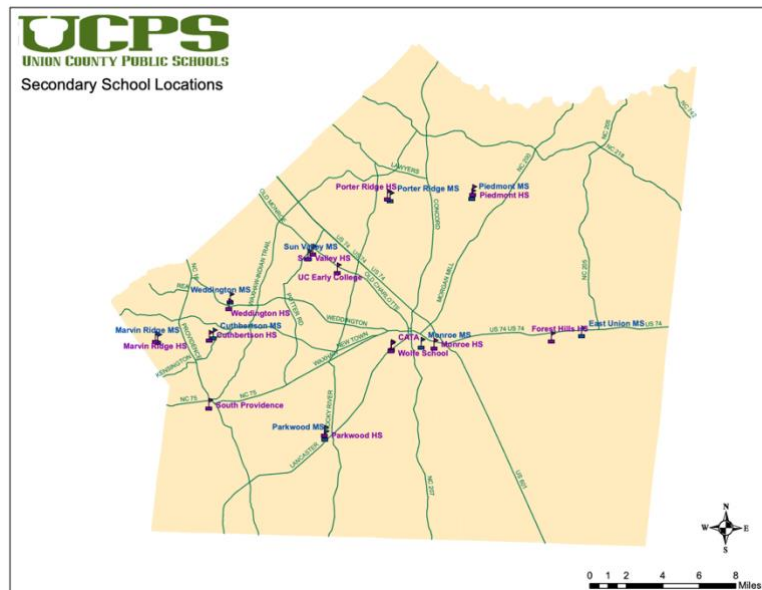
Union County Public schools have data available on the following measures of school quality (Table 1): average total SAT score, graduation rate, post-secondary enrollment, ACT composite score of 17 or higher, number of short-term suspensions, acts of crime committed per 1000 students, and number of dropouts.

Table 1: School Quality Measures by School

	Average Total SAT Score	Post secondary enrollment	Graduation Rate	ACT composite score of 17 or higher	Number of Short Term Suspensions	Acts of Crime comitted per 1000 students	Dropouts
Forest Hills	863	51%	80.40%	31.80%	151	21.62	17
Porter Ridge	991	71%	91.30%	60.30%	218	11.65	11
Parkwood	982	62%	95.00%	50.50%	204	11.25	1
Marvin Ridge	1207	87%	95.00%	88.30%	46	1.09	2

After conducting a correlation test of these measures of school quality (Table 2), three variables were chosen for a regression analysis as they have low correlation: ACT composite score of 17 or higher, graduation rate, and the number of short-term suspensions.

Table 2: Correlation Table for School Quality Measures

	Property Value	Average Total SAT Score	Graduation Rate	Post secondary enrollment	ACT composite score of 17 or higher	Number of Short Term Suspensions
Property Value	1.0000					
Average Total SAT Score	0.7690	1.0000				
Graduation Rate	0.4682	0.7513	1.0000			
Post secondary enrollment	0.7551	0.9715	0.7374	1.0000		
ACT composite score of 17 or higher	0.7616	0.9863	0.7557	0.9972	1.0000	
Number of Short Term Suspensions	-0.6281	-0.6994	-0.1109	-0.5918	-0.6201	1.0000
Acts of Crime comitted per 1000 students	-0.7235	-0.9809	-0.8640	-0.9615	-0.9761	0.5561
Dropouts	-0.4488	-0.7318	-0.9533	-0.6466	-0.6860	0.2304

*Highlighted cells indicate highly correlated variables.

Two regression analyses have been conducted. One analysis is based on whether a certain property value is located next to a certain school. The analysis consists of three binary variables and takes its starting point in properties around Parkwood. The three variables are the three remaining schools, and if a property is located next to a school, its corresponding variable value is 1. The second regression analysis is using the three school quality measures to attempt to estimate how much of the variation in property values is explained by these variables.

RESULTS

The first regression analysis is taking its starting point in Parkwood. The regression analysis is summarized in Table 3. Property Value = \$227,445.50 – (61, 534.91 * Forrest Hill) + (72,819.83 * Porter Ridge) + (384,355.83 * Marvin Ridge).

Table 3: Regression Analysis Based on School

School Locations	
	3 Variable Regression
Intercept	227445.50*
Forest Hills	-61543.91*
Porter Ridge	72819.83*
Marvin Ridge	384355.83*
Note: Cell entries are regression coeffecients. Statistical significance is denoted by * = P < .01	

Based on this equation, a property located near the starting point of Parkwood has an average taxable value of \$227,445.50. If the property is located by Forest Hills the average tax value would be \$61,534.91 less. If the property is located by Porter Ridge the average tax value would be \$72,819.83 higher. If the property is located by Marvin Ridge the average tax value is \$384,355.83 higher. This regression analysis resulted in an R^2 value of 0.6188, which means that 61.88 percent of the variation in property values is explained by which school the property is located nearby.

The second regression analysis is summarized in Table 4 and resulted in the following equation: Property Value = \$255,420.84 – (\$275,624.03 * Graduation Rate) + (\$735,233.79 * ACT composite score of 17 or higher) – (\$673.65 * number of short-term suspensions). The graduation rate has a p-value of 0.16 which means the graduation rate is not significant. Therefore, another regression analysis was conducted excluding the graduation rate. This

analysis resulted in the following equation: Property Value = $80,188.83 + ($645,057.51 * ACT composite score of 17 or higher) – ($815.48 * number of short-term suspensions).

Table 4: Regression Analysis Based on School Quality

School Quality Regression		
	3 Variable Regression	2 Variable Regression
Graduation Rate	-275624.03*	
ACT Score	735233.79*	645057.51*
Number of Suspensions	-673.65	-815.48*
Note: Cell entries are regression coeffecients. Statistical significance is denoted by * = P < .01		

The starting property value is an average of $80,188.83. If the nearby school's percentage of students scoring higher than 17 on the ACT the property value increases, the property value decreases by around $815.48 for every short-term suspension. This equation resulted in an R^2 value of .6188, which means that 61.88 percent of the variation in property values is explained by the three variables.

LIMITATIONS

There are several limitations to this study. The most significant limitations include the fact that the results from IMPLAN are estimates based on the input/output model, meaning that the results are not as accurate as results from computer-generated equilibrium models. Furthermore, it must be noted that all IMPLAN results are estimates. There is no available data that specifies the exact number of employees for Union County Public Schools which is why the estimate of 5,000 was used. The limited number of property values and the lack of data associated with each property's tax is a major limitation of the regression analysis and the study in general; however, there is simply no better data available on Union County's official website. Furthermore, it

would have been beneficial for the analysis if there was school measure data on the individual level instead of averages; however, this data is not available.

CONCLUSION

According to the IMPLAN analysis, the construction projects analyzed added approximately $76 million to the GDP and supported 936 jobs. The ongoing operations added approximately a total of $20 million and supported approximately 5,235 jobs. Furthermore, the regression analyses showed that property values vary depending on which school they are located next to. The average property value is the lowest if the property is located next to Forest Hills, second-lowest next to Parkwood, second highest next to Porter Ridge, and highest next to Marvin Ridge. The school's average performance on the ACT and the number of short-term suspensions both have an impact on the average property values in the neighborhoods located close to the schools. The better the school performs on the ACT the higher the average property values and the more short-term suspensions a school has the lower the average property values next to the school tend to be. These results can be beneficial for policymakers when making decisions on how to allocate public resources.

REFERENCES

Bae, J., Dall'erba, S. (2016). The economic impact of a new solar power plant in Arizona: Comparing the input-output results generated by JEDI vs. IMPLAN. *Regional Science Policy & Practice*, 8(1-2), 61-73.

The Business Economic and Community Outreach Network at Salisbury University, "The Economic and Community Value of the Wicomico County Public Schools" https://www.wcboe.org/cms/lib/MD50000151/Centricity/Domain/2520/BEACON%20Wicomico%20Schools%20Final%20Report.pdf (Accessed December 5th, 2021)

Cambong, J., Brown, H. N., Miller, C. (2021, June). *Neshoba County Campus Improvements Economic Impact Report*. Neshobacentral. https://www.neshobacentral.com/about/news/1719097/neshoba-county-school-district-economic-impact-report

Carroll, M. C., Smith, B. W. (2006). Estimating the economic impact of universities: the case of Bowling Green State University. *The Industrial Geographer*, 3(2), 1-12.

Chiodo. Abbigail J., Hernández-Murillo, Rubén, Owyang, Michael T. (2010). Nonlinear Effects of School Quality on House Prices. *Federal Reserve Bank of St. Louis Review*, 92, 185-204.

Chisholm-Burns, M., Chang, C. F., Cooper, S., B.S. (2020). Economic Contributions of a College of Pharmacy to the Surrounding Communities and State. *American Journal of Pharmaceutical Education*, 84(2), 250-257.

Courtney, A., Murray J., Osman, M. (2013, December). *The Economic Impact of Williamsburg-James City County Public Schools.* College of William and Mary. https://www.wm.edu/as/publicpolicy/documents/prs/wjcc13.pdf

Crompton, J. (2020). Uses and abuses of IMPLAN in economic impact studies of tourism events and facilities in the United States: a perspective article. *Tourism Review of AIEST - International Association of Scientific Experts in Tourism*, 75(1), 187-190.

IMPLAN Group LLC. IMPLAN 2021. Huntersville, NC. IMPLAN.com.

Jud, G. D., Watts, J. M. (1981). Schools and Housing Values. *Land Economics*, 57(3), 459.

Khalaf, C., Jolley, G. J., Clouse C. (2021). The Economic Impact of Small Colleges on Local Economies: A Guide to Attainable Data and Best Practices. *Economic Development Quarterly.*

Mulligan, G., Jackson, R., Krugh, A. (2013). Economic base multipliers: A comparison of ACDS and IMPLAN. *Regional Science Policy & Practice*, 5(3), 289-303.

North Carolina Department of Public Instruction. (n.d.). Testing Results (Green Book) https://www.dpi.nc.gov/districts-schools/testing-and-school-accountability/school-accountability-and-reporting/testing-results-green-book

North Carolina Department of Public Instruction. (n.d) The North Carolina SAT and AP Reports. https://www.dpi.nc.gov/districts-schools/testing-and-school-accountability/school-accountability-and-reporting/north-carolina-sat-and-ap-reports

Schuyler, G. (1997). The assessment of community college economic impact on the local community or state. *Community College Review*, 25(2), 65.

Seo, Y., Simons, R. A. (2009). The Effect of School Quality on Residential Sales Price. *The Journal of Real Estate Research,* 31(3), 307-327.

Union County Government. (n.d.). Downloadable GIS Data. https://www.unioncountync.gov/departments/gismaps/resources/downloadable-gis-data

Union County Board of Education. (2020). Comprehensive Annual Financial Report. https://www.ucps.k12.nc.us/cms/lib/NC01910453/Centricity/Domain/110/Union%20County%20Public%20Schools%202020%20CAFR%20FINAL.pdf

Union County Public Schools. (n.d.). District Attendance Maps. https://www.ucps.k12.nc.us/Page/2786