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ELAIA

Volume 1 Issue 1 Summer 2018



The Honors Journal of Olivet Nazarene University

The Effects of Heart Medication on the Heart Rates of *Drosophila Melanogaster*

Vocal Processing with Spectral Analysis

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The name Olivet comes from Mount Olivet, or the Mount of Olives, a hill outside Jerusalem known in ancient times for its olive groves and which featured prominently in Christ's life and ministry. Olives have been cultivated for thousands of years and hold rich theological symbolism (the olive branch as a symbol of peace, for instance, or anointing with olive oil). In selecting a name for this journal, we wanted a title that drew upon the symbolism and history in Olivet's name itself.

ELAIA (el'AYE'ah) is the phonetic spelling of the Greek word for olive. The symbolism is apt in more ways than one: olive trees take years to mature and bear fruit, and the research contained in this journal is likewise the fruit of these students' years of labor. Like the olive tree, we pray these students continue to grow, cultivate deep roots, and bear the fruits of peace and holiness in all their scholarly endeavors.

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PROGRAM

Each fall, the Honors Program at Olivet Nazarene University admits a small number of academically-gifted students into its freshman class. From the moment they set foot on our campus, these women and men join a community of scholars, and together they read, reflect upon, and discuss the most important ideas of the past and present—all within a Christian fellowship. The first two years of the program involve a series of Honors courses, taught by a team of faculty and modeled on the historic “old-time college,” where small class relationships, interdisciplinary discussion, and debate prevailed.

In the junior and senior years, the Honors program shifts its focus away from the classroom to the laboratory or library. There, students work on a capstone scholarship project within their major that involves original research and writing. Honors students gain experience comparable to what happens at large research institutions, as they work one-on-one with a faculty mentor and alongside their classmates in research seminars to conceive and complete their individual projects. For our graduates—many of whom go on to advanced study in medicine, law, or other fields—scholarship becomes a deeply personal, transformative, and spiritually meaningful act. Throughout their four years, Honors students ultimately learn how to love God with their minds, as well as their hearts.

Over the years, the Program has continued to grow and flourish, and the depth of its research continues to increase. This inaugural journal represents the fruits of that development, containing capstone research projects from the 2018 Honors Program senior class and their faculty mentors. The Table of Contents is diverse, and in that way it is a crystal clear reflection of our program’s community of scholars.

I, along with the members of the Honors Council, am gratified by the work of each student and faculty mentor printed within these pages. Congratulations, everyone!

- Stephen Lowe, *Honors Program Director*

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**The Effects of Heart Medication
on the Heart Rates of *Drosophila Melanogaster***

Felicia A. Baer

ACKNOWLEDGEMENTS

This research was made possible by funding from the Olivet Nazarene University Honors Program and materials made available by the Biology Department. I would like to thank Dr. Dwight Ginn for mentoring me as I completed this research and Dr. Dan Sharda for his input and help in revising my work.

ABSTRACT

Background

Current animal models of human cardiac disease may be similar in anatomy and physiology but are often expensive and tedious to work with. The current need is for a model organism that is more efficient to work with in the lab but that still provides an accurate model of human cardiac disease. *Drosophila melanogaster* (*D. mel*) is such a candidate. While 74% of the genes coding for protein are conserved between *D. mel* and human hearts, it is unknown if cardiac medication used in humans, such as atropine and propranolol hydrochloride, similarly affect heart rate. I hypothesized that administration of atropine and propranolol hydrochloride to third instar larvae would cause an increase and decrease respectively in the heart rates of *D. mel*.

Methods

After larvae hatched and reached the second instar larval phase, they were moved to fresh vials. The control group larvae were transferred to vials containing no medication, and the experimental group larvae were transferred to vials with 1mM atropine or 1mM propranolol hydrochloride. The larvae inhabited the new vials for twenty-four hours to reach the third instar larvae stage. Larvae were removed, placed individually on a microscope slide, and observed using the 4X objective lens of a Leica compound microscope. Heart rates of fifty larvae per group were recorded in triplicate over fifteen second intervals.

Results

We observed elevated heart rates of 406 ± 3.18 beats per minute in atropine treated larvae when compared to rates of 388 ± 2.07 in control larvae, a 4.83% increase. Moreover, heart rates were slowed to an average of 274 ± 2.70 beats per minute in propranolol hydrochloride treated hearts, a 29.18% decrease. Both changes in heart rate when compared to the control were found to be statistically significant ($p < 0.001$).

Conclusion

Administration of propranolol hydrochloride and atropine increased and decreased the heart rates of *D. mel* respectively. This data supports the hypothesis that *D. mel* can serve as an experimental model for human cardiovascular disease. Future work should build on this study and focus on the use of *D. mel* in preliminary pharmaceutical testing for new medication treating cardiovascular conditions.

Keywords: *drosophila melanogaster*, atropine, propranolol hydrochloride, heart rate

treatment of cardiac disease are areas of active research. Organisms of mammalian origin are predominantly chosen for studying cardiac disease and include baboons, pigs, sheep, dogs, rabbits, rats, and mice (Hasenfuss, 1998). Mammals are typically used due to their similarity in physiology, making them candidates to research new treatment methods (Patel et al., 2001). Smaller mammals, such as rats and mice, are also useful because they can be genetically manipulated and subsequently used to determine the effects of mutations in genes relating to cardiac function (Rosenthal and Brown, 2007). Similarly, zebrafish have recently emerged as another model for human cardiac disease research and have been used to model congenital heart defects and cardiomyopathies, as well as to determine mechanisms that can lead to cardiac disease (Bakkers, 2011).

These organisms have been pivotal in developing current knowledge regarding the physiology of the human cardiovascular system as well as the development and treatment of cardiovascular disease. Unfortunately, many of these organisms are inefficient to use in the laboratory. Costs to obtain and maintain these organisms in a laboratory setting are not trivial. According to the Jackson Laboratory website, purchasing mice for research would cost approximately \$10.75 per mouse (“Jax mice pricing information,” 2018). Genetically modified mice are even more expensive, and, according to the Cyagen Biosciences website, could range from \$250 to nearly \$7,000 depending on the desired method of inserting genes into the mouse genome (“Regular transgenic mice,” 2018). Rabbits, according to the Charles River website, range from \$160 to \$330 per rabbit depending on weight (“New Zealand white rabbit,” 2018). In addition, many of these animals can be difficult to handle and manage within the lab to ensure they are cared for humanely throughout research. Mice, for example, can move quickly and may attempt to bite the hands of researchers while handling (Buerge and Weiss, 2004). Dogs and primates, according to the Johns Hopkins University Animal Care and Use Committee, may require tranquilization if they are aggressive and difficult to work with. To advance research in this area, it would be advantageous to find an organism that is more efficient to utilize in the lab but that still provides an accurate model of human cardiac disease.

Drosophila melanogaster

D. mel is a candidate for modeling human cardiac disease and is an efficient organism to use in the lab for many reasons. One reason is the well documented short lifespan of the organism (Linford et al., 2013). *D. mel* is used as a model of aging due to its short life span of approximately fifty days from fertilization of the egg to the death of the adult fly. This lifespan is significantly lower than those of typical mammals. A rat, for example, has an estimated mean life expectancy of twenty-two months (Baati et al., 2012). A shorter lifespan allows researchers to study organisms in different stages of life over a shorter amount of time.

Another contributor to the efficiency of using *D. mel* is the immense research that has been done to sequence the genome of the organism (Pandey and Nichols, 2011). Scientists know many of the genes in the DNA of *D. mel* and are thus able to use this information to determine whether genes code for disease or for resistance to disease. Further, *D. mel* can incur mutations in genes homologous to genes of human disease

INTRODUCTION

Models of Cardiovascular Disease

According to the American Heart Association, one American dies of cardiac disease every forty seconds (Mozaffarian et al., 2015), and the causation, prevention, and

naturally or by manipulation (Bier and Bodmer, 2004). This ability allows *D. mel* to be a model for discovering the outcome of mutations in genes necessary for physiological function and maintenance of health.

Further benefits include the feasibility of raising the organisms in lab and the inexpensive cost (Doke and Dhawale, 2015). *D. mel*, in contrast to the prices of mammalian models, can be purchased for \$8.10 per vial containing twenty-five to thirty flies for most wildtype and mutant strains. In addition, all of the materials necessary for culturing can be purchased for \$65.50 (“Carolina easy fly drosophila cultures, living,” 2018).

In addition to being a practical organism to work with, *D. mel* is a candidate for human cardiac disease research. A study performed by Cammarato et al. (2011) determined that the proteome, or complete protein makeup, of the *D. mel* heart contains 498 genes vital to heart function. Seventy-four of these genes (15%) were protein products that are also produced in humans. Additionally, 73% of the genes were determined to be orthologs of genes found in humans and mice. Other experiments researching the genetic makeup of the *D. mel* heart have shown that they are physiologically similar to human hearts. The hearts of *D. mel* can develop structural defects and suffer from arrhythmias (irregular heartbeats) or cardiomyopathies (hereditary cardiac disease) (Pandey and Nichols, 2011). Another study used *D. mel* to model the development of age-related heart failure (Ocorr, Akasaka, and Bodmer, 2007). Due to genetic similarity, *D. mel* hearts develop heart failure caused by errors in pacing as well as arrhythmias, key factors in researching age-related heart failure. This research suggests that *D. mel* has a promising future in determining genetic contributions to cardiac disease. Anatomically, however, the heart of *D. mel* is different from that of a human (**Figure 1**).

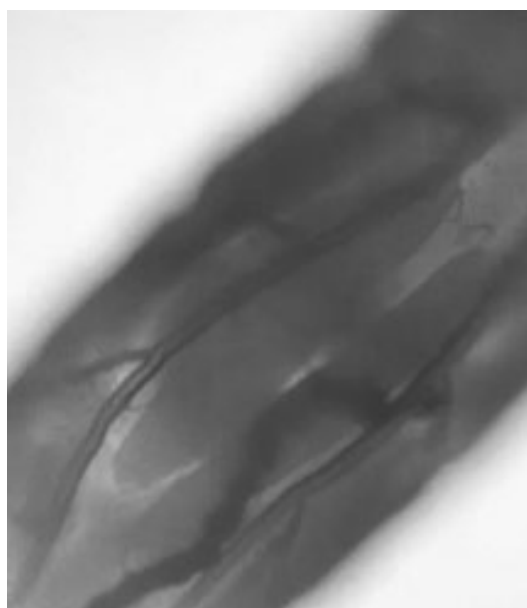


Figure 1: *D. mel* cardiac tube captured during the recording of heart rate.

The image was taken of a *D. mel* larvae under the 4X objective lens of a Leica compound microscope. The outline of the head of the cardiac tube can be observed. Surrounding the cardiac tube is a single layer of cardiomyocytes.

In a study identifying the genetic components of heart development and function, it was noted that the *D. mel* heart has only one chamber, referred to as the cardiac tube, and lacks coronary arteries (Seyres, Röder, and Perrin, 2012). This structure is different than that of a human heart, in which there are four highly vascularized chambers. Another study notes that the *D. mel* heart has only one layer of cardiomyocytes (heart cells) whereas the human heart has two sections of cardiomyocytes, the myocardium and endocardium (Medioni et al., 2009). Despite the anatomical differences though, the similarity in genetics and proteome present a convincing case for *D. mel* as a model of human cardiovascular disease.

Despite current advances in understanding genomic similarities between *D. mel* and other organisms typically used as models, it is still not known whether the *D. mel* cardiovascular system is capable of responding similarly to heart medication. Understanding physiological responses of the *D. mel* heart to heart medication could further qualify it as a model of human cardiovascular disease and open new doors of research.

Atropine

Atropine is frequently prescribed to increase heart rate in instances of hypotension (“Atropine,” 2014). The drug is administered to those suffering from bradycardia, a condition of extremely low heart rate to increase firing of the SA node in the heart (Al, 2014).

Atropine increases heart rate in humans by preventing acetylcholine from binding to sinoatrial and atrioventricular nodes. It does this by blocking muscarinic acetylcholine receptors (mAChRs) and, as a result, contraction of these pacemaking nodes increases (Kinkade, 2012). For this drug to effect *D. mel* in a similar fashion, the organism must have conserved receptors with the same capability of being blocked by atropine. One study determined which G-protein receptors in *D. mel* were coupled to mAChRs, the active site of atropine (Ren, Folke, Hauser, Li, and Grimmlikhuijzen, 2015). They found that mammals have five mAChRs and that *D. mel* has only two, an A-type and B-type. Specifically, the A-type mAChRs in *D. mel* were determined to have a similar structure to the mammalian receptors. For these receptors to function similarly to human mAChRs, the *D. mel* heart would need to be similarly innervated. It was originally thought that the *D. mel* heart was not innervated. It was discovered, however, that the *D. mel* heart is indeed innervated (Dulcis and Levine, 2003). At the larval stage, heart rate is controlled by a pacemaker structure thought to be located in the caudal region of the heart. With all of this in mind, it is still not known whether these receptors can invoke the same response on the innervating structure of the *D. mel* heart. If the mAChRs of *D. mel* can respond to atropine similarly to humans, this would support our knowledge of *D. mel* as a model for human cardiac disease and medicinal research.

Propranolol hydrochloride

While the effect of atropine increases heart rate, propranolol hydrochloride is a β -blocker that decreases heart rate and is prescribed by physicians for patients suffering

from heart failure (Coppola, Froio, and Chiumello, 2015). Results of one study suggest that β -blockers benefit patients by reducing heart rate and thereby inducing relaxation which may have an effect on diastolic filling of the heart (Dobre et al., 2007).

β -blockers reduce heart rate by blocking *beta1-adrenergic receptors* (β ARs), reducing sinoatrial node automaticity and therefore heart rate (Gibson and Raphael, 2014). A study measuring the effects of β ARs agonists versus antagonists on *D. mel* showed that antagonist β -blockers, such as propranolol hydrochloride, decreased mortality by 6.4% (Spindler et al., 2013). The same study also showed that *D. mel* does not have β ARs but possesses a family of G-protein receptors that are structurally and functionally related to β ARs. However, it is not known whether treatment with propranolol produces an effect on heart rate. If the β AR-like receptors in *D. mel* can respond to propranolol hydrochloride in a similar way to humans, this would further support *D. mel* as a model for human cardiac disease and medicinal research.

The Effects of Heart Medication on *D. mel*

While there is proteomic support for a conserved mechanism of regulation, it is unknown if heart medications such as atropine and propranolol hydrochloride have a similar effect on heart rate. We hypothesized that atropine and propranolol hydrochloride in the growth media of third instar larvae would cause an increase and decrease respectively in the heart rates of *D. mel*. Characterization of the pharmacologic activity of these two drugs on *D. mel* cardiac activity would further clarify if there are conserved mechanisms of heart rate regulation between the invertebrate *D. mel* and mammals and would lend further support to using *D. mel* as a model for human cardiac disease.

METHODS

Materials and *D. mel* Culturing

Wild type *D. mel* were purchased from Carolina Biological and atropine and propranolol hydrochloride were purchased from Sigma Aldrich. To maintain a constant living environment, the flies were kept at 22°C and transferred to new vials to mate. The vials were made by mixing equal amounts of Instant Drosophila Medium purchased from Carolina Biological with sterile deionized water in drosophila culturing vials with sponge plugs. The flies were given several days to lay eggs, and once larvae were seen in the vials the adults were moved to new vials to repeat the process and obtain stock vials of adult flies.

Treatment with Atropine and Propranolol Hydrochloride

The experimental procedure required a time span of seven days to complete (Figure 2) and was completed three times in succession. On the first day, adult flies from stock vials were placed in three new vials to mate. Over the following four to five days, the adult flies laid eggs in the medium that hatched into larvae and began to mature. By the sixth day, the larvae reached the second instar larval phase. Larvae at this stage were preferred due to their burrowing nature in which they tunnel deep into their growth media, thereby increasing exposure to medication in the media. Second instar larvae were transferred to

fresh vials that contained the experimental treatment. For the control group, fifty second instar larvae were moved to vials containing media made with sterile deionized water. For the first experimental group, fifty second instar larvae were transferred to vials with 1mM propranolol hydrochloride in the media. For the second experimental group, fifty second instar larvae were transferred to vials with 1mM atropine in the media. 1mM concentrations of each medication were utilized after performing a preliminary test of a tenfold range of molar concentrations, of which 1mM concentrations were found to display an effect on heart rate without mortality of the larvae. The second instar larvae inhabited the new vials for twenty-four hours, maturing into third instar larvae during this time at which point heart rates were observed.

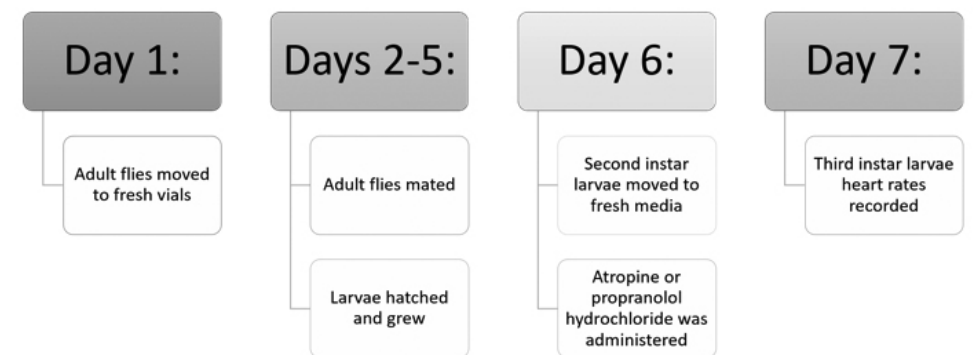


Figure 2: Timeline of procedure detailing when flies were moved to new vials and when heart rates were recorded. On Day 1, adult flies were moved to new vials to mate. Over the next few days, the flies were left to lay eggs. Once the larvae hatched from the eggs and entered the second instar larval stage of development, they were moved to fresh media. At this stage, atropine or propranolol hydrochloride were administered to the media. After twenty-four hours, the third instar larval heart rates were recorded.

Heart Rate Assessment of Third Instar Larvae

The heart rate of each third instar larvae of the control and treatment groups was observed three times in fifteen second intervals. Larvae were removed, placed individually on a microscope slide, and observed using the 4X objective lens of a Leica compound microscope. Beating hearts were visualized through the transparent skin of the dorsal side when larvae were placed on their ventral side. Once the microscope was properly focused on the larva, a fifteen second timer with a ten second interval of rest was started. A tap counter was used to count the number of times the heart beat during each fifteen second interval. After a ten second rest, the process was repeated until a total of three heart rates had been recorded for the larva.

Values were averaged for each larva, with the resulting fifty averages used to calculate the average heart rate and standard error for the group. To determine the significance of the two experimental groups from the control group, a T-Test was performed. The statistical analysis was done using Microsoft Excel.

RESULTS

Second instar *D. mel* larvae were treated with 1mM atropine or 1mM propranolol hydrochloride for twenty-four hours, after which the heart rates of fifty larvae were assessed at the third instar larval stage for each treatment. These averages were compared to that of untreated control larvae (**Figure 3**). Atropine treatment resulted in a heart rate of 406 ± 3.18 beats per minute, which represented a 4.83% increase in heart rate over control hearts (388 ± 2.07 bpm). On the contrary, propranolol hydrochloride treated larvae had heart rates of 275 ± 2.70 beats per minute, which was a 29.18% decrease in heart rate when compared to controls. Both changes in heart rate were statistically significant ($p < 0.001$). These results indicate that both heart rate medications influenced a change in the heart rates of *D. mel* larvae.

DISCUSSION

This research aimed to test whether *D. mel* larvae can respond similarly to humans when treated with heart medication. We observed a significant difference in the heart rates of the larvae that were treated with propranolol hydrochloride. Control larvae had an average heart rate of 388 ± 2.07 beats per minute while the propranolol hydrochloride treated larvae had an average heart rate of 275 ± 2.70 beats per minute ($p < 0.001$). This supports the hypothesis that propranolol hydrochloride decreases the heart rate of third instar larvae and demonstrates that *D. mel* responds to heart medication similarly as humans. Further, we also observed a difference in the heart rates of the larvae treated with atropine. Larvae treated with atropine had an increased heart rate of 406 ± 3.18 beats per minute ($p < 0.001$). This supports the hypothesis that atropine increases the heart rate of third instar larvae and demonstrates that *D. mel* can respond to heart medication similarly as humans.

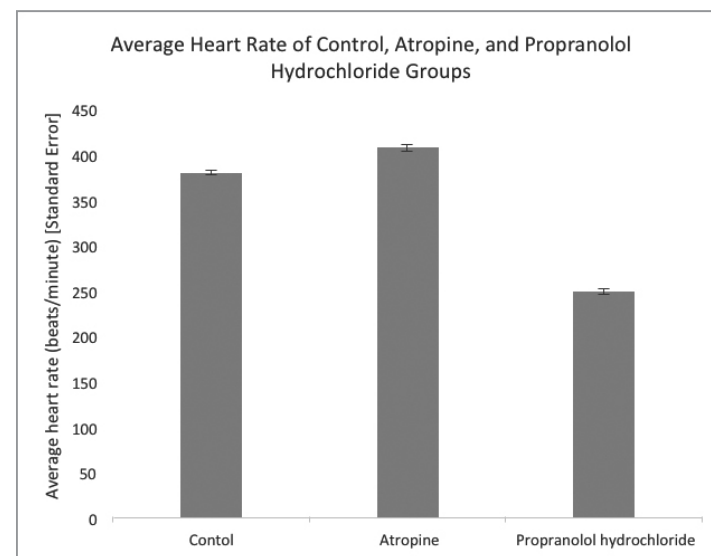


Figure 3: Atropine and Propranolol Hydrochloride act as respective positive and negative chronotropes of *D. mel* heart rates.

Average heart rates of treated third instar larvae in the control, atropine (1mM), and propranolol hydrochloride (1mM) groups were assessed by observing the heart rate using the 4X objective lens of a *Leica* compound microscope. Heart rates were recorded in triplicate for fifty in each group. Data are presented as mean plus and minus the standard error. Respective increase and decreases of heart rate due to atropine and propranolol hydrochloride treatments were statistically significant ($p < 0.001$).

The effects of the two medications on the heart rates of *D. mel* support previous work that demonstrated the conserved mAChRs and β ARs in *D. mel*. One study determined the protein content of the two mAChRs found in the *D. mel* genome (Ren et al., 2015). They concluded that one of the two mAChRs is pharmacologically similar to that of humans; however, they did not demonstrate that *D. mel* was capable of responding to atropine in a similar way to humans. The increase in heart rate due to atropine supports this research, further supporting the notion that the mAChRs in *D. mel* are pharmacologically similar to human mAChRs. Another study used propranolol hydrochloride to study the effects of β -blockers on the lifespan of *D. mel* (Spindler et al., 2013). In this research, it is explained that *D. mel* do not have the same β ARs as humans but rather utilize a family of G-protein receptors that may function very similarly to that of humans. The decrease in heart rate due to propranolol hydrochloride that was observed in this study would support this research and suggest that the heart of *D. mel* has conserved receptors capable of responding to the β -blocker propranolol hydrochloride in a similar manner to humans.

To evaluate the similarity between the effects of atropine and propranolol on the heart rates of *D. mel* larvae and humans, it is necessary to compare the results of this experiment to those found by other studies. After the administration of atropine, there was an observed 4.83% increase in larval heart rate. One study, which measured heart rate after the endobronchial administration of atropine in humans, found a 16% increase in heart rate at a dosage of 0.02 mg/kg (Paret et al., 1999). The difference between this 16% increase in heart rate from the observed 4.83% increase in heart rate is due to differences in atropine dose as well as the method of delivering the medication. Another study found that atropine increased heart rate in humans by 13.1 beats per minute (bpm) after 80 minutes at a dosage of $0.15 \mu\text{g}/\text{kg}/\text{min}$ (Bruck, Ulrich, Gerlach, Radke, and Brodde, 2003). The difference between the 13.1 bpm increase from the approximately 18 bpm increase observed in this experiment is due to differences in dose. There was also an observed 29.18% increase in larval heart rate after the administration of propranolol hydrochloride. In one study, participants who received 40mg of propranolol hydrochloride were found to have resting heart rates of 62 bpm after five hours as opposed to 72 bpm in participants who received the placebo (Joannides et al., 2006). The difference in recorded decrease in heart rate is due to the difference in dose administered and time between doses. Larvae in this experiment were exposed to propranolol hydrochloride for twenty-four hours before heart rate was recorded; however, participants in the study described above were exposed to the drug for only five hours. This difference in time could influence the observed decrease in heart rate in the two experiments. Through comparing the results of this experiment to those of other studies, it is apparent that the effects of the drugs on the heart rates of *D. mel* larvae are similar. To confirm this conclusion, future studies need to employ concentrations of the drugs that more closely parallel those used in human studies.

There were limitations in the study design that should be recognized and improved upon in future research. Most importantly, the research should have been completed as a blinded study. This would allow for experimental recording of heart rates while

unaware of the group being observed. Performing the experiment in this way would eliminate the possibility of bias. Further, there are more precise methods of recording heart rate that could have been used. At heart rates as rapid as that of *D. mel*, human error in manually counting is inevitable. With additional funding, software could be purchased which would allow for computer-generated analysis of videos of the beating larval heart that would more accurately assess the heart rate (Vogler and Ocorr, 2009).

While further research will add to our knowledge in this area, the data collected in this experiment suggest that the *D. mel* heart responds to atropine and propranolol hydrochloride as the human heart does. If this is indeed the case, future research could be performed to discover whether *D. mel* has the capability of responding to other heart medications. In the future, *D. mel* could be used as a model organism for research being performed on the effects of these heart medications.

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Vocal Processing with Spectral Analysis

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ABSTRACT

A well-known signal processing issue is that of the “cocktail party problem,” which refers to the need to be able to separate speakers from a mixture of voices. A solution to this problem could provide insight into signal separation in a variety of signal processing fields. In this study, a method of vocal signal processing was examined to determine if principal component analysis of spectral data could be used to characterize differences between speakers and if these differences could be used to separate mixtures of vocal signals. Processing was done on a set of voice recordings from thirty different speakers to create a projection matrix that could be used by an algorithm to identify the source of an unknown recording from one of the thirty speakers. Two different identification algorithms were tested. The first had an average correct prediction rate of 15.69%, while the second had an average correct prediction rate of 10.47%. Additionally, one principal component derived from the processing provided a notable distinction between principal values for male and female speakers. Males tended to produce positive principal values, while females tended to produce negative values. The success of the algorithm could be improved by implementing differentiation between time segments of speech and segments of silence. The incorporation of this distinction into the signal processing method was recommended as a topic for future study.

Keywords: vocal processing, spectral analysis, principal component analysis

INTRODUCTION

The digital age has produced a demand for signal processing techniques in various areas of study [1,2]. One such demand that has proved to be particularly difficult to address has been in the area of signal source separation; specifically, there is a call for a solution to the “cocktail party problem” [1, 3]. The cocktail party problem refers to the phenomenon experienced by humans in instances of a large gathering. When in a crowded room, one may be holding a conversation with another in the midst of various other voices speaking in the same vicinity. Little is known about the brain processes occurring during the processing of speech with background noise, yet unimpaired individuals are able to separate and group different sounds according to their origin while focusing on a single vocal signal [3]. This paper proposes a vocal analysis method that seeks to emulate this process through the production of a data projection matrix. This matrix is used to characterize unknown vocal signals with the goal of identifying their sources from within a set of recorded voices.

REVIEW OF LITERATURE

Linguistic Theory

Much of modern linguistic theory contains discussion on the components of speech, both from the perspective of production and of perception. Bowers, Kazanina, and Andermane explain that the traditional view is that a group of linguistic units known as phonemes can be used to represent the basic units of speech in a language [4]. The English language, for example, is commonly represented as having forty-four attributed phonemes, including

sounds created by each letter of the English alphabet in addition to some sounds created by the combination of letters, such as /sh/, /th/, or /ch/ [4]. It is the arrangements of these phonemes that form the words available in a language.

While this is the traditional starting point from which speech is studied, it is not accepted without question as the ultimate representation of linguistic patterns. Phonemes can be broken down further into phones, which represent the unique ways in which a single phoneme can be pronounced, namely due to its orientation in a word [4]. For instance, the phoneme /t/ is articulated differently when oriented at the beginning of a word, as in “two,” and in the middle of a word, as in “steak.” Most arguments against phoneme theory advocate for a greater complexity and contextual nature of phoneme recognition [4, 5]. For instance, it has been shown that phoneme recognition does not occur as clearly outside of the context of speech as within speech [5]. Further, the perceptual learning of phonemes can be specific to the voice of the speaker and the ear of the listener, adding more to the complexity of phoneme recognition [4]. However, despite these challenges, this needn’t lead to the full dismissal of phoneme theory. Indeed, phoneme recognition is still used by researchers as a basis for measuring the quality of speech identification algorithms [6]. The fact that phoneme recognition can be altered depending on the context of the voice speaking implies a difference in phoneme production among speakers [5]. The goal of the method presented in this paper was to identify components of uniqueness among different speakers for the purpose of source separation; these components were identified through signal processing techniques including blind source separation and spectral analysis.

Blind Source Separation

The history of analysis techniques used to approach issues regarding speech processing has been relatively inconsistent and scattered. Researchers Hu and Loizou described how the comparison of algorithms developed for speech enhancement is highly difficult due to the variety of ways in which these methods are presented [7]. Inconsistencies between methodologies and the absence of a common speech database reference are just a couple of the issues that stand in the way of clear comparison. In general, however, algorithms developed for the purpose of addressing the cocktail party problem do tend to fall under a common approach known as blind source separation [8]. Practical use of an algorithm that separates vocal signals, especially in real-time applications, requires the use of this approach. In blind source separation, a system receives a mixture of signals in a single input. The goal of the approach is to determine the original signals that have been mixed together. This approach has applications in many areas of signal processing, including those surrounding processing speech [8]. In the case of mixed voices, the mixed input signal’s original source signals are the vocal signals from each individual. The practicality of blind source separation comes from its ability to perform source separation without prior knowledge of the signal sources.

Buchner and Aichner explained the standard approach to blind source separation [1]. Typically, blind source separation problems are approached with the assumption of a reverberant environment [1]. For example, this would hold true for many instances of

the cocktail party problem, where sound waves from multiple speakers will bounce off of walls or objects and be projected around the room accordingly. Signals are often measured via multiple inputs, allowing the system to utilize reverberation as an aid in the process of separating signals. These are often referred to as multiple-input-multiple-output (MIMO) systems [1]. Such processes have practical application in certain areas of signal processing, including those related to imaging, due to the greater likelihood that certain systems may practically allow for multiple data input streams. However, in the area of speech processing, a multitude of data inputs is not always practical. Applications such as voice recognition on mobile devices require the use of a single microphone, or at best, multiple microphones in very close proximity. Because of this, alternative source separation and spectral analysis methods must be examined in order to meet the needs of real-world applications.

One way in which this single input-stream requirement can be addressed is by measuring signals against a predetermined set of standard signal components. The work of Sirovich and Kirby, who worked in the field of facial recognition, is a classic example of this method [9]. They developed a methodology in which singular value decomposition was utilized to develop a set of images that formed the “building blocks” of constructing the picture of a face [9]. In terms of their usage of singular value decomposition, these images could be classified as eigenvectors. By this definition, the eigenvector images could be summed together, each scaled by a corresponding eigenvalue, to form an image of a face. Over time these eigenvector images came to be known as “eigenfaces.” In a similar way, this research aims to develop a set of eigenvectors which may be used to separate and reconstruct human speech.

Spectral Analysis

Often, the data available from the initial form of a signal may be insufficient for the purposes of source recognition or separation. In such cases, it becomes beneficial to perform certain operations on the signal that allows them to be observed from different perspectives. One widely used technique is known as the Fourier transform [10]. The namesake of this operation, Joseph Fourier, postulated that any signal could be observed as the summation of a set of sinusoidal waves [11]. The Fourier transform allows a signal to be decomposed into a set of cosine or sine waves that vary in amplitude and frequency, according to the original signal [11]. This operation transforms the original signal from the time domain to the frequency domain. The resulting frequency components are known as spectra. These spectra can be observed and analyzed to determine significant pieces of information about the composition of the original signal. It is the observation of these frequency spectra that is known as spectral analysis. Nakatsuji and Omatu demonstrated the use of the Fourier transform as a preprocessing technique used in real-time spectral analysis [10]. In their research, spectral analysis was repeated as new samples were added to the initial set, which would consistently update the spectral data to reflect the current data set [10]. This approach provides an expansion on traditional spectral analysis, which typically utilizes a single initial instance of a Fourier or equivalent transform to produce frequency data that is then compared with all later data [10].

Despite its usefulness across a wide variety of fields, the Fourier transform in its classic

form is a relatively lengthy computational process by modern standards. This has posed an issue with the use of the Fourier transform in fields where computational speed and efficiency are limiting factors [2]. Such fields include that of speech processing, where research on signal separation is done for the benefit of devices such as hearing aids or speech-to-text applications. In such devices, which often utilize embedded systems, the allowable complexity of an algorithm is limited by memory storage and computational speed.

In these applications it becomes useful to analyze data using a modified Fourier transform known as the fast Fourier transform [2]. This transform requires fewer computations than the traditional discrete Fourier transform, making it a commonly used technique. Paèz and Garzòn demonstrated that the fast Fourier transform could be used in a spectrographic analysis application that offered a reduced computation time when compared to equivalent applications used by traditional entities such as MATLAB [2]. In doing so, they showed that the fast Fourier transform has practical use in increasing the efficiency of spectral speech analysis in embedded systems [2].

Independent and Principal Component Analysis

Among researchers addressing issues of signal processing and source separation there are two widely used techniques known as Principal Component Analysis (PCA) and Independent Component Analysis (ICA). Both of these techniques have seen use in the field of data processing, namely for the purpose of data reduction and analysis [12]. It is important to distinguish between these two techniques in order to understand the specific benefits of each in different applications. Both techniques aim to decompose a set of data into generalized components. In PCA, correlation between portions of the original data set is used to determine the most common, and thus most significant, components of the set [12,13]. These components are compiled and listed with the goal of eliminating redundancy by containing as much significance as possible within each component [12]. The most significant difference between this approach and that of ICA is that PCA utilizes some sample data or prior knowledge to be used in the decomposition process. It requires previous information about the sources being separated, making it a difficult technique to be utilized effectively in a true problem of blind source separation [12]. ICA was developed in response to this shortcoming as a more adaptable form of PCA [12]. ICA performs a similar function, but begins only with the assumption that the data set to be examined is a linear mixture of independent source signals [12]. The lack of a necessity for prior information on the sources of the mixture has made ICA a widely utilized technique in the field of blind source separation [14]. However, ICA alone tends to be insufficient in particularly complex problems, as the number of mixture signals usually must be greater than the number of source signals [14]. Most methodologies involving the use of this technique require pairing it with an additional signal-identification technique [8]

Mori et al. described a step in the right direction by utilizing independent component analysis with a binary masking technique in order to overcome reliance on a MIMO system [8]. They implemented ICA based on a single-input-multiple-output (SIMO)

model and experimented with the algorithm's ability to separate mixtures of vocal signals [8]. This methodology was shown to significantly improve upon the performance of ICA alone [8]. Interestingly, the process still utilized multiple microphones to produce the greatest accuracy, but each input was analyzed individually using the SIMO model [8]. Mori et al. described the algorithm as being effective when the number of mixed vocal signals was less than or equal to the number of microphones utilized as inputs [8]. Thus, the ability to perform vocal source separation with fewer—and ideally, a single—microphone remained an unanswered problem.

Lu et al. made significant progress in terms of developing a blind source separation algorithm truly meant for a single stream of input data [14]. Their process combines ICA with higher-order statistics to extract significant component data from a single input stream [14]. The process was shown to be a successful starting point for algorithms seeking to answer the call for single-channel blind source separation [14]. It was noted that the algorithm was limited, like most, on the number of original source signals that could be separated [14]. Although this was a step forward for the problem of blind source separation, this particular process is not necessarily aimed towards the issues surrounding the cocktail party problem specifically. To create successful blind source separation algorithms for more specific applications, it is necessary that work be done to bridge the gap between the particular nuances of such applications and generalized source separation techniques such as this.

One of the key inspirations of this research was a study performed by Makarewicz and Makarewicz in the field of source separation [13]. In their study, they examined a possible method of addressing the problem of remotely determining the mineral content of pyroxene mixtures. The goal of such research was to develop methods by which the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) might analyze the mineral content of soils with which it comes into contact. They examined light spectra produced by pyroxene mixtures, using PCA to decompose this information into eigenvectors. Examination of these eigenvectors as principal components was used to correlate them with properties of the mixture, such as the percent clinopyroxene. This correlation was then used to create a projection matrix by which unknown samples could be characterized. This process was successful in characterizing composition and grain size of unknown mixtures. Here, we sought to enact a similar methodology directed towards the separation of vocal signals. In a manner similar to the studies put forward by Makarewicz and Makarewicz [13], as well as by Kirby and Sirovich [9], an algorithm was created to identify a set of speech eigenvectors which can be used to characterize unknown speech samples and mixtures via a projection matrix.

METHODS

Participants

Vocal samples gathered for this research were obtained voluntarily from thirty individuals, including sixteen male and fourteen female participants. Twenty-eight of the participants fell within the range of eighteen to twenty-three years of age, while two were forty-seven years of age. Twenty-nine of the participants were Caucasian,

and one female was of Hispanic heritage.

Materials

The script used in all voice recordings was obtained from a short story titled “Arthur the Rat” [15]. This story was used by the Dictionary of American Regional English (DARE) in the collection of voice samples from across the United States during fieldwork completed between 1965 and 1970 [15]. The passage is specifically designed to include phonetic representation of all phonemes present in American English [15].

All voice samples were recorded on a Samsung Galaxy S7 smartphone using the microphone of a standard Samsung headset. Windows Movie Maker was used to edit recordings, and VLC Media Player software was used for the conversion of audio files. All further data processing and analysis was completed using FreeMat, a free, open source coding environment similar to products such as MATLAB.

Data Collection

Participants were recorded in a quiet room and asked to read the entirety of the “Arthur the Rat” passage with a natural tone, comfortable pace, and slightly raised volume for the sake of producing clear recordings. The microphone was held by the speaker at a distance of approximately six inches in front of the mouth. The speakers were told to not stop due to any mistakes in pronunciation or reading that may occur during the recording.

Any mistakes which caused the speaker to deviate from the given script as well as additional comments made by the speaker were later removed from the recording using Windows Movie Maker and saved as an mp4 file. These files were then imported into VLC Media Player to be converted to a standardized audio format. Each mp4 file was converted to a waveform audio file (WAV) format with a single channel, a bitrate of 88 kilobytes per second, and a sample rate of 11025 Hertz. The bitrate and sample rate were chosen to increase efficiency by limiting the amount of data that would need to be processed for each vocal sample.

WAV files were imported into FreeMat for processing as follows. The data from the recording was divided into time segments with a 90% overlap between consecutive segments and 4096 data samples per segment. This segmenting of the recording provided small samples of data of identical sizes for all recordings; all remaining processing of the recordings was done according to these segments. The fast Fourier transform of each time segment was calculated to produce a frequency spectrum for the sample and was stored in an n by 2048 spectra matrix, where n represented the number of time segments created for the audio file. Each spectrum was normalized before being stored into the spectra matrix in order to account for differences in volume between speakers. **Figure 1** illustrates sample spectra of one recorded speaker.

Singular Value Decomposition

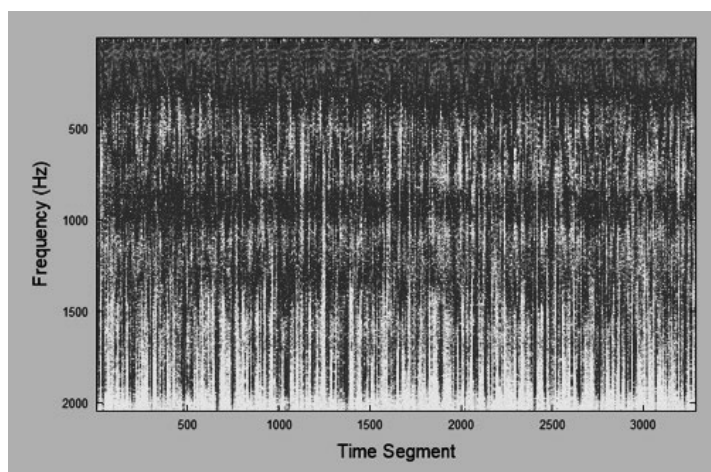


Figure 1. Normalized frequency spectra for each time segment from Speaker #1. Frequency spectra represent the results of the Fast Fourier Transform on the original recording's time segments.

Following the production of the frequency spectra for each recording, the spectra were each individually processed. Singular Value Decomposition (SVD) was performed on each set of spectra representing the thirty speakers, producing thirty unique sets of principal values and principal vectors. The SVD function orders the produced principal vectors in order of significance, placing the most significant vectors at the front of the matrix. The first fifty, and thus the fifty most significant vectors, were saved for each of the thirty spectra sets.

This process was completed for all thirty recordings, producing thirty sets of fifty principal vectors. These matrices were then concatenated into a single matrix on which SVD was completed a final time. This produced a set of 1500 principal vectors representing the full span of the thirty recorded vocal signals. This set was saved as the final principal vectors matrix, Matrix A; this matrix was not changed from this point forward in the process. Matrix A was then used to create a projection matrix of principal values that characterized each original speech signal in terms of these principal vectors. This was completed by reproducing the spectra for each speaker in the same manner described before. A principal value matrix was calculated by multiplying a speaker's spectra matrix by the Matrix A. Then, for every speaker, the principal values corresponding to a single principal vector were averaged and the standard deviation found. Matrix B stored the averaged principal values for each speaker, and Matrix C stored the standard deviations of the principal values for each speaker. Data from Matrix B and C for the ten most significant principal vectors is presented in the Appendix. This completed the preprocessing of data into projection matrices that would then be used to predict speakers.

Speaker Prediction – Algorithm 1

The difference between the two algorithms came in how weights were applied to each principal value in the set. A principal value that has a larger general magnitude among all the speakers is one that is more significant and prevalent throughout all the samples. Because of this, the weight given to each principal value in Algorithm 1 was calculated by summing the magnitudes of a particular principal value for all speakers, shown

as the vertical columns in Appendix A. Then, Z-scores were calculated by dividing the difference between the measured principal value and the average principal value of a known speaker from the Appendix A database by the standard deviation of that principal value for a known speaker from the Appendix B database. These Z-scores were calculated for each measured principal value and possible speaker combination. Finally, the measure used to compare speaker possibilities was calculated by summing the weight multiplied by the Z-score of every principal value for a known speaker. The calculation used to produce these values is shown in **Equation 1**,

$$M = \sum a - \mu / \sigma * W$$

where:

M = Z-score sum,

α = measured time segment principal value,

μ = average speaker principal value,

σ = speaker's principal value standard deviation, and

W = principal vector weight.

This produced a single, weighted Z-score sum M for every possible speaker from the database. The speaker with the lowest value M was chosen as the predicted speaker for the given time segment. This process was completed for each time segment in the spectra set, allowing the algorithm to predict which speaker produced the recording for every time segment.

Speaker Prediction – Algorithm 2

Algorithm 2 followed a similar methodology to the first. The spectra and principal values for the unknown speaker were produced in the same manner. However, the weights applied to each principal vector were removed. As stated, the SVD function used to calculate the final principal vectors matrix arranges the principal vectors in order of significance, placing the most significant vectors at the front of the matrix. Thus, the first columns in the principal value database represent the most significant data. For this algorithm, it was chosen to only calculate Z-scores for the first ten sets of principal values from the database. Z-scores were calculated and summed in the same manner as in Algorithm 1. No weights were applied to the calculated Z-scores. The calculation used to produce the Z-score sum to be compared between speakers is shown in **Equation 2**,

$$M = \sum_{(i=1)}^{10} a - \mu / \sigma$$

The speaker with the lowest Z-score total was again chosen as the algorithm's predicted speaker for a given time segment. Algorithm 2 was completed for the full duration of each original voice recording to determine its effectiveness at determining source speakers.

RESULTS

Thirty speakers were recorded while reading the given script. These recordings were processed by performing singular value decomposition on the spectral data from each recording to produce a projection matrix, which was used in the development of two speaker identification algorithms. The algorithms were developed in FreeMat to predict a speaker for every time segment of a given recording. Both algorithms were run for the full duration of all thirty recorded speakers. A plot of the predictions made by the algorithm for a given time segment of a recording is shown in **Figure 2**. The accuracy of Algorithm 1 was computed for each speaker and displayed in Table I. The correct prediction rate represents the percentage of time segments of a given speaker for which the algorithm correctly predicted the identity of the speaker. Overall, the algorithm had an average correct prediction rate of 15.69%, with a standard error of 3.93%. These rates ranged from 0.22% to 85.69%. Algorithm 1 performed notably well with Speaker 8, which produced the highest correct prediction rate of 85.69%. The accuracy of Algorithm 2 was computed for each repetition and displayed in Table II. Overall, the algorithm had an average correct prediction rate of 10.47%, with a standard error of 2.82%. These rates ranged from 0.00% to 65.83%. As with Algorithm 1, this algorithm performed the best with Speaker 8, producing the highest Algorithm 2 correct prediction rate of 65.83%.

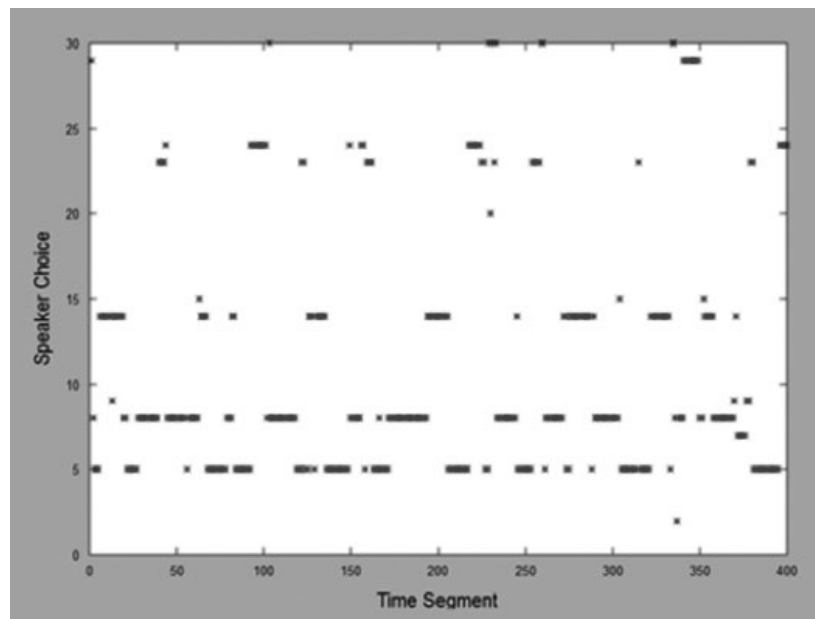


Figure 2. Algorithm 1 speaker predictions for first 500 time segments of Speaker 5. The vertical axis identifies Speaker choices 1-30, while the horizontal axis identifies the segment of time analyzed. Each “*” symbol shown on the plot represents a speaker prediction made by the algorithm during an iteration for each time segment.

TABLE 1. ALGORITHM 1 ACCURACY.

Table 1 notates the percentage of time segments Algorithm 1 correctly predicted the recording's speaker for each of the thirty recordings. Algorithm 1 had a correct prediction rate greater than 10% for only twelve recordings.

Recording	Correct Prediction Rate	Recording	Correct Prediction Rate
1	0.64%	16	1.04%
2	11.18%	17	3.34%
3	1.67%	18	15.91%
4	1.13%	19	2.04%
5	24.87%	20	1.23%
6	0.36%	21	6.61%
7	1.65%	22	23.20%
8	85.69%	23	43.59%
9	8.49%	24	73.88%
10	0.22%	25	9.69%
11	1.14%	26	2.04%
12	0.00%	27	24.38%
13	1.99%	28	1.68%
14	36.97%	29	36.82%
15	16.11%	30	18.14%

TABLE 2. ALGORITHM 2 ACCURACY.

Table 2 notates the percentage of time segments Algorithm 2 correctly predicted the recording's speaker for each of the thirty recordings. Algorithm 2 had a correct prediction rate greater than 10% for only nine recordings.

Recording	Correct Prediction Rate	Recording	Correct Prediction Rate
1	0.00%	16	1.87%
2	3.41%	17	5.35%
3	4.51%	18	10.67%
4	0.97%	19	4.72%
5	30.31%	20	3.88%
6	0.58%	21	3.18%
7	1.46%	22	14.59%
8	65.83%	23	40.79%
9	0.74%	24	38.45%
10	0.58%	25	17.25%
11	0.39%	26	1.37%
12	0.08%	27	14.56%
13	0.97%	28	0.12%
14	24.60%	29	8.43%
15	3.43%	30	18.14%

DISCUSSION

This research had two objectives, the second of which depended upon the success of the first. The first objective was to determine whether principal component analysis of spectral voice data could be used to identify differences between speakers. Success in this objective would be characterized by an algorithm predicting the correct speaker for at least 70% of the recording for each of the thirty speakers, which was not met with the utilized methods. Algorithm 1 was unable to correctly guess the speaker of a recording for a majority of the total time segments analyzed. For eighteen out of the thirty recordings, the algorithm correctly guessed the speaker less than 10% of the recording. Algorithm 2 resulted in a slight decrease in performance when compared to Algorithm 1, still yielding an insufficient success rate. For nineteen out of the thirty recordings, Algorithm 2 correctly guessed the speaker less than 10% of the recording. Thus, both algorithms yielded similar results, with neither being able to consistently identify an unknown speaker for more than 70% of the speaker's recording.

It is worth noting that in both algorithms, a few individual speakers were guessed overwhelmingly often no matter which speaker was actually present in the recording. For instance, Algorithm 1 had a strong tendency to guess Speaker 8 and 24 for all of the recordings. This is likely the reason these few speakers had higher percentages of accuracy. This would imply that the few higher-performing recordings likely resulted from the tendency of the algorithm to become too focused on some characteristic of a certain speaker rather than a legitimate recognition of the speaker.

The poor performance of the speaker identification algorithms was consistent with the data calculated and stored in the principal value and standard deviation Matrices B and C. By comparing the data in these two matrices, shown in Appendices A and B, it can be seen that the standard deviations for many principal value-speaker pairs were relatively high. In many cases, the standard deviation and average principal value for a principal vector and speaker were on the same order of magnitude, indicating a high level of variance in most principal value representation throughout an individual recording. This increases the likelihood that principal values between speakers will overlap, which increases the difficulty of attempting to classify the speaker based on these values. **Figure 3** illustrates a comparison of the representation of a particular principal value between two speakers. As is shown, a majority of the points from each speaker fall in the same general area on the plot. Ideally, the speakers would produce more separated clusters, which would indicate that the principal vector involved was a useful principal component to be used in recognizing differences between speakers.

One piece of useful and interesting information was obtained from the results of the study. Participants were grouped by gender in the speaker order, such that Speakers 1 through 16 represented males and Speakers 17 through 30 represented females. Upon examination of the average principal values for principal vector #4, shown in Appendix A, it was discovered that a distinction can be made between the average principal values for males and for females. This is illustrated in **Figure 4**. Male speakers tended to have negative values corresponding to principal vector #4, while females tended to have positive values. Table 3 compares the prediction of the gender of each speaker

to the actual genders of the speakers after using the sign of the average of principal vector #4 values to predict the speaker. This method correctly identified the gender of 87.5% of male speakers and 85.7% of female speakers. An analysis of the implications of this principal vector would be an interesting subject for future research. It could prove beneficial to further examine the specific representation of the vector across the spectrum of speakers in order to determine if there is an identifiable characteristic that is described by the principal component. No clear correlation between these values and a specific speech characteristic was found in the brief analysis of this principal vector.

TABLE 3. GENDER PREDICTION USING PRINCIPAL

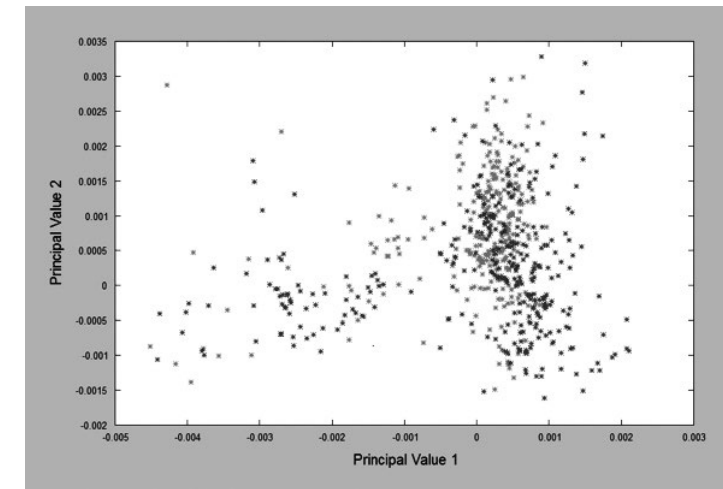


Figure 3. Interaction of two principal values for Speaker 1 (light) and Speaker 2 (dark). The principal values overlap between the two speakers for most of the region, making it difficult to use the interaction of the principal values to separate the speakers.

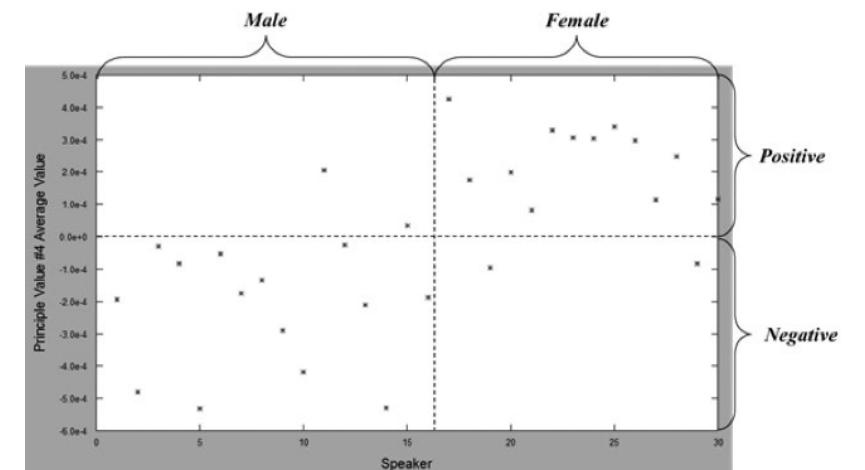


Figure 4. Comparison of principal values of principal vector #4 between males and females. Males tend to have negative principal values while females tend to have positive principal values, showing a potential for distinction between genders using principal vector #4.

VALUES OF PRINCIPAL VECTOR #4.

Table 3 shows the results of classifying the gender of a speaker according to the average principal value of principal vector #4 for the speaker.

Predicted \ Actual	Male	Female	Total
Male	14	2	16
Female	2	12	14
Total	16	14	30

A positive average value predicted a female, while a negative average value predicted a male. This method correctly identified fourteen out of sixteen males and twelve out of fourteen females.

Because the first objective of identifying spectral differences between speakers was unsuccessful, the second objective of separating mixed vocal signals was not attempted. This is because the developed algorithms were unable to correctly predict the source of a single-speaker recording, so they would not be effective in guessing the sources of mixed recordings. One potential source of error from the implemented methodology was the failure to distinguish between time segments where the speaker was talking as opposed to segments of no speech. The methods took no consideration of the difference between these two potential conditions, treating segments of silence identically as segments of speech. Addressing this issue would be a beneficial focus for continued study. Revising the computation method by eliminating the inclusion of data from segments of silence may prove better at highlighting the identifiable traits of different speakers.

A method of identifying differences between vocal signals through principal component analysis of spectral data was studied. The method was not successful in identifying differences such that they could be used to identify different speakers. One of the principal vectors created showed a difference between the corresponding principal values for males and females, identifying the vector as a potentially useful tool in identifying the gender of a speaker. More analysis of this vector is recommended to determine if it can be correlated with a specific characteristic of speech. In addition, future work is recommended in which the method used here be modified to better account for segments of silence from the speaker in a recording.

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APPENDIX A – PRINCIPAL COMPONENT VALUE AVERAGES DATABASE

Speaker	Principal Value Averages (x10 ⁻³)									
	PV #1	PV #2	PV #3	PV #4	PV #5	PV #6	PV #7	PV #8	PV #9	PV #10
1	0.0452	0.5662	0.2814	-0.1934	-0.2749	-0.7227	-0.8242	-1.1918	-0.7681	0.3751
2	0.3190	0.1517	0.2028	-0.4807	-0.4787	-0.7702	-0.8529	-1.4408	-1.1884	-0.3927
3	0.3015	0.0707	0.3259	-0.0300	-0.1625	-0.5728	-1.1162	-0.9804	-0.6102	0.8653
4	0.0366	0.0209	0.5392	-0.0824	0.0017	-0.2281	-0.6325	-0.8196	-0.5445	0.5931
5	-0.0791	0.0824	-0.1112	-0.5312	-0.4871	-1.2939	-0.8713	-1.4575	-1.3701	-0.2886
6	0.1408	-0.5756	0.3126	-0.0529	-0.2599	-0.1516	-0.9759	-0.8654	-0.6473	0.4085
7	0.0804	0.2443	0.2125	-0.1756	-0.0777	-0.5396	-0.6809	-0.8179	-0.7653	0.8757
8	-0.4990	0.4669	0.5833	-0.1350	-0.2172	-0.7032	-0.9615	-1.2488	-0.8943	0.8484
9	-0.1322	0.4463	0.2742	-0.2904	0.0078	-0.8190	-0.7830	-1.0411	-0.8435	0.7497
10	0.4358	0.5125	0.0233	-0.4175	-0.2654	-0.6145	-0.6428	-0.9316	-0.6248	0.6519
11	-0.1462	-0.0188	0.6042	0.2503	-0.1013	-0.6089	-1.074	-1.0096	-0.6970	0.4292
12	-0.0103	-0.0125	0.3685	-0.0251	-0.2963	-0.5653	-0.9377	-1.3918	-0.8695	0.3473
13	-0.0483	0.3446	0.0204	-0.2110	0.0100	-0.4681	-0.6503	-1.0603	-0.4283	1.0583
14	-0.0408	0.4588	0.0779	-0.296	-0.1844	-0.8445	-0.6822	-1.3058	-0.9105	0.6539
15	-0.0610	0.6312	0.5220	0.0341	0.0128	-0.4754	-0.7025	-1.0465	-0.5791	1.0855
16	0.0576	0.0695	0.1062	-0.1882	0.0251	-0.4045	-0.6532	-0.9177	-0.3895	0.8304
17	-0.2538	-0.1426	0.8340	0.4246	0.2567	-0.3045	-1.1500	-0.7884	-0.7352	0.8307
18	0.0391	0.3136	0.7607	0.1750	0.2813	-0.5528	-0.9035	-0.9280	-0.4043	1.1424
19	-0.1309	0.4559	0.6917	-0.0952	-0.2631	-0.8418	-1.1642	-1.0935	-0.8034	0.0357
20	0.2611	0.3345	0.8030	0.1986	0.0233	-0.3189	-0.8086	-0.6824	-0.5099	0.5250
21	-0.1126	0.6196	0.5727	0.0817	-0.2174	-0.6005	-0.8510	-1.3072	-0.6940	0.5982
22	0.0428	-0.1171	0.5259	0.3287	0.1095	-0.2379	-0.9821	-0.8300	-0.4992	0.7498
23	-0.0729	-0.1303	0.9140	0.3052	0.0291	-0.5530	-1.2767	-1.1022	-0.5934	0.5312
24	0.0172	-0.1421	0.8481	0.3033	0.3141	-0.5593	-1.0014	-0.8723	-0.8292	0.5440
25	-0.2938	0.2973	0.6129	0.3403	0.0004	-0.7349	-0.8868	-0.9079	-0.8620	0.1419
26	0.0114	0.0532	0.7529	0.2974	0.0350	-0.6165	-0.9651	-1.0628	-0.6633	0.3192
27	-0.9182	0.6916	0.7484	0.1126	0.1586	-0.0925	-0.3902	-0.3826	-0.5065	0.2003
28	-0.1699	0.2432	0.6477	0.2471	-0.0772	-0.3912	-0.9046	-0.7085	-0.5124	0.5651
29	-0.0668	0.1830	0.5966	-0.0833	0.0670	-0.6030	-0.7233	-0.9447	-0.5394	0.7478
30	-0.2687	0.7080	1.0226	0.1161	0.1683	-0.4511	-0.7025	-0.7335	-0.6888	0.2551

APPENDIX B – PRINCIPAL COMPONENT

VALUE AVERAGES DATABASE

Speaker	Principal Value Standard Deviation (x10 ⁻³)									
	PV#1	PV #2	PV #3	PV #4	PV #5	PV #6	PV #7	PV #8	PV #9	PV #10
1	1.1412	0.7337	0.7714	1.0605	0.8183	0.8736	1.1658	1.1392	1.1003	0.9014
2	0.9338	0.6400	0.6436	1.0013	0.7168	0.8664	1.0100	0.9494	0.9206	0.8596
3	1.3529	0.8303	1.0941	1.1936	0.9362	0.8367	1.3238	1.3067	1.3802	1.0125
4	0.9075	0.6293	0.6241	0.7811	0.8254	0.7272	0.9662	0.7528	0.9605	0.8383
5	1.2082	0.8143	0.7827	1.2978	0.8307	0.9203	1.3625	1.0379	1.4875	1.5200
6	0.9251	0.6792	0.5827	0.790	0.6861	0.6951	0.9524	0.8367	0.9797	0.8102
7	1.0907	0.8110	0.8357	1.3787	0.8713	0.9114	1.0779	0.9742	1.1179	0.7900
8	1.3874	1.0686	1.1201	1.4416	1.4362	1.2135	1.5373	1.5681	1.4813	1.3884
9	1.4497	0.8425	0.9118	1.3445	0.9625	1.0606	1.3920	1.2423	1.1751	1.0378
10	1.1252	0.6813	0.8175	1.1064	0.8218	1.0169	1.1782	1.1261	0.9978	0.8240
11	1.2407	0.916	0.8153	0.9668	0.9108	0.9010	1.4049	1.0943	1.3122	1.3668
12	0.9074	0.729	0.6289	0.9130	0.7141	0.6945	0.9137	0.9808	1.1010	1.1019
13	1.0916	0.8474	0.9174	1.0768	1.0417	0.9622	1.1328	1.0701	1.2463	0.8847
14	1.3222	0.8700	0.8489	1.5190	0.9987	1.1433	1.3402	1.1237	1.2353	1.1093
15	1.2529	0.8668	0.9741	1.2885	1.2176	1.0453	1.2751	1.1630	1.2363	0.9948
16	1.0230	0.7618	0.8534	1.2875	0.8644	0.9927	1.0653	1.0863	1.1876	0.8357
17	1.2422	0.9537	1.0037	1.0528	1.2919	1.0360	1.3629	1.2937	1.2299	1.1686
18	1.3224	0.8589	1.0925	1.5525	0.9669	1.3602	1.2417	1.5642	1.2074	1.0593
19	0.9760	0.7898	0.8882	0.9932	0.8899	1.2505	1.2028	1.0380	0.8923	1.0680
20	1.2615	0.9881	0.9593	1.2814	1.1996	1.3500	1.4718	1.4165	1.1257	0.9915
21	0.9673	0.8482	1.0705	1.4054	1.0254	1.3736	1.1511	1.4147	0.8843	1.0021
22	1.5144	0.9601	1.0178	1.5375	0.9787	1.3772	1.500	1.4284	1.3047	0.8619
23	1.4231	1.1126	1.0759	1.3719	1.3906	1.3676	1.6692	1.5724	1.4062	1.3103
24	1.4031	1.0358	1.1526	1.5463	1.2673	1.2452	1.592	1.5680	1.2194	1.0835
25	1.1523	1.0978	0.9455	1.0323	1.1598	1.0777	1.3552	1.2845	1.1695	1.1632
26	1.2364	0.8998	0.9090	1.2904	1.0969	1.2965	1.3391	1.3153	1.1160	1.0253
27	1.1827	0.8524	0.8138	0.9016	1.273	1.1157	1.2143	1.2279	0.9792	0.9708
28	0.9511	0.7838	0.6751	0.7797	0.9532	0.7541	0.9813	0.9664	0.8991	0.9255
29	0.9358	0.8013	0.8805	1.2616	0.9327	1.2047	1.0931	1.1953	0.8972	0.9335
30	2.5023	1.1500	1.2359	0.9633	0.8473	0.8513	0.7499	0.5440	0.5739	0.5422

APPENDIX C – “ARTHUR THE RAT” SCRIPT [15]

“Once there was a young rat named Arthur, who could never make up his mind. Whenever his friends asked him if he would like to go out with them, he would only answer, ‘I don’t know.’ He wouldn’t say ‘yes’ or ‘no’ either. He would always shirk making a choice. His aunt Helen said to him, ‘Now look here. No one is going to care for you if you carry on like this. You have no more mind than a blade of grass.’

One rainy day, the rats heard a great noise in the loft. The pine rafters were all rotten, so that the barn was rather unsafe. At last the joists gave way and fell to the ground. The walls shook and all the rats’ hair stood on end with fear and horror. ‘This won’t do,’ said the captain. ‘I’ll send out scouts to search for a new home.’

Within five hours the ten scouts came back and said, ‘We found a stone house where

there is room and board for us all. There is a kindly horse named Nelly, a cow, a calf, and a garden with an elm tree.’ The rats crawled out of their little houses and stood on the floor in a long line. Just then the old one saw Arthur. ‘Stop,’ he ordered coarsely. ‘You are coming, of course?’ ‘I’m not certain,’ said Arthur, undaunted. ‘The roof may not come down yet.’ ‘Well,’ said the angry old rat, ‘we can’t wait for you to join us. Right about face. March!’

Arthur stood and watched them hurry away. ‘I think I’ll go tomorrow,’ he calmly said to himself, ‘but then again, I don’t know; it’s so nice and snug here.’

That night there was a big crash. In the morning some men—with some boys and girls—rode up and looked at the barn. One of them moved a board and he saw a young rat, quite dead, half in and half out of his hole. Thus the shirker got his due.”

APPENDIX D – FREEMAT CODE

```
%Initial Processing.m
%Takes .wav audio files of all 30 speakers and performs FFT & SVD on
each

for i=1:30      %process recordings for Speakers 1 through 30
    %load individual speaker
    speaker = ['Person_' num2str(i) '.dat'];
    load(speaker);

    %read audio file
    [z, SR, bits] = wavread(speaker);
    time_chunk_length = 4096;    % number of samples
    overlap = 0.9;    %90 percent overlap

    %calculate number of time segments and size of spectra matrix
    num_of_time_chunks = floor((length(z)-time_chunk_length*overlap)/
    (time_chunk_length*(1-overlap)));
    spectra = zeros(num_of_time_chunks, time_chunk_length/2);

    %process each time segment
    for i=1:num_of_time_chunks
        time_chunk = z(((i-1)*time_chunk_length*(1-overlap)+1):(i-1)*
        time_chunk_length*(1-overlap)+time_chunk_length));
        %fast fourier transform of time segment
        spectrum = abs(fft(time_chunk));
        spectrum = spectrum/sum(spectrum);    %normalize spectrum
        %add spectrum to total spectra matrix
        spectra(i,:) = spectrum(1:time_chunk_length/2);
    end

    %perform singular value decomposition
    [U,S,V] = svd(spectra);
    values = U*S;
    vectors = V';

    %save data for each speaker
    save_name = ['Person_' num2str(i) '_decomp.dat'];
    save(save_name, 'values', 'vectors', 'spectra')

end
```

```
%Combine Vectors.m
%combines first 50 principal vectors from each speaker and performs
SVD on total set

all_vectors = [];    %initialize blank matrix
for i=1:30    %load all speakers
    speaker = ['Person_' num2str(i) '_decomp.dat'];
    load(speaker);
    %add first 50 principal vectors from speaker to total set
    all_vectors = [all_vectors; vectors(1:50, 1:2048)];
end

%perform SVD on total set
[U,S,V] = svd(all_vectors);
values = U*S;
vectors = V';

%save vectors from SVD
save final_vectors.dat vectors
```

```
%Value Compute.m
%Computes principal values for each speaker based on previously
defined principal vectors

load final_vectors.dat;    %load final vectors

%compute for all 30 speakers
for i=1:30
    speaker = ['Person_' num2str(n) '_decomp.dat'];
    load(speaker);
    %calculate values
    values = spectra*inv(vectors);
    %save values for each speaker
    save_name = [speaker '_Values.dat'];
    save(save_name, 'values');

end
```

```
%Values Database.m
%Computes averages and standard deviations of values for each speaker
%and stores in matrix databases

%initialize database matrices
v_average_DB = [];
v_stdev_DB = [];
%compute for all 30 speakers
for i=1:30
    speaker = ['Person_' num2str(n) '.dat_Values.dat'];
    speaker=speaker
    load(speaker)
    %calculate averages and standard deviations
    v_averages = sum(values)/size(values, 1);
    v_stdevs = std(values);
    %add to databases
    v_average_DB(n,:) = v_averages;
    v_stdev_DB(n,:) = v_stdevs;

end

%save databases
save('value_DB.dat', 'v_average_DB', 'v_stdev_DB');
```

```

%Algorithm_1.m
%Computes sum of weighted z-scores comparing principal values to make
prediction of which speaker is talking

    %load values database and final principal vectors
load value_DB.dat;
load final_vectors.dat;

for i=1:30
    speaker = ['Person_' num2str(n) '_Values.dat'];
    speaker=speaker
    load(speaker)
        %initialize blank people vector
    people = zeros(1,size(values,1));
        %predict speaker for each time segment
    for i=(1:size(values,1))
        measure = sum(repmat(sum(abs(v_average_DB)), [30,1])'.*
            abs(((repmat(values(i,:), [30,1]) - v_average_DB)./
            v_stdev_DB)')));
        [measure_min, person] = min(measure);

        people(1,i) = person;
    end
        %calculate percentage of correct predictions
    num_correct = 0;
    for i=1:size(values, 1)
        if (people(i) == n)
            num_correct = num_correct + 1;
        end
    end
    percent_correct = num_correct / size(values, 1)
        %save results
    save_name = ['Person_' num2str(n) '_Results_1'];
    save(save_name, 'people');
end

```

```

%Algorithm_2.m
%Computes sum of unweighted z-scores comparing principal values to
make prediction of which speaker is talking

    %load values database and final principal vectors
load value_DB.dat;
load final_vectors.dat;

for i=1:30
    speaker = ['Person_' num2str(n) '_Values.dat'];
    speaker=speaker
    load(speaker)
        %initialize blank people vector
    people = zeros(1,size(values,1));
        %predict speaker for each time segment
    for i=(1:size(values,1))
        measure = sum(abs(((repmat(values(i,1:30), [30,1]) -
            v_average_DB(:, 1:30))./v_stdev_DB(:, 1:30))')));
        [measure_min, person] = min(measure);
        people(1,i) = person;
    end
        %calculate percentage of correct predictions
    num_correct = 0;
    for i=1:size(values, 1)
        if (people(i) == n)
            num_correct = num_correct + 1;
        end
    end
    percent_correct = num_correct / size(values, 1)
        %save results
    save_name = ['Person_' num2str(n) '_Result_2'];
    save(save_name, 'people');
end

```



**The Effects of Inulin and Galactooligosaccharides on
the Production of Reuterin by *Lactobacillus Reuteri***

Micah Dwight Forshee

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ABSTRACT

The microbiome is a dynamic community that can positively and negatively influence host health. *Lactobacillus reuteri* is a probiotic that has received much attention for its ability to inhibit pathogens such as *Salmonella typhimurium*, *Escherichia coli*, and *Clostridium difficile*. It does so by its unique ability to metabolize glycerol into the antimicrobial compound 3-HPA, which is commonly referred to as reuterin. The ability to secrete reuterin is dependent not only on glycerol availability but also the concentration of glucose. In fact, there appears to be a “goldilocks” ratio between glucose and glycerol as either too much or too little glucose significantly diminishes reuterin production. Since *L. reuteri* primarily resides in distal regions of the intestine and colon where most of the glucose has already been absorbed, it seems unlikely that reuterin production would be promoted at the physiological level via this mechanism.

Prebiotics are carbohydrates that are indigestible by the host and remain for enzymatic digestion by intestinal probiotics. Inulin and galactooligosaccharides (GOS) are two widely studied prebiotics that are known for their ability to promote the growth of a wide range of *Lactobacilli*, and have been shown to promote *L. reuteri* growth to varying degrees. Here, we asked if prebiotics such as inulin and GOS promote the production of reuterin in the absence of glucose. *L. reuteri* were cultured in TSB with or without glycerol in the presence of glucose, inulin, or GOS and assessed for their ability to produce reuterin. While inulin did not enhance the production of reuterin, GOS induced reuterin production, although 45% less than that of glucose. Moreover, unlike the dose-dependence observed with glucose, incubation with GOS induced similar reuterin production regardless of concentration. This suggests that an enzymatic equilibrium may exist where glucose/galactose is cleaved from GOS only as needed by *L. reuteri*.

Finally, to confirm the biopotency of reuterin production, we cultured *S. typhimurium* with supernatants from *L. reuteri* that were grown with various carbohydrates. Supernatant dilutions as low as 1:15 were able to significantly retard growth of *S. typhimurium* with ratios of 1:1 completely inhibiting growth. Together, these results suggest that prebiotics such as GOS may be able to elicit physiologically relevant production of reuterin, which may shape the flora of the microbiome and reduce incidence and severity of pathological infections. Further, as GOS are particularly abundant in breast milk, it suggests a possible link for early immunoprotection from intestinal pathogens while the infant is still immunologically naïve.

Keywords: *Lactobacillus reuteri*, *Salmonella typhimurium*, inulin, *Galactooligosaccharides* (GOS), reuterin, metabolism, prebiotics, probiotic

INTRODUCTION

Microbiome: Influence on health

The interaction and effects between human health and the microbiota have been widely studied (D’Argenio and Salvatore, 2015; Gibson and Roberfroid, 1995). Improper balances in the composition of the microbiome can lead to deleterious effects on the

host. For example, obesity has been correlated with elevated levels of pathogenic bacteria in the gut (Fei and Zhao, 2013). One study in particular looked at the ratio of one specific probiotic, *Bifidobacteria*, and one specific pathogen, *Escherichia coli*. They found that a group of obese school aged children had a significantly lower ratio of probiotics to pathogens (Gao et al., 2015). Other negative outcomes associated with unhealthy gut bacterial composition are inflammatory bowel disease (D’Argenio and Salvatore, 2015) and chronic heart failure (Pasini et al., 2016). In contrast to poor gastrointestinal conditions, symbiotic bacteria have been seen to ameliorate certain conditions such as constipation (Bekkali, Bongers, Van den Berg, Liem, and Benninga, 2007; Coccorullo et al., 2010; Ojetti et al., 2014), obesity (Chen et al., 2014; National Human Genome Research Institute, n.d.), and can inhibit pathogens in vitro (De Weirdt et al., 2012) and in vivo (Uraipan and Hongpattarakere, 2015). These beneficial bacteria are referred to as probiotics which can be defined as living organisms that convey beneficial health to the host or support a proper equilibrium of autochthonous microbes within the gastrointestinal tract (Uraipan and Hongpattarakere, 2015). Two genera most commonly studied for their favorable effects upon human health are *Bifidobacteria* and *Lactobacilli*.

Probiotics generally exert their positive shift in a balanced microbiome in one of two ways. The first is through occupying a physical niche within the gut by which they are able to limit any pathogen adherence to intestinal walls. The second mechanism involved in probiotic activity is in the secretion of an antimicrobial substance that inhibits pathogen (De Weirdt et al., 2012; Kšonžeková et al., 2016). The secreted antimicrobial typically either kills pathogenic organisms, or it prevents adherence to intestinal walls thereby preventing niche establishment; both mechanisms effectively shift the gastrointestinal equilibrium. Probiotics produce microbial inhibition by excreting exopolysaccharides, which are polysaccharides on the cell surface of gram positive bacteria that then can be released into the adjacent vicinity (Chapot-Chartier and Kulakauskas, 2014), or other compounds with antimicrobial properties including bacteriocin peptides (De Weirdt et al., 2012; Kšonžeková et al., 2016; Schaefer et al., 2010; Silva Sabo, Converti, Todorov, Domínguez, and Souza Oliveira, 2015). Because of probiotics’ anti-pathogenic properties and correlation with health, much research has focused on elucidating how they may be promoted.

In the pursuit of enhancing the endogenous probiotic populations of the gut, a new category of compounds was outlined. Gibson and Roberfroid first coined the term prebiotics to describe these compounds, and characterized them as nondigestible food stuffs that selectively promote the growth or activity of bacteria that beneficially impact the health of the host (Gibson and Roberfroid, 1995). Further delineation can be made between fibers and prebiotics by clarifying that fibers cannot be fermented in the gut while prebiotics are fermentable compounds (Stewart, Savarino, and Slavin, 2009). There are a number of prebiotics that have been studied including different types of fructans and oligosaccharides. These prebiotics selectively promote the growth of probiotics (Chung et al., 2016), mainly the genera *Bifidobacteria* and *Lactobacillus* (Kneifel, 2000; Kolida, Tuohy, and Gibson, 2002).

Changes in the microbiome have also been observed in human subjects due to prebiotics. For example, Costabile et al. administered different prebiotics to humans to see how they would impact the microbiota. While the overall number of bacteria remained the same, specific probiotics such as *Bifidobacteria* and *Lactobacillus* increased depending on the particular prebiotic used. At the same time, bacteria associated with an unhealthy gut, such as *Clostridia* and *Bacteroides*, decreased in number (Costabile et al., 2010). Not only do prebiotic effect bacterial distribution, but Tarr et al. have also observed a significant impact on health. Mice were subjected to a variety of conditions involving stress and prebiotics, and not only did the addition of prebiotics help modulate the gut composition to keep it within a range of normal, but it also prevented the mice from becoming anxious when placed in stressor situations. (Tarr et al., 2015).

***Lactobacillus reuteri* and the antimicrobial production of reuterin**

Lactobacillus reuteri (*L. reuteri*) is a probiotic with promising potential. It has already been demonstrated to have health benefits in human studies, and studies like the ones conducted by Ojetti et al. and Coccorullo et al. have shown the potential advantageous outcomes associated with the *L. reuteri* (Coccorullo et al., 2010; Ojetti et al., 2014). In a double-blind, randomized, placebo controlled study by Coccorullo et al., supplementation of *L. reuteri* was investigated for its ability to alleviate the functional chronic constipation that some infants experience. Not only did they see a significant increase in bowel movements, but there was also no observed adverse effect resulting from the added probiotic (Coccorullo et al., 2010). Another essential benefit of *L. reuteri*'s probiotic capacity is in its inhibition of pathogenic activity. One mechanism of inhibition is by limiting the ability of pathogens to adhere to gut epithelial cells. One group of researchers demonstrated that *L. reuteri* produced an exopolysaccharide that limited *E. coli*'s ability to adhere to porcine epithelial cells in vitro (Kšonžeková et al., 2016). This is in addition to the general characteristic of probiotics ability to occupy space that could otherwise be utilized by pathogens (Uraipan and Hongpattarakere, 2015). Another intriguing aspect of *L. reuteri*'s probiotic ability is its unique metabolism of glycerol into reuterin. De Weirdt et al. observed that the supernatant from *L. reuteri* produced antimicrobial effects against *Salmonella typhimurium*. In this study, they subjected *S. typhimurium* to a variety of supernatant concentrations from *L. reuteri* cultures. A 1:10 concentration of supernatant from *L. reuteri* grown in the presence of glycerol not only limited the adhesion of *S. typhimurium* to other cells, but it also inhibited the actual growth of *S. typhimurium* in vitro. Moreover, it was determined that reuterin was the active antimicrobial compound that was secreted by *L. reuteri* (De Weirdt et al., 2012).

Reuterin (**Figure 1**) is also known as 3-hydroxypropional or 3-hydroxypropionaldehyde (Schaefer et al., 2010), and is produced by a glycerol dehydratase that is B12 dependent (Talarico and Dobrogosz, 1990). It also is clear that reuterin is metabolized from glycerol and not due to an overall increased metabolism. The precise mechanism of reuterin's inhibition is not fully understood though it has been seen to have a wide range of antimicrobial inhibition (Talarico and Dobrogosz, 1989), including the pathogenic *Clostridium difficile*, which is responsible for many extended hospitalizations

(Schaefer et al., 2010). There are currently two hypotheses to explain its broad range of inhibition. The first is that reuterin inhibits the activity of ribonucleotide reductase (Cleusix, Lacroix, Vollenweider, Duboux, and Le Blay, 2007; Talarico, Casas, Chung, and Dobrogosz, 1988), the enzyme that synthesizes ribonucleotides in bacteria (Torrents, 2014). The other proposed, and more widely accepted, hypothesis is that reuterin introduces oxidative stress to the cells. Schaefer et al. investigated the mechanism by which reuterin may exert its potential oxidative stress. They showed that *E. coli* that had mutations for a specific gene involved with oxidative stress were more susceptible to reuterin's effect. Moreover, they also showed that excess cysteine in the media could mitigate the antimicrobial effects of reuterin, suggesting that reuterin is interacting with thiol groups (Schaefer et al., 2010).

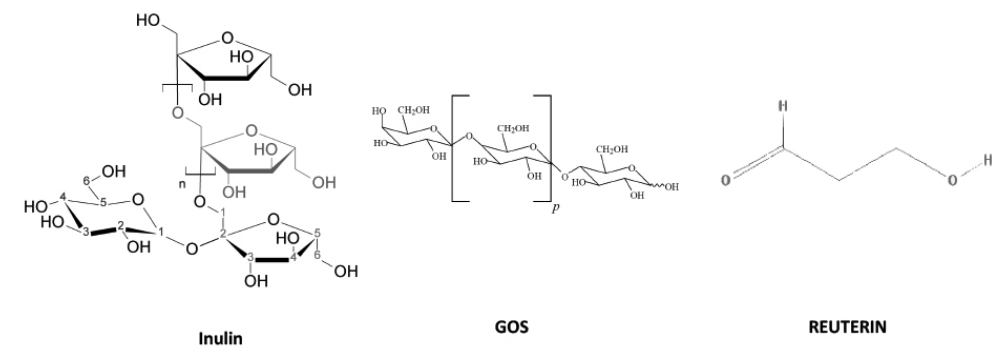


Figure 1: Structures of Inulin, GOS, and Reuterin. Each of the three compounds of interest for this research is depicted in this image. The β-glycosidic bonds between the monomers in inulin and GOS can be seen. As well, the varying degree of polymerization for both carbohydrates is illustrated with the brackets. Reuterin is easily observed as a modified version of glycerol by its molecular structure. (Fisch, 2006; Klaas, n.d.; Pubchem, n.d.)

Inulin and Galactooligosaccharides

Inulin (**Figure 1**) is a known prebiotic that can promote the growth of probiotics. Found in foods such as chicory root, Jerusalem artichoke, banana, onion, and garlic, inulin is a polymer of carbohydrate that varies in its degrees of polymerization (Costabile et al., 2010). It is mainly composed of fructose monomers linked by 1-2 β-glycosidic bonds of repeating length. This β-glycosidic linkage is what makes it indigestible by human gastrointestinal enzymes. An α-glucose moiety sometimes starts the polymer (Roberfroid, 2005). Chung et al. meticulously verified the prebiotic activity of inulin for various probiotics in vitro (Chung et al., 2016). These prebiotic effects have been observed in vivo as well. One such study was conducted with humans where half of the subjects were given inulin and half were given a placebo of maltodextrin. Fecal samples taken before and after revealed that the number of *Lactobacilli* and *Bifidobacteria* increased in the experimental group. This suggests that prebiotics, and specifically inulin, can be used to actively modulate the composition of the microbiome (Costabile et al., 2010). Not only can inulin be given directly, but it also can be incorporated into baked goods while still retaining its prebiotic activity, thus expanding its realistic use (Kleessen B

et al., 2007). *L. reuteri* is known to respond to various prebiotics of which inulin is one (Kassim, Baijnath, and Odhav, 2014).

Galactooligosaccharides (**Figure 1**) (GOS) are naturally occurring carbohydrates in breast milk that are synthesized via a β -galactosidase enzyme (Macfarlane, Steed, and Macfarlane, 2008). GOS have β -glycosidic linkages between galactose moieties of repeating length, and most often terminate with a single glucose monomer (Austin, Bénet, Michaud, Cuany, and Rohfritsch, 2014). GOS can often vary in degrees of polymerization from 2 to 10 (Macfarlane et al., 2008), and are known to be metabolized by at least two different strains of *L. reuteri* (Kneifel, 2000).

Bacterial metabolism

Although prebiotics such as inulin and GOS are known to enhance the growth of some strains of *L. reuteri*, it is uncertain how, or if, the metabolism of these prebiotics impacts the overall metabolic state of the organism. Some evidence demonstrates that prebiotics have increased the amount of antimicrobial substances produced by probiotics. If this is true, the ingestion of prebiotics could become a medicinally important part of a person's diet. Our research set out to determine if metabolism of prebiotics by *L. reuteri* significantly increase reuterin production. Specifically, we hypothesized that the addition of prebiotics such as inulin or GOS to *L. reuteri* would increase reuterin secretion and inhibit *S. typhimurium* growth.

METHODS

Bacteria, compounds, and storage

Lactobacillus reuteri PTA 6475 were obtained from the American Type Culture Collection (Manassas, VA) and were maintained by anaerobic culture on Tryptic Soy Agar plates. *Salmonella typhimurium* was obtained from Presque Isle Cultures (Erie, PA), and incubated aerobically on TSA plates. Plated cultures were stored at 4° C for use throughout experiments. Further, 10% and 20% glycerol stocks were created for long-term storage of cultures. Inulin from chicory root was obtained from Chem-Impex International, while GOS was acquired from Bimuno. Tryptic Soy Broth (TSB) was supplemented with 0.1% Tween 80 in all conditions as a surfactant to aid the growth of *L. reuteri* [2].

Reuterin production by supplementation of inulin

L. reuteri was inoculated in TSB and twelve hours later, tubes were measured by spectrophotometry (OD 596). Next, 180 μ L of working solution (OD596 close to 0.1) was inoculated in triplicate into Falcon tubes containing 9 mL of either TSB, TSB with 5.0% inulin (w/v), TSB with 20 mM glycerol, or TSB with 20 mM glycerol and 5.0% inulin. Cultures were incubated with shaking for sixteen hours at 37°C. Following this incubation, *L. reuteri*, were pelleted by centrifugation at 1500 rcf for ten minutes. The supernatant was removed, and the pellets were suspended in 9 mL of the corresponding broth (TSB, TSB with 5.0% inulin, TSB with 100 mM glycerol, and TSB with 100 mM glycerol and 5.0% inulin). They were then incubated again for two hours at 37°C with

shaking. Subsequently, each sample was centrifuged at 8000 rcf for ten minutes. From every tube, 4.5 mL of the supernatant was placed into another tube that had 4.5 mL of TSB to render a 1:1 ratio of supernatant to TSB. Additional controls included 9mL of broth that had not been cultured with *L. reuteri* (TSB, TSB with 100mM glycerol, and TSB with 5.0% inulin). All samples were inoculated with 180 μ L from the *S. typhimurium* working solution which consisted of *S. typhimurium* culture with an OD596 of 0.1. Initial experiments were then incubated in a 37°C shaking water bath for two, three, four, five, six, and seven hours. At each time point, a 1 mL aliquot was taken from each of the samples and measured by spectrophotometry for their OD596. All conditions were performed in triplicate.

Titration of Reuterin

To titrate reuterin production, the previously described protocols were repeated except the supernatant was diluted to 1:1, 1:5, 1:10, and 1:15. Further, a 1 mL aliquot was removed only after four hours (during *S. typhimurium* growth) for spectrophotometric analysis.

GOS as primary carbon source

Assays were carried out in a similar manner as stated previously. However, carbon restricted (glucose free) TSB media was used to make the GOS media. In the carbon restricted TSB, the following four concentrations of GOS (w/v) were added: 0.25% GOS, 0.25% GOS with glycerol, 2.0% GOS, and 2.0% GOS with glycerol. As previously noted, glycerol concentration started at 20 mM and then was brought up to 100 mM for each control that had glycerol. The positive control was standard TSB, which contains 13.9 mM of glucose, supplemented with glycerol. The negative control in this experiment lacked *L. reuteri* supernatant, and it was used to assess uninhibited growth of *S. typhimurium*. A 1:5 ratio of supernatant to TSB was used to incubate the *S. typhimurium*. After four hours, the *S. typhimurium* cultures were assessed by the spectrophotometer at 596 nm.

Statistical Analysis

The statistical analysis for all experiments was done with a student's t test to determine significance of results. A p value < 0.05 was considered to be statistically significant.

RESULTS

Effect of inulin on reuterin production

S. typhimurium was first grown in the experimental supplements inulin and glycerol to determine if in the absence of *L. reuteri*, any inhibition of growth would be observed. As shown in Figure 2, inulin and glycerol did not inhibit *S. typhimurium* growth. Furthermore, as others have reported, Figure 2 demonstrates that when *L. reuteri* was grown without glycerol, and the supernatant was used to culture *S. typhimurium*, there is an absence of any detectable antimicrobial effect. Each *S. typhimurium* sample was measured for growth with the spectrophotometer at an OD596. These results indicate that inulin alone does not inhibit *S. typhimurium* growth (**Figure 2**). However, as shown in Figure 3, supernatants at a 1:1 dilution from *L. reuteri* cultured in the presence of glycerol inhibit *S.*

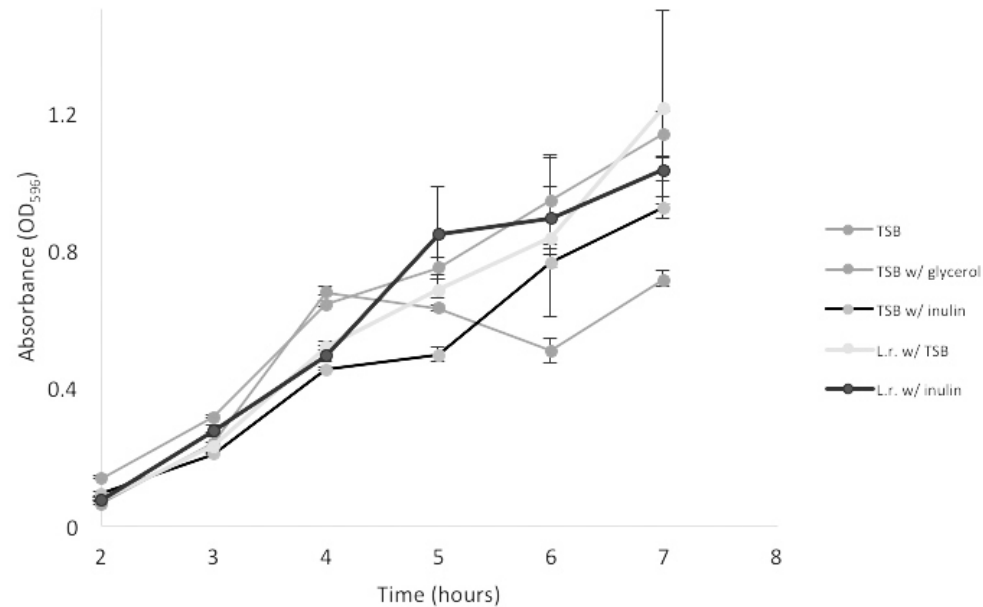
Controls had negligible effects on *S. Typhimurium* growth

Figure 2: Glycerol and inulin alone do not inhibit the growth of *S. typhimurium*. *S. typhimurium* was cultured with or without glycerol and inulin and assessed for growth over seven hours of log growth. Supernatants from *L. reuteri* (*L.r.*) that were cultured with or without inulin were also cultured with *S. typhimurium* to assess inhibitory capacity. All samples were completed in triplicate.

typhimurium growth for every time point after hour 2 ($p < 0.001$). And while the addition of glycerol significantly inhibited the growth of *S. typhimurium*, the addition of inulin did not further affect this response at a 1:1 dilution as can be seen in **Figure 3**.

Dilution assay

As a 1:1 dilution of supernatants from *L. reuteri* cultures resulted in complete inhibition of *S. typhimurium*, we asked if effects of inulin would be observed over smaller dilutions in order to more precisely understand reuterin production. Dilutions for glycerol cultured *L. reuteri* with and without inulin were made at 1:1, 1:5, 1:10, and 1:15. *S. typhimurium* was then cultured in these varied supernatant dilutions, and the optical density of *S. typhimurium* was assayed after four hours of incubation. The steady increase of absorbance indicates that the dilutions were within the sensitive range of our assay's measurable limits. There was no significant difference observed between supernatant from *L. reuteri* grown with glycerol or glycerol plus inulin for any of the dilutions (**Figure 4**). These data further suggest that the addition of inulin does not increase reuterin production. Moreover, the dose dependent increase of absorbance indicates that the dilutions were within the sensitive range of our assay's measurable limits. The data obtained from **Figure 4** further suggests to reject the first hypothesis that suggested the addition of inulin would increase reuterin production.

Glycerol with inulin did not increase inhibition

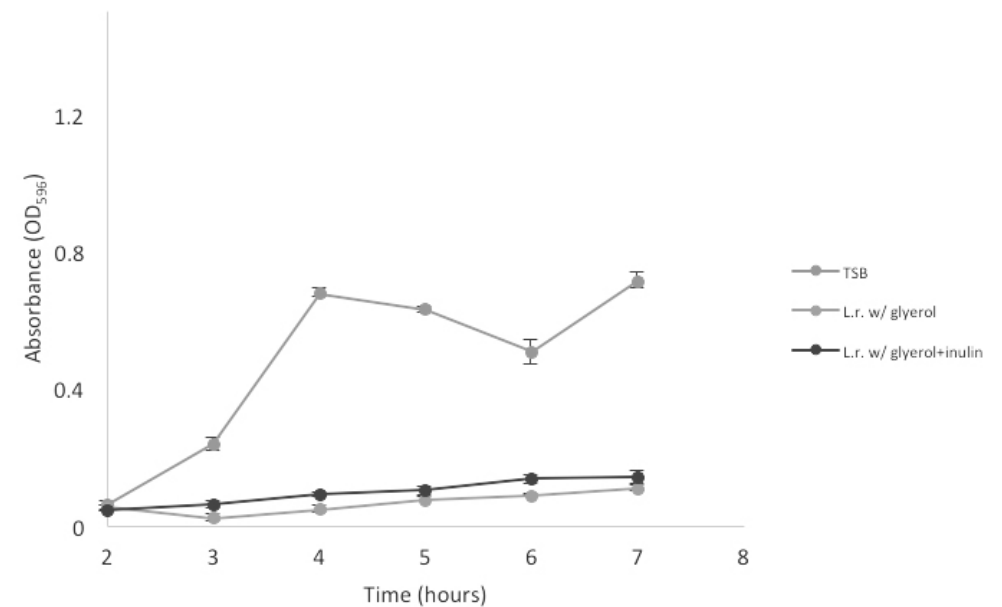


Figure 3: Glycerol with inulin supplementation did not increase inhibition of *S. typhimurium*. *S. typhimurium* growth was significantly inhibited by both supernatants that were extracted from *L. reuteri* cultures incubated with glycerol. However, there was no difference in inhibition between supernatants with glycerol vs glycerol with inulin at a 1:1 ratio.

GOS as a primary carbon source

The supernatant of *L. reuteri* grown with GOS as the primary carbon source was used to determine whether *L. reuteri* can produce reuterin while metabolizing other carbohydrate sources other than glucose. As expected, the supernatants without any glycerol supplementation did not inhibit *S. typhimurium* growth significantly (**Figure 5**).

While not as potent as TSB that contains glucose as a carbon source, the addition of glycerol to GOS as a primary carbon source, at both concentrations tested, significantly inhibited the growth of *S. typhimurium* ($p < 0.01$). This suggests that *L. reuteri* can secrete reuterin while metabolizing carbohydrates other than glucose. Also, the negative control of the supernatant from glucose with glycerol was significantly lower than every other sample ($p < 0.05$).

DISCUSSION

We were able to consistently recreate the study of reuterin's inhibitory effects on *S. typhimurium* by De Weirdt et al. (De Weirdt et al., 2012). While no inhibitory ability was observed by the addition of inulin, it cannot be entirely ruled out that the metabolism of inulin does not promote any inhibitory reuterin production. Even though it is known that some *L. reuteri* species can use inulin (Kassim et al., 2014), metabolism was not confirmed in this study, and it is possible that the *L. reuteri* did not metabolize

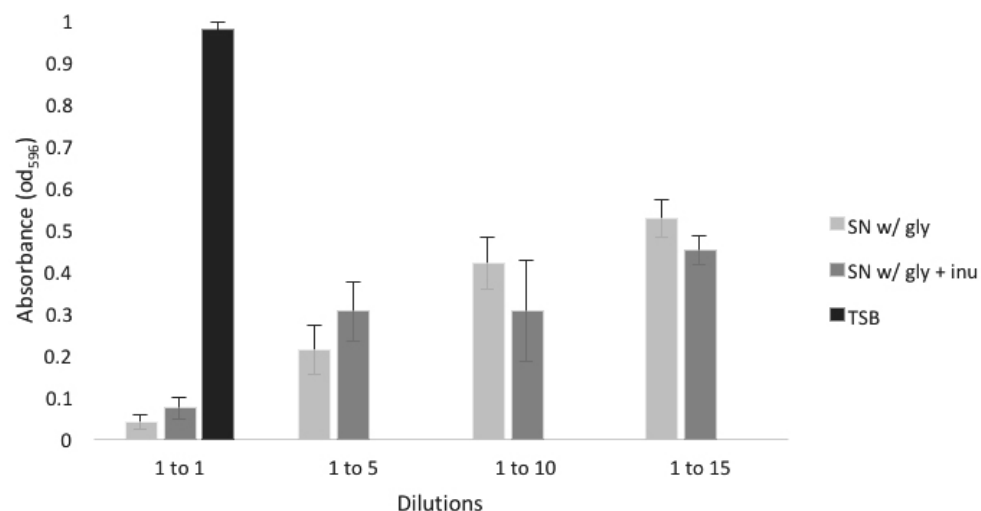


Figure 4: Titration of reuterin production is not influenced by inulin supplementation. Supernatants were prepared from *L. reuteri* grown with glycerol and glycerol with inulin. Each were diluted accordingly before use in the assay. All dilutions inhibited *S. typhimurium* growth. There was no significant difference between glycerol and glycerol with inulin at any dilution ($p>0.05$).

Reuterin production with GOS as carbon source

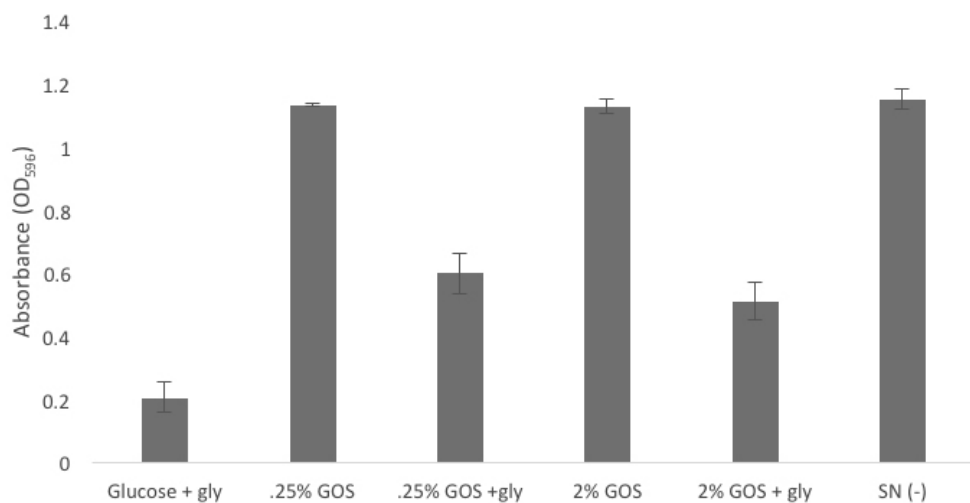


Figure 5: Reuterin production with GOS as primary carbon source is sufficient to inhibit *S. typhimurium* growth. Both concentrations of GOS at 0.25% or 2% allowed for reuterin production to occur thus the inhibition. Though significant inhibition was observed, there was also significant difference between the negative control and the GOS with glycerol supernatants.

the inulin much, if at all, because it was in the glucose rich carbon environment of the TSB. Furthermore, other studies have shown that certain strains of *L. reuteri* do not metabolize inulin at all (Adebola, Corcoran, and Morgan, 2014; Kneifel, 2000). So while this research demonstrated for the first time that an addition of inulin does not increase the production of reuterin with or without glycerol, it did not answer the question of whether it would have if inulin were the sole carbon source in the media. To better construct a more thorough assay would require a carbon restricted media, which would shift *L. reuteri* to metabolize the inulin. However, the promise of continuing this line of experimentation may be limited. Two studies looked at the effect that a variety of prebiotics had on different probiotics. These studies seem to imply that prebiotic utilization is strain specific. For example, inulin was not well metabolized by the strains used in at least two studies (Adebola et al., 2014; Kneifel, 2000). Contrarily, Kassim et al. showed that some *L. reuteri* strains did metabolize inulin (Kassim et al., 2014). Nevertheless, the PTA 6475 strain used in this study has not been tested for its ability to metabolize inulin in any literature reviewed. Therefore, the PTA 6475 strain cannot yet be said with great confidence to metabolize inulin when in a carbon restricted media. This is one area of study yet to be explored.

A new direction to be followed is how other carbohydrate sources effect reuterin production. One study particularly has already shown that carbohydrate sources can act synergistically to impact antimicrobial production (Tzortzis, Baillon, Gibson, and Rastall, 2004). A logical continuation of this would be to extend a study to incorporate different prebiotics to see if they impact antimicrobial output. This is precisely what was done with the GOS assay. The carbon restricted media (no glucose) was supplemented with GOS as the primary carbon source for *L. reuteri*. A study conducted by Lüthi-Peng, Dileme, and Puhan demonstrated that reuterin synthesis is optimized at a particular glycerol to glucose ratio. Following this line of thought, their study would seem to insinuate that carbohydrate metabolism does indeed impact reuterin output (Lüthi-Peng, Dileme, and Puhan, 2002). Supported by this study, the GOS assay was completed in order to better determine the relationship between reuterin synthesis and carbohydrate metabolism. This research showed for the first time that reuterin can be metabolized without glucose being the primary carbon source.

Discovering *L. reuteri*'s ability to continue to synthesize reuterin with GOS as a primary carbon source brought great insight into this unique form of metabolism (Figure 5). Going forward, there is still much to continue to study. For one, there was a significant difference in inhibition between glucose and GOS as the carbon source. It is known that different factors impact the production of different metabolites. (Årsköld et al., 2007). One study by Liu and Yu revealed that the ratio of glucose to glycerol can be optimized for the production of reuterin. (Liu and Yu, 2015). Future studies should determine whether there is an optimal ratio of GOS to glycerol in the same way that others have previously found the ideal glucose to glycerol ratio.

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The Relationship Between Musicianship, Academic Motivation, Academic Achievement, and Self-Esteem

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ABSTRACT

Background

Past research suggests that students involved in music are intrinsically motivated. For example, Diaz (2010) showed that undergraduate musicians possessed high levels of academic intrinsic motivation. Intrinsic motivation is a predictor of high academic achievement as well. Additionally, past research indicates that music education is positively correlated with academic achievement and self-esteem. This study continues to investigate the relationships between musicianship and academic motivation, academic achievement, and self-esteem, but it does so using a post-secondary sample and an expanded classification system for musicianship.

Methods and Procedures

A survey link was emailed to all undergraduate students at a small, Christian university in the Midwest. Participants were asked to share their past music experience along with demographic information such as major, GPA, and ACT score. They also completed twenty-eight items from Vallerand's Academic Motivation Scale (Vallerand, 1992) as well as ten items from Rosenthal's Self-Esteem Scale (Rosenthal, 1965). Five hundred fifty-eight students completed the survey. The participants were categorized into four levels of musicianship: music majors/minors ($n=40$), non-music majors/minors in collegiate level music ensembles ($n=216$), non-music majors/minors not in collegiate level music ensembles ($n=136$), and non-musicians ($n=164$). Inferential statistics were used to compare the academic motivation, academic achievement, and self-esteem of the groups.

Results

Using an independent samples t-test, it was found that musicians (both music majors/minors and non-music majors/minors in collegiate level ensembles) had higher academic motivation and ACT scores than non-musicians. No other statistically significant differences were found between any other groups on academic motivation, academic achievement, and self-esteem.

Keywords: Academic motivation, academic achievement, intrinsic motivation, self-esteem, music education, musicianship

INTRODUCTION

Students in the United States are falling behind other modern nations in mathematics and science. Of American high school seniors, only 16% are mathematically proficient and pursuing STEM careers. Furthermore, 81% of Asian-American and 71% of white secondary students have access to a complete mathematics and science department that offers critical courses like calculus or physics for success in STEM fields. The availability of these courses for minority students is even lower (U.S. Department of Education). U.S. leaders are concerned with these alarming statistics that might put the American economy and its technology behind other global leaders. This has led to an

emphasis on training STEM educators and encouraging kindergarten through twelfth grade students to study STEM subjects (Rosenthal, 2013). Due to this emphasis, arts programs are often the first to be cut or reduced in schools during economic struggles (Catterall, 2014). The research on music education and academic achievement suggests that cutting music programs will, in the long run, damage not only student performance in STEM subjects but in overall academic performance (Catterall, 2014). If it can be shown that music education is a unique contributor to the academic achievement of students at all levels, then perhaps the education system will place greater emphasis on the importance of learning music.

Positive correlations have been found between musicianship and academic motivation (Asmuc, 1986; Diaz, 2004; Schmidt, 2005; Stoeber and Eismann, 2007; Willie, 2014), musicianship and academic achievement (Bobbett and Dorothy, 1990; Evans, 2018; Fehr, 2016; Gouzouasis, Guhn, and Kishor, 2007; Graziano, Peterson, and Shaw, 1999; Harris, 2008; Santos-Luiz, Coimbra, and Fernandes da Silva, 2009), and musicianship and self-esteem (Costa-Giomi, 2004; Henley, Caulfield, Wilson, and Wilkinson, 2012; Yücesan and Sendurur, 2018). However, no research studies explore musicianship, academic motivation, academic achievement, and self-esteem in regards to students at the university level. In addition, many of these studies treat musicianship as a dichotomous variable. Because musicianship may be more nuanced than this, the present study will focus on relationships between musicianship, academic motivation, academic achievement, and self-esteem but with stratified levels of musicianship within a large sample of college students.

Academic Motivation

According to Young (2005), a successful student is characterized as being a self-regulated learner, and one aspect of achieving self-regulated learning is a student's academic motivation. Motivation is broken into two strands: intrinsic motivation and extrinsic motivation. Intrinsically motivated students are motivated by internal factors like self-determination, curiosity, and effort, whereas extrinsically motivated students are motivated by external factors like rewards and consequences (Santrock, 2014). The current consensus among education professionals is that intrinsic motivation is key to successful education. However, it is important to note that extrinsic motivation and intrinsic motivation are not in opposition to each other. The two are both at work in a student's academic career (Santrock, 2014). The motivation students possess is important to their overall success in their studies.

Asmus (1986) found that elementary and secondary students in music programs attributed their success or failure to internal motivation. Another study found similar results. Middle and high school band students characterized their performance ability, effort, and practice time as stemming from intrinsic motivation (Schmidt, 2005). Still other researchers looked at the motivational aspects of high school band students' skills, finding that effort and achievement in music was associated with intrinsic motivation whereas performance anxiety and emotional stress from music involvement was associated with extrinsic motivation (Stoeber & Eismann, 2007). Willie (2014)

implemented a “band bucks” program in his high school band classes in which students were awarded band bucks for completing practicing goals. He found that the students were at first extrinsically motivated to practice for the reward of band bucks. However, over time, the students began to practice for the enjoyment and achievement of playing a piece well instead of simply for the reward of band bucks. The successful students were ultimately intrinsically motivated to practice (Willie, 2014).

Much of the current study is inspired by Diaz’s (2010) work with post-secondary musicians. He analyzed intrinsic and extrinsic motivation among instrumentalists in college. The participants consisted of 169 undergraduate and graduate students in band or orchestra ensembles. Researchers collected gender, academic major, primary instrument, graduate status, year in school, and a motivation survey. Results indicated that intrinsic motivational factors contributed to students’ overall music motivation. It is important to note that the participants in his study were collegiate musicians.

The current study now seeks to compare the academic motivation of collegiate musicians and collegiate non-musicians. Diaz (2010) focused on music majors and the differences between distinct music majors. However, there has been little to no research done on the academic motivation of music majors, non-music major musicians, and non-musicians.

Academic Achievement

Educators assert that children involved in band or music lessons are more productive and engaged students than those not learning to play music (Fehr, 2016). Research studies have affirmed that music involvement is a positive contributor to success in school. On the elementary level, one study found that children receiving piano and spatial video game training scored significantly higher on a spatial and mathematics skills test than children only receiving video game training or no training (Graziano, Peterson, and Shaw, 1999). Harris (2008) found that a musically enriched classroom in a Montessori school scored higher on the Test of Early Mathematics Ability 3 than the traditionally taught Montessori classroom, shedding light on integrated music instruction.

Another study focused on students receiving piano lessons over five to seven years and over three to four years compared to students receiving no training. Results indicated that the longer students persevered in piano lessons, the higher their spatial and numerical reasoning (Santos-Luiz, Coimbra, & Fernandes da Silva, 2009). Continued music education is more successful than short exposure to music training. It is important to note the distinction between several years of music training and short-term exposure to music training. In the current study, participants are divided into groups of different musical levels of post-secondary musicians who have either spent many years practicing and performing in their music concentrations, simply taken a few music lessons in elementary school, or anywhere in between.

Furthermore, music was a unique contributor to academic success according to a study that examined high school band students’ scores in core subjects like math, biology and

English to non-music students and students in visual arts classes. The band students’ scores were significantly higher than non-music students and visual arts students, which implies that the benefits of music education are not the same as benefits offered by art education (Gouzouasis, Guhn, and Kishor, 2007). While other artistic endeavors like the visual arts are valuable, the benefits of music education is not equivalent with art education.

Individual musical training is important in developing techniques and musical IQ, but music is an art to be shared and performed through social music-making. Many teachers have concluded that students in band are high achieving students in their other core classes. In fact, according to 96% principals interviewed across the United States, music programs encourage and motivate students to stay in school. Furthermore, 86% said that high-quality music education contributed to high graduation rates (Fehr, 2016). In most schools, playing in band is a privilege given to students who achieve above average grades, which in turn benefits students’ chances of graduation. In fact, schools with music programs have higher graduation rates than schools without music programs (Fehr, 2016).

Studies linking music education to academic achievement do not stop short at the elementary and secondary grades. One study focused on students taking music and sound design classes in an undergraduate program. The participants reported that they benefited academically from the music and sound design classes. The study recommended further evidence-based research be done on the effect of arts education on the academic achievement of postsecondary students (Evans, 2018).

Bobbett and Dorothy’s (1990) study on musical independence looked at the high school and college experiences of musical students. They found that musical independence enhanced the Scholastic Achievement Test scores of participants and established four factors needed and developed by students with musical independence: natural intellectual skills, musical experiences, time on task, and effective study habits (Bobbett and Dorothy, 1990). The study supports the idea that the skills a student develops at both the secondary and postsecondary levels when learning music can be beneficial to other areas of academia and, therefore, his or her overall academic achievement.

Self-Esteem

Another aspect of music education is the personal benefits it provides to students. Costa-Giomi (2004) studied the effects of piano lessons for fourth-grade students over the course of four years. He found that their self-esteem was positively affected by the piano lessons as compared to students not receiving any training. Another group of researchers (Henley, Caulfield, Wilson, and Wilkinson, 2012) analyzed the benefits that the Good Vibrations Project (a social music making program specializing in Javanese music) provided to adult prison inmates. Their results showed that the program had a direct positive relationship with the inmate’s social well-being as well as their self-esteem and overall emotional stability.

Students participating in bands, choirs, and orchestras are developing positive social and personal skills while learning music. However, Costa-Giomi's (2004) study simply focused on elementary students while Henley et al.'s (2012) project was removed from education. Some research has been done on music and self-esteem at the undergraduate level. It was found that college students who participated in music therapy, poetry therapy, and creative drama had higher percentages of self-esteem than the control group who did not participate in any of the three outlets (Yücesan and Sendurur, 2018). Though the current study does not explore the effects of music therapy on college students, it is important to note that music participation, whether it be through social music making or music therapy, has a positive relationship with self-esteem. Therefore, looking at self-esteem among musicians and non-musicians is relevant to the existing body of research.

Current Study

The current study investigated three dependent variables, academic motivation, academic achievement, and self-esteem, at the undergraduate level. Academic motivation was being explored because it is an important aspect to the overall self-regulation and academic outcome of students from elementary school to higher education. Furthermore, Diaz (2010) showed that postsecondary musicians have positive levels of academic motivation. Academic achievement was important to this study not only because academic motivation is a key component to a student's overall academic success but because music education has been shown to be a factor in the academic success of students both in elementary school, high school, and college. The current study sought to connect academic motivation and academic achievement of college students. Finally, self-esteem was included because past research shows it has a positive relationship with music education and social music-making.

No research has compared academic motivation, academic achievement, and self-esteem of undergraduate students with differing levels of music experience. The present study compared the academic motivation, academic achievement, and self-esteem of music majors/minors, non-music major musicians of varying years of experience (see Table 1), and non-musicians.

Research Questions and Hypothesis

Three research questions drove the research:

1. Is there a significant difference between Music Majors/Minors and Non-Music Major/Minors Musicians in academic motivation, academic achievement, and self-esteem?
2. Is there a significant difference between Musicians and Non-Musicians in academic motivation, academic achievement, and self-esteem?
3. Is there a significant difference between Musicians of different music experience in academic motivation, academic achievement, and self-esteem?

It was hypothesized that there would be a significant difference between each of the above listed groups in academic motivation, academic achievement, and self-esteem. Furthermore, in general, it was hypothesized that college students with more musical experience would have high levels of academic motivation, academic achievement, and self-esteem such that each level of musicianship represented in Table 1 would have subsequently higher levels of academic motivation, academic achievement, and self-esteem. For example, music majors would have significantly greater academic motivation, academic achievement, and self-esteem than other musicians and non-musicians while non-music-major musicians in collegiate ensembles would have significantly greater academic motivation, academic achievement, and self-esteem than non-music-major musicians not in collegiate ensembles with greater than five years of music experience.

METHODS

Participants

Undergraduate students from a private, Christian university in the Midwest were asked to participate. A total of 558 participants took the survey. Based on their own self-report, participants were sorted into one of four different groups: music majors/minors ($n=40$), non-music majors/minors in collegiate ensembles ($n=136$), non-music-majors/minors not in collegiate ensembles ($n=164$), and non-musicians ($n=218$). All groups and sub-groups are listed in Table 1. The non-music majors/minors in collegiate ensembles subgroups were determined based on the ensemble requirements at the sampled university and another university with similar size, mission, and demographic.

Materials

Academic motivation was measured using Vallerand's Academic Motivation Scale (Vallerand et al., 1992). The scale measured self-determination, which determines positive or negative academic motivation. The scale utilized a seven-point Likert scale with endpoints that range from 1 (does not correspond) to 7 (corresponds exactly) and consisted of twelve intrinsic motivation items, twelve extrinsic motivation items, and four amotivation items. The scale combined each aspect of motivation, intrinsic, extrinsic, and amotivation, to determine a participant's self-determination or overall academic motivation. Each participant received an overall self-determination index with 16 being high self-determination and -16 being low self-determination. The mean and standard deviation for this scale can be found in Table 2.

Academic Achievement was measured using participants' ACT or SAT score. ACT and SAT scores have been shown to be predictors of collegiate success both in terms of program success and collegiate perseverance (Burton and Ramist, 2001; Zwick and Sklar, 2005; Radunzel and Noble, 2012; Westrick, 2017). Twenty participants reported SAT scores, and scores were converted to ACT scores using College Board's online Instructions for Converting New SAT Scores to Old SAT Scores.

However, it is understood that ACT and SAT score represents the past academic achievement of postsecondary students. Therefore, current college GPA was also collected to reflect the current academic achievement of postsecondary students. Since

all GPAs were compared on a 4.0 scale, any reported GPA score was converted from a 5.0 scale to a 4.0 scale using Texas Southern University's GPA converter (<http://em.tsu.edu/registrar/gpacc.php>). The mean and standard deviation for GPA and ACT score can be found in Table 2.

Self-esteem was measured using Rosenberg's Self-Esteem Scale (Rosenberg, 1965). Participants completed five positive and five negative self-esteem items on a five-point Likert scale with endpoints that range from 1 to 5. The mean and standard deviation for this scale can be found in Table 2.

Participants reported their level of musicianship through a series of single answer and open response prompts. First, each participant shared whether or not he or she played an instrument or sang, the instrument(s) he or she played, and whether or not he or she considered himself or herself a proficient musician. The open response section asked participants who played an instrument to describe their past musical experience, their participation in collegiate ensembles, and their participation in collegiate-level music lessons. The date of graduation, gender, major, and minor (if applicable) was also reported by each participant.

Procedures

All participants were asked to complete a survey via campus email. Each undergraduate student received an email with a brief description of the project and a link to an informed consent page. After giving consent, participants answered prompts about their music experience and involvement. In this section, students listed the instrument or instruments they played. The participants who played instruments used free response to communicate all music experience including lessons, ensemble experience, and history of playing.

Next, participants completed Vallerand's Academic Motivation Scale (Vallerand et al., 1992) and Rosenberg's Self-Esteem Scale (Rosenberg, 1965) before moving on to the demographics section of the survey.

RESULTS

Table 2 shows the correlation between the dependent variables of academic motivation, ACT score, current college GPA, and self-esteem. There were significant positive correlations between GPA and academic motivation, GPA and ACT score, self-esteem and academic motivation, and self-esteem and GPA.

For research question 1, independent samples t-tests were used to compare music majors/minors to all students who played an instrument or vocal students and did not major or minor in music on academic motivation, academic achievement, and self-esteem. Table 3 shows that there were no significant differences between the academic motivation, ACT scores, GPA, and self-esteem of music majors/minors and non-music-major musicians.

For research question 2, participants who played an instrument or sang were compared to participants who did not play an instrument or sing on academic motivation, academic achievement, and self-esteem. Independent samples t-tests were used to do this. Table 4 shows that there were no significant differences between musicians and non-musicians on GPA and self-esteem. However, there was a significant difference between musicians and non-musicians on academic motivation and ACT score. Table 4 shows that musicians score significantly higher than non-musicians on academic motivation and ACT scores.

For research question 3, one-way ANOVAs were used to compare five different levels of musicianship on academic motivation, academic achievement, and self-esteem. The five groups compared were: music majors/minors, musicians in collegiate ensembles, musicians not in collegiate ensembles with five or more years of experience, musicians not in collegiate ensembles with three to five years of experience, and musicians not in collegiate ensembles with less than three years of experience (see Table 1). Table 5 shows that there were no significant differences between any of the groups, and Table 6 gives the mean and standard deviation of the groups' academic motivation, ACT score, GPA, and self-esteem.

DISCUSSION

The purpose of this study was to explore relationships between musicianship, academic motivation, academic achievement, and self-esteem using an expanded classification scheme for musicianship within a large sample of college students. No significant differences occurred between the music majors/minors and non-music-major musicians in terms of academic motivation, ACT score, GPA, and self-esteem. Both groups had similar levels of academic motivation with positive academic motivation scores. Furthermore, when comparing different levels of non-music-major musicians, in ensembles and not in ensembles, no differences were found between the four variables. This was not surprising since Diaz (2010) found that the motivation of separate groups of musicians did not vary greatly from the larger set of musicians. In other words, the overall motivation and academic achievement of Diaz's musicians did not differ depending on experience and expertise.

However, when comparing musicians and non-musicians the outcome was different. While GPA and self-esteem did not significantly vary, the academic motivation and ACT scores of the two groups was significantly different. While both groups had positive academic motivation, the musicians had significantly higher scores than non-musicians. In other words, musicians had higher academic motivation than non-musicians. Again, this supports past research conclusions in which musicians and music students possessed high academic and intrinsic motivation (Diaz, 2010; Schmidt, 2005; Willie, 2014). Specifically, Diaz found that his participants, all musicians, had high intrinsic motivation. However, it is important to note that Diaz concluded that musicians had high intrinsic motivation instead of simply high academic motivation. Diaz focused specifically on intrinsic and extrinsic motivation factors, not academic motivation in general. Furthermore, the musicians had significantly higher average ACT scores than non-musicians

which is consistent with past research that shows a positive relationship between musicianship and academic achievement (Gouzouasis, Guhn, and Kishor, 2007; Graziano, Peterson, and Shaw, 1999; Harris, 2008; Santos-Luiz, Coimbra, and Fernandes da Silva, 2009). However, these studies did not address academic achievement and musicianship of post-secondary students, and many were longitudinal studies.

The study was limited by the method of classification used to define musicians and non-musicians. During data collection, participants answered survey questions about music experience and involvement. Most questions were open-ended so that participants had complete control over the description of their music history. This limited the study due to each individual's potentially biased definition of their own skills causing some participants to over-qualify or under-qualify their experience and expertise.

Another limitation was that the number of participants in the subgroups of non-music majors/minors not in collegiate ensembles was significantly smaller than other groups. Specifically, non-music majors/minors not in collegiate ensembles with three to five years of experiences and with less than three years of experience only included twenty-five and twenty-four participants respectively.

This study was further limited by the definition of academic achievement. ACT score and current GPA was used to measure academic achievement. However, perhaps academic achievement is not purely defined by quantitative achievements. For example, it might be useful to view academic achievement in terms of grit (Duckworth, Peterson, Mathhews and Kelly, 2007). It is recommended that the idea of academic achievement be explored in future research by including leadership, communication abilities, and rigor of study as components of academic achievement.

It is recommended that further research more clearly defines the classification of musicians and non-musicians by asking about level of theory knowledge, sight-reading skills, performance experience, chair level, classical training or the lack thereof, and instrument diversity. Future studies could use more sophisticated tools to quantify participants' musical skill and look at the academic motivation, academic achievement, and self-esteem of participants. More precise definitions of musicianship will help to better the understanding of how academic motivation is linked to level of musicianship.

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TABLE 1
Groups and subgroups of musicians based on music experience

Groups	Music Majors/Minors (n=40)	Non-Music Majors/Minors in Collegiate Ensembles (n=136)	Non-Music Majors/Minors Not in Collegiate Ensembles (n=164)	Non-Musicians (n=218)
Subgroups	N/A	N/A	1. 5 years of experience with instrument/voice AND/OR high school band, choir, or lesson experience AND/OR collegiate/professional level experience (n=106) 2. Between 3-5 years of experience with instrument/voice AND/OR only middle school/elementary band, choir, or lesson experience (n=25) 3. Less than 3 years of experience with instrument/voice AND/OR only church experience AND/OR other (n=24) 4. Unknown (n=8)	N/A

TABLE 2
Descriptive statistics and intercorrelations for academic motivation, ACT score, GPA, and self-esteem

Variable	M	SD	Alpha	1 Academic Motivation	2 ACT	3 GPA	4 Self-Esteem
1 Academic Motivation	6.59	3.76	.91				
2 ACT	26.37	4.14	N/A	.05			
3 GPA	3.50	.42	N/A	.22**	.41**		
4 Self-Esteem	38.26	6.99	.86	.38**	-.04	.12**	

Note: Ns = 472 – 501. **p < .01

TABLE 3
Descriptive statistics and independent-samples t-test results comparing music majors/minors and non-music-major musicians on academic motivation, academic achievement, and self-esteem.

Variables	t	df	p	Music Majors/Minors		Non-Music-Major Musicians	
				Mean	SD	Mean	SD
Academic Motivation	1.75	304	.08	8.13	2.87	7.05	3.54
ACT	-.99	314	.32	26.14	5.74	26.87	3.99
GPA	1.10	328	.27	3.59	.35	3.51	.45
Self-Esteem	.91	318	.36	39.16	6.45	38.07	6.99

Note: Ns for the Music Majors/Minors group ranged from 36 to 38. Ns for the Non-Music-Major Musicians group ranged from 270 to 292.

TABLE 4
Descriptive statistics and independent-samples t-test results comparing musicians and non-musicians on academic motivation, academic achievement, and self-esteem

Variables	t	df	p	Musicians		Non-Musicians	
				Mean	SD	Mean	SD
Academic Motivation	4.37	499	.00	7.17	3.48	5.69	4.02
ACT	2.79	517	.01	26.78	4.23	25.75	3.95
GPA	.89	539	.37	3.52	.44	3.48	.39
Self-Esteem	-.28	526	.78	38.20	6.97	38.37	7.05

Note: Ns for the Musicians group ranged from 306 to 330. Ns for the Non-Musicians group range from 195 to 211.

TABLE 5
One-way ANOVA comparing musicians of differing experience on academic motivation, academic achievement, and self-esteem.

Variables	Sum of Squares	df BG	df WG	Mean Square	F	Sig.
Academic Motivation	86.43	4	294	21.81	1.85	.12
ACT	7288.86	4	303	1822.22	.57	.68
GPA	.272	4	317	.068	.34	.85
Self-Esteem	177.73	4	306	44.43	.91	.46

TABLE 6
Academic motivation, academic achievement, and self-esteem descriptive statistics for musicians of differing experience.

Variables	Music Majors/Minors	Musicians in Collegiate Ensembles	Musicians Not in Collegiate Ensembles (5+ years of experience)	Musicians Not in Collegiate Ensembles (3-5 years of experience)	Musicians Not in Collegiate Ensembles (less than 3 years of experience)
Academic Motivation	8.13 (<i>sd</i> =2.87)	7.23 (<i>sd</i> =3.45)	7.29 (<i>sd</i> =3.41)	5.58 (<i>sd</i> =4.45)	7.19 (<i>sd</i> =2.93)
ACT	26.14 (<i>sd</i> =5.74)	26.86 (<i>sd</i> =4.09)	37.09 (<i>sd</i> =99.38)	27.18 (<i>sd</i> =2.92)	25.90 (<i>sd</i> =3.96)
GPA	3.59 (<i>sd</i> =.35)	3.53 (<i>sd</i> =.49)	3.49 (<i>sd</i> =.45)	3.54 (<i>sd</i> =.44)	3.51 (<i>sd</i> =.35)
Self-Esteem	39.16 (<i>sd</i> =6.75)	37.76 (<i>sd</i> =7.09)	38.54 (<i>sd</i> =6.79)	38.17 (<i>sd</i> =8.06)	35.95 (<i>sd</i> =6.23)



**Noncontact Anterior Cruciate Ligament Injuries
in Collegiate Female Soccer Players:
The Effects of a Four-Week Prevention
Program on Landing Kinematics**

Tess K. Marcordes

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ABSTRACT

Background

Noncontact anterior cruciate ligament (ACL) injuries are highly prevalent in soccer as quick changes in direction, stop-start motions, as well as jumping and landing often paired with shooting or passing a ball, are occurring continuously throughout a practice or match. Exaggeration of these movements with extreme joint motions increases the likelihood of ACL injury. An ACL injury can be detrimental to an athlete's career, as 25% of athletes with such injuries do not return to their pre-injury level of play (Padua, DiStefano, Beutler, de la Motte, DiStefano, and Marshall, 2015). Myer, Ford, McLean, and Hewitt (2006), Garcia (2011), and Pollard, Sigward, Ota, Langford, and Powers (2006) have all conducted research showing programs consisting of a combination of plyometric, balance, and strengthening exercises constitute effective prevention of ACL injury.

Methods

This study tests a new, unestablished ACL injury prevention program designed using elements of previous successful programs, increasing in difficulty as the weeks progressed. Three soccer players participated in the program three days a week for four weeks. The Modified Lower Extremity Scoring System (LESS) was used to determine the risk of ACL injury of each participant. Participants were members of a varsity women's soccer team at a small Christian university in the Midwest. None of the participants had sustained any knee injury prior to participation. The control group consisted of five participants, while the experimental group consisted of three participants. Both groups performed vertical drop tests as their landings were evaluated with the Modified LESS prior to implementation of the prevention program on the experimental group. After the program concluded, each group was retested. We hypothesized that after participation in the progressive four-week ACL prevention program, the experimental group would display a decrease in their Modified LESS scores, thus indicating a decrease in the possibility of noncontact ACL injury, whereas the control group would see little to no differentiation of scores.

Results

The hypothesis that a four-week progressive ACL injury prevention program would lower ACL injury risk factors on the Modified LESS was confirmed. A similar decreasing trend was observed in five of the ten categories on the Modified LESS of the experimental group.

Conclusion

The decrease of the Modified LESS scores occurred in the same five of the ten categories. This could be due to the auditory cues that were given while the participations were performing. The results found in the study correlated with other research.

Keywords: anterior cruciate ligament, ACL, injury, ACL injury, knee, soccer, landing, LESS

INTRODUCTION

In 2011, 127,000 ACL reconstructive surgeries were performed in the United States (Bates, Nesbitt, Shearn, Myer, and Hewett, 2016). ACL tears often lead to reconstructive surgery that can require up to twenty-four months of recovery (Otzel, Chow, and Tillman, 2015). Re-tearing the previously injured ACL as well as the tearing of the contralateral ACL post-reconstructive surgery is not an uncommon occurrence either. An injury to the ACL could be detrimental to one's athletic career. About 25% of athletes who sustain an ACL injury and undergo reconstructive surgery may never return to the level of play they were at prior to injury (Padua et al., 2015).

An athlete not only is susceptible to an ACL injury when a force comes in direct contact with the knee, but ACL injury can also occur without any contact to the knee; the latter is known as a noncontact injury. Noncontact ACL injuries have two basic factors that increase injury risk for an athlete: extrinsic factors, including playing surface, shoes, weather, and other elements outside the body that can affect the knee and its movements; and intrinsic factors, including gender, anatomy, age, joint movements, and biomechanics (Garcia, 2011). Weiss and Whatman (2015) state that athletes who participate in sports that "involve stop-start movements, changes in direction, jumping and landing both with and without passing and/or shooting a ball" (p. 1326) are more at risk for a noncontact ACL injury. All elements stated by Weiss and Whatman (2015) are fundamental aspects of soccer; therefore, it can be concluded that soccer players are at an increased risk of ACL injury. Several studies have discovered the most common movements in soccer that create a high risk are acts of pressing, or defending, as opposed to kicking, dribbling, or trapping the soccer ball (Kaneko, Sasaki, Hirose, Nagano, Fukano, and Fukabayashi, 2017). Exaggeration of these movements paired with extreme joint motions increase the likelihood of ACL injury.

Structure of the Knee

As the largest joint of the body, the knee connects the lower leg to the femur with a series of cartilage, bone, and ligaments (Dupler, 2011). Being a hinge joint, the knee works cohesively alongside the muscles surrounding it, moving the body in all directions (Lerner and Wilmoth, 2007c). The four ligaments of the knee joint that connect the femur to the lower leg are the medial collateral ligament (MCL), the lateral collateral ligament (LCL), the anterior cruciate ligament (ACL), and the posterior cruciate ligament (PCL). These ligaments are subject to injury in situations that require quick, explosive, or twisting motions (Lerner and Wilmoth, 2007a). In addition, the medial and lateral menisci create a surface for the knee joint to move smoothly.

The menisci - The medial and lateral menisci are the cartilage that are a part of the knee's anatomy. Anteriorly, the medial meniscus attaches to the tibial plateau by meniscal roots (Koo, Choi, Lee, and Wang, 2015). As it forms a semicircle, the medial collateral ligament (MCL) connects the middle of the medial meniscus to the femur and tibia. The posterior intercondylar fossa is the location of the other end of the meniscus (Goldblatt and Richmond, 2003). The lateral meniscus is circular in shape,

covering the majority of the tibial plateau with the anterior portion in connection with the ACL. To prevent the grinding of the bones, the menisci keep the joint lubricated for easy movement as well as acting as a cushion to absorb shock (Koo et al. 2015). Both menisci have the secondary role of restraining the rotation and translation of the knee (Halewood and Amis, 2015).

The collateral ligaments - The medial collateral ligament (MCL) and the lateral collateral ligament (LCL) are located on the either side of the knee joint: the MCL on the medial side of the knee and the LCL on the lateral side (Lerner and Wilmoth, 2007a). Both collateral ligaments are tight while the knee is in flexion, indicating how the two ligaments provide stability to the knee. From zero to thirty degrees, the LCL is in control of the varus rotation (displacement towards the body's midline) of the knee at all angles (Halewood and Amis, 2015). When the knee is in full flexion, tibial rotation occurs because the LCL is slack (Goldblatt and Richmond, 2003).

The MCL is often referred to in two different parts: the deep MCL (dMCL) and the superficial MCL (sMCL). The sMCL is very important in knee stability as well as being the primary restraint to valgus (displacement away from the body's midline) rotation and external rotation (Goldblatt and Richmond, 2003). When the knee is in flexion, the prevention of internal and valgus rotation is the primary role of the dMCL; however, the sMCL is the most effective to preventing valgus rotation. The dMCL is not as strong as the sMCL due to smaller fibers that compromise the ligament, making it more likely to rupture (Halewood and Amis, 2015). Because of its connection to the dMCL, the medial meniscus is highly susceptible to injury when the dMCL is torn.

The cruciate ligaments - The determination of the motions of the knee is due to the insertions, lengths, and linkages of the ACL and PCL (Halewood and Amis, 2015). If either the ACL or the PCL is injured, the athlete will not be able to perform movements that require twisting and explosion (Goldblatt and Richmond, 2003). The ACL connects the femur to the tibia providing the knee with a large source of stabilization (Lerner and Wilmoth, 2007a). This connection provides stabilization, restraining anterior translation of the tibia on the femur (Goldblatt and Richmond, 2003). If the ACL is functioning properly, the tibia should not be able to be pushed forward when the knee is in flexion. The secondary roles of the ACL include being a restraint to varus-valgus movements, internal rotation, and hyperextension (Goldblatt and Richmond, 2003).

Prevention of posterior translation of the tibia on the femur when the knee is in flexion is the primary restraint of the PCL, but this role is transferred to the MCL as the knee moves to extension (Bates and Sekiya, 2009). This means that when the knee is in flexion, if the PCL is still intact, the tibia will not be able to move backwards. The secondary role of the PCL is the assistance in the natural external rotation of the tibia as well as a restraint to varus-valgus movements. However, studies have shown that without the PCL, the LCL still acts as a restraint to these movements (Goldblatt and Richmond, 2003).

Female Susceptibility to ACL Injury

In comparison to men, women are two to five times more susceptible to ACL injuries (Padua and Marshall, 2006). Females' quadriceps and hamstrings are not as strong as a male's, contributing to the higher rate of risk, in addition to the female anatomy revealing a smaller tissue structure of the ACL as well as a smaller intercondylar notch on which the ACL connects (Lerner and Wilmoth, 2007a). The smaller tissue tears more easily. Biomechanically, the hips and knees of females move in such a way that increases ACL injury risk (Sakaguchi et al., 2014). Women anatomically have a wider hip structure than men do, creating a "Q" angle in which the positioning of the pelvis, femur, and knee become risk factors ("ACL injury prevention," 2012). The "Q" angle is a result of the femur making an inward angle from the hip to the knee; therefore, the leg is no longer in a perpendicular position to the ground (Lerner and Wilmoth, 2007a). Risk of ACL injury has a direct relationship with the "Q" angle: as the angle increases, the risk of ACL injury increases. A knee that is not fully stabilized while performing motions involving cutting, change in speed, and jumping is at high risk for injury.

High school and college-aged female athletes are at the greatest risk for ACL injury (Padua and Marshall, 2006). It has been suggested that the increased susceptibility to injury at this age may be due to past training as a child. The average training level of a young girl is often lower in intensity in comparison to that of young boys' training. As girls progress to higher levels of competition, with its increasing demands on the body, research suggests that there is a lack of proper training for the average girl participating in soccer. Due to the inadequacy of training, athletes' bodies do not transition properly to the elevated intensity. The body's reactions to quicker movements and harder landings and hits are underdeveloped, often resulting in incorrect movements of the body, particularly in the knee, thus increasing the risk of ACL injury ("ACL Injury Prevention," 2012). An ACL injury prevention program for female athletes at this age must take into consideration this lack of training. Therefore, the program must include movements and proper instruction for accurate execution of the movements to provide further preparation to the athletes as they progress to higher levels of completion.

Risk Factors of Noncontact ACL Injuries

Kaneko et al. (2017) report that 70 to 84% of ACL injuries in athletes are noncontact. Noncontact injuries are very complex because several risk factors come into play when an injury occurs. These risk factors include environmental, hormonal, anatomical, genetic, biomechanical and neuromuscular factors. However, the biomechanical and neuromuscular factors are risk factors that can be manipulated in order to decrease injury risk, whereas it is not possible to change the other factors listed (Sugimoto et al., 2015). In the current study, the focus is placed upon two biomechanical/neuromuscular risk factors: the movement and reactions of the hips and the movements and reactions of the quadriceps and the hamstrings.

The quadriceps and hamstrings -The quadriceps and hamstrings play a key role in the degree of knee flexion when landing from a jump. If the quadriceps are weak, the reduction in the knee flexion ultimately increases the likelihood of ACL rupture (Otzel

et al., 2015). In the rehabilitation process after surgery, quadriceps lose strength, making it more likely for the patient or athlete to re-rupture the same ligament. Therefore, quadriceps strengthening is important when preventing initial injury as well as in rehabilitation process after ACL reconstructive surgery. In reference to the hamstrings, extreme quadriceps contraction without equivalent contraction of its counterpart, the hamstrings, puts extreme stress on the knee when landing with an extended leg (Myer et al., 2006). This action often occurs naturally when the quadriceps are significantly stronger than the hamstrings. Injury risk increases due to the muscle fatigue causing internal rotation of the hip resulting in severe knee abduction (movement away from the body's midline). However, even if the quadriceps and the hamstrings work together, fatigue results in a change in the movements of the hip and ankle joint, indicating another risk factor of ACL injury (Thomas, Villwock, Wojtys, and Palmieri-Smith, 2013).

The hip - In more recent studies, it has been discovered that a decreased range of motion (ROM) of the hip may also be a contributor to noncontact ACL injuries (Lopes, Gomes, and Spinelli, 2016). In general, knee adduction (movement toward the body's midline) is an indicator of ACL injury. If the ROM of the hip is limited, weight is not distributed properly on the knee, creating more stress on the joint and the ligaments associated, including the ACL, thus increasing the risk of injury. A study of male and female soccer players concluded that the range of motion of the hip of the athletes that re-tore their ACL was significantly smaller (about twenty degrees) than that of the athletes who did not re-rupture their ACL (Gomes, Humberto, and Ruthner, 2014). If the hip is constricted and has little ROM or too small of a degree of flexion, a load placed on the knee will not be properly distributed, therefore placing unnecessary stress on the tendons of the knee and escalating the injury risk (Arendt and Dick, 1995). Aside from a decreased ROM, weakness of the muscles and tendons in association with the hip joint also places an athlete at risk for injury. The hip abductor muscle influences the proximal control of the hip (Park, Kim, and Kim, 2016). If the muscle is weak, the hip compensates by internally rotating and abducting, consequently causing the knee to adduct (Sakaguchi et al., 2014). An increased adduction of the knee joint is another indication to ACL injury. Gomes et al. (2014) performed a study of healthy male soccer players with no ACL injuries or male soccer players with history of rupturing one ACL on two separate occasions. Of the subjects with history of injury, over half had experienced an ACL tear in both knees. Gomes et al. (2014) discovered that the re-occurrence of ACL injuries of the athletes with re-ruptured ligaments or contralateral tears correlates to the weakness of the muscles and tendons that are a part of the knee. With the proper training, the risk of injury can decrease.

Prevention

Measures can be taken in order to change and control the movements of an athlete's knee, to neutralize the uneven contractions of the quadriceps and hamstrings, and to minimize other risk factors. Training the quadriceps and the hamstrings together, encouraging both muscle groups to work collaboratively, will improve the possibility of injury. Strengthening these muscles using coordinating actions provides greater

benefit in injury prevention than strengthening of individual muscles (Thomas, Palmieri-Smith, and McLean, 2011). As the individual moves throughout a game or practice, the previous collaborative training of the quadriceps and hamstrings endorses co-contraction, thus decreasing the risk of ACL injury.

During the rehabilitation process after ACL reconstruction, isokinetic strengthening of both muscles is an effective training option (Otzel et al., 2015). Isokinetic strengthening is a form of resistance training that includes the combination of tension and speed incorporated into exercises, furthering the strength of the targeted muscles ("Isokinetic," 2003). Researchers find that isokinetic training produces positive results in injury prevention; however, the improvement is limited (Ratamess et al., 2016). Most isokinetic programs are primarily used for the general population; therefore, basic one joint movements are the focus. As the majority of sports require multiple-joint movements, isokinetic training in an ACL injury prevention program would be most effective in developing muscular control when multiple-joint exercises are used (Otzel et al., 2015). Soccer requires a wide variety of irregular movements of several different joints; for that reason, isokinetic training alone may not acknowledge all of the possible movements that can occur in competition. Benefits are still available to an athlete in this type of training; thus the combination of isokinetic and other forms of training may prove to be advantageous for injury prevention and overall athletic performance.

Myer et al. (2006) conducted a study to compare the effects of plyometric and dynamic exercises on the knees of women, observing the reaction of the knee and its flexion angle when landing from two different jumps: vertical and horizontal. The plyometric exercises used, specifically continuous jumping, are especially effective in training the knee to properly react to the force placed on the knee itself when landing. At the completion of the study, it was concluded that plyometric training increases the flexion angle of the knee when landing from a vertical jump but had no effect on the flexion angle of the knee during the horizontal jump. The effect of the stabilization and balance training's effect was the opposite: it did not improve the flexion angle of the vertical landing, but it did decrease the likelihood of injury to the ACL from a sideways jump (Myer et al., 2006). The study concluded that combining both plyometric and stabilization training is best in ACL injury prevention because the movements in each decrease injury risks in separate ways, when landing from vertical and from horizontal jumps, both of which are used in several sporting events (Myer et al., 2006).

In addition to plyometric and stabilization exercises, physical therapist Amado Garcia (2011) suggests three additional factors to include in the development of ACL injury prevention programs: flexibility, agility, and strength. Flexibility, whether it be achieved by static or dynamic stretching, is important because it allows the muscles and joints to move more freely. Static stretching requires the athlete to maintain a particular position, extending a particular muscle or muscle group that will be involved in the upcoming competition, for twenty to thirty seconds (Lerner and Wilmoth, 2007d). Dynamic stretching combines walking/jogging to increase heart rate and blood flow to the muscles and simple stretches held for around three to five seconds before returning to walking or jogging.

Balance, agility, plyometrics, and strength all have overlapping benefits and purposes when preventing injury. Research shows strength and plyometric training positively increase the abduction angle in the hip, allowing an athlete to have more control over hip and knee movements (Pollard et al., 2006). These exercises translate directly to soccer, as large quantities of energy and motions are required. When playing in a soccer match, athletes use their lower bodies to move in one direction as their upper bodies move in the opposite direction to deceive and shield off their opponents (Gomes et al., 2014). The athletes must be placed in situations that are game-like while training, enabling their bodies to learn the correct way to move and adjust in moments that the upper and lower body are moving in different directions.

McNair, Prapavessis, and Callener (2000) discovered the importance of proper instruction (technical, auditory, and metaphoric imagery) during an ACL injury prevention program. The purpose of different cues given to participants is to correct the joint kinematics of the participants when landing from a vertical jump. The current study implements both the technical and auditory cues, as they were the most beneficial in McNair et al.'s (2000) study. Specifically, McNair et al. (2000) compared three experimental groups, all receiving a different form of verbal instruction to improve ground reaction force, with a control group that received no instruction on their jumps. Technical instruction consisting of biomechanical prompts, such as "position yourself on the balls of your feet with bent knee just prior to landing" (p. 294), were given to the first group. The second experimental group was instructed by auditory cues. By listening to the sound of their landing, the participants were told to use this information to create less sound when landing from future jumps. The third and final experimental group received instruction via metaphoric imagery perspective. Participants were asked to visualize "bubbles floating down toward the ground" (p. 294) or similar imagery.

After the experimental groups went through the specific training assigned to them, every participant was retested. The researchers discovered that the second experimental group, those that received auditory cues, presented the greatest decrease in ground reaction force, thus indicating a decrease in risk of knee injury. The group receiving technical instruction also displayed a decrease in ground reaction force as well, however not as significant as the decrease presented by the auditory group. In the current study, when participants were performing vertical jumps throughout the program, technical instruction used by McNair et al. (2000), such as "position yourself on the balls of your feet with bent knee just prior to landing" (p. 294), as well as auditory cues, including instruction to listen to the volume of their landings, were used.

MATERIALS AND METHODS

Participants

A sample of ten female soccer players from a small Christian university in the Midwest participated in this study. Each participant signed a written informed consent, approved by the university's Institutional Review Board before testing. The inclusion criteria

of the study required that the participants were part of the women's varsity soccer team during the fall 2016 season, were currently participating in the team's offseason training, and had no prior ACL injury.

Materials

Red, green, and blue TheraBands™ of increasing resistance were used in the prevention program. They were cut and tied into loops with the circumference of approximately sixty centimeters. These loops were placed around the legs, proximal to the patella, as the participants performed three exercises (Duck Walks, Bridges, and Clams) throughout each progression of the program in order to provide resistance to strengthen the muscles that were being activated during the exercise.

Yes4All™ balance pads were used when performing the sport-specific exercise as well as a cushion for other exercises in order to prevent sliding on the carpet of the room in which the program was held. In the first progression, the participants began by jumping with two feet laterally onto the balance pad and landing with one foot. The second progression moved onto the participant balancing on one foot on the pad as a soccer ball was tossed to participants, which they would volley back towards the thrower with the free foot. Finally, both exercises of the first and second were combined in the third progression: starting on both feet, jumping laterally and landing on one foot, immediately progressing to volleying the soccer ball with the free foot. When performing Russian hamstrings in all three progressions, participants placed the balance pads underneath their knees in order to minimize the sliding of their knees on the carpet, possibly resulting in "rug burns" on their knees. Participants also placed their forearms on top of the balance pad when performing planks for similar reasons.

Procedure

In the current study, the goal of the four-week ACL injury prevention program was to improve the participants' landing kinematics, resulting in a decrease in the scores of the Modified Landing Error Scoring System (LESS). An established ACL injury prevention program was not used in this study. However, a compilation of neuromuscular exercises and strengthening exercises are included in the progressive four-week program as displayed in Tables 1A, 2A, and 3A. A program consisting of a variety of exercises produces a greater reduction in injury risk (Nessler et al., 2017). Each session occurred after the participants had taken part in their off-season training for the soccer program in order to ensure the muscles were fatigued for each of the sessions. As fatigued muscles increase the risk of ACL injury, training the participants in a safe environment when their muscles are in this state encourages the improvement of muscular strength and endurance, consequently decreasing injury risk (Thomas et al., 2013). To ensure a safe, minimal risk environment for the participants, high intensity and dynamic exercises were performed first during each session, encouraging endurance and strength development as well as fatiguing the muscles even more to provide a challenge when performing the stabilization and strengthening exercises. The sessions concluded with stabilizing and strengthening exercises, challenging the muscles to activate in a less demanding environment than that of a plyometric exercise.

Overall improvement of landing kinematics was the primary goal of this study. To elicit positive results in landing, teaching the muscles associated to the knees, hips, and core to react properly when landing from a jump is key. The program used in the current study consists of plyometric, stabilization, and strengthening exercises, each with the objective of training the body to land properly. The program includes nine exercises, progressing from high intensity jumping plyometric exercises to lower intensity stability and strengthening exercises. Over four weeks, three overall progressions occurred, beginning with simple exercises and advancing into more complex versions or increasing resistance of the exercises as the participants became acclimated and accomplished with the easier exercises. Each progression was performed in three separate sessions before the participants moved onto the next progression, adding up to a total of twelve sessions performed over the course of four weeks. The program was designed to last no longer than one hour each session. After performing a ten-minute warm-up, including a combination of dynamic stretches and static stretches, the participants began the program. A demonstration of each exercise was given to the participants directly before being asked to perform it. Technical and auditory cues were given to each participant before, during, and after every exercise in order to ensure proper execution.

Alongside proper instruction, the selection of exercises to implement in an ACL injury prevention program is crucial. The current study implements the use of stability and plyometric exercises based upon the discoveries made by Myer et al. (2006). A comparison of the effects of two single-component injury prevention programs, plyometric versus balance training, on the landing kinematics of female athletes was performed by Myer et al. (2006). Aside from the primary goal of differentiating the effects of a plyometric program versus a balance program on injury risk, the secondary goal of the study was to properly instruct and teach the female athletes on the correct and most safe way to land from a jump. As plyometric and stabilization exercises are very different, different forms of instruction and cues are needed to ensure proper execution. The teaching strategies varied between the two groups: the balance group received feedback while performing a particular task. By doing so, participants were able to make adjustments immediately, whereas feedback given to participants in the plyometric program was after an exercise was performed. Due to the high-paced nature of plyometric exercises, this was the best time to give instruction to the participants in order to help them make a conducive response. Several of the exercises used by Myer et al. (2006), stabilizing and plyometric, were used in the current study, as they were shown to be successful in decreasing injury.

As ACL injuries have become more prevalent in recent years, several injury prevention programs have been created in order to maximize the reduction of injuries. The current study does not use an established ACL injury prevention program; therefore, several existing ACL injury prevention programs were studied to generate the best results. The discoveries from a review of ACL injury prevention programs conducted by Alentorn-Geli et al. (2009) were implemented in the current study. The purpose of Alentorn-Geli et al.'s study (2009) was to determine what techniques were more effective than others

when preventing noncontact ACL injuries. Alentorn-Geli et al. (2009) drew three conclusions from their review. First, not one specific program that is established and standardized works for all soccer players in order to reduce noncontact ACL injuries. Second, when comparing the results of programs that consist of multiple exercise components versus the programs consisting of a single component, the researchers discovered that the studies assessing multi-component programs elicit a more significant decrease in noncontact ACL injury susceptibility. Multi-component programs consist of several different forms of exercises (e.g. plyometrics, agility, strengthening, etc.), as opposed to a single component program that focuses on one particular type of exercise. Finally, Alentorn-Geli et al. (2009) concluded that the majority of noncontact ACL injury prevention programs have a duration of six to eight weeks, resulting in a decrease of injury risk. Due to these findings, the program created for the current study consists of multiple components: plyometric, strengthening, and stabilization exercises. However, as most programs last six or more weeks, the current study aims to observe the effects of a multi-component program in a shorter time period of four weeks.

In the current study, the analysis of the level of risk when landing from a vertical jump was performed using the modified Landing Error Scoring System (LESS). Participants' landings from a vertical jump off of a surface twelve inches above the ground were analyzed using the modified LESS. The jump was performed a minimum of four times following instruction and a practice trial, thus enabling the researcher to view the participants' landing kinematics from the front and the side of the participant.

RESULTS

Assessment of the vertical jumps occurred prior to participation in the ACL injury prevention program and after the program concluded. Out of the fifteen possible points attainable when using the modified LESS, the average score of the three participants of the experimental group prior to participation in the prevention program was 7.25. This is a 48.33% risk factor for ACL injury. Of the control group, the average LESS score was slightly lower, at 6.33, resulting in a risk factor incidence of 42.2%. After participation in the four-week ACL injury prevention program, the average LESS score for the experimental group decreased drastically by 38.33%, as the average score was 1.5 on the modified LESS, with a 10% risk factor incidence. A slight variation was observed in the data between the pre- and post-testing LESS scores of the control group, the post-testing average score being 6.25 and a 41.67%. Comparison of average LESS scores of both groups are demonstrated in **Figure 1**.

DISCUSSION

The primary finding of this study was that a progressive, multi-faceted ACL injury prevention program implemented over four weeks decreases several ACL injury risk factors based upon the Modified LESS. A key factor contributing to the decreased

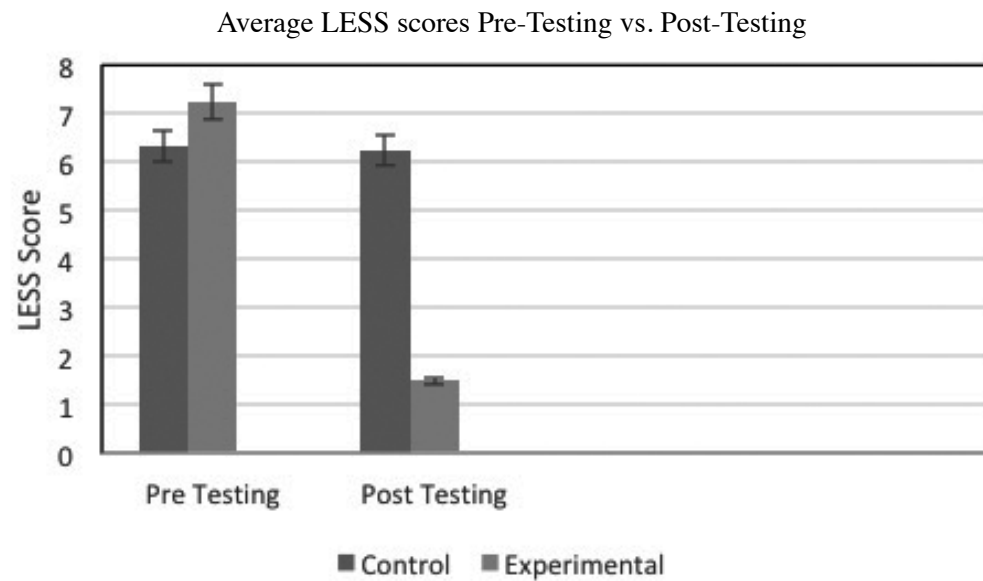


Figure 1. Comparison of average Modified LESS scores of the control and experimental groups before and after implementation of ACL injury prevention.

injury risk is the instruction given to the participants throughout the program. Exercises were explained and demonstrated to the participants before execution. The current study used the same auditory cues as McNair et al. (2000), instructing the participants to listen to their landings in order to guide them to land more softly in the following jumps. Technical cues were also used in the current study, however not as thorough as those used by McNair et al. (2000). In congruence with the research of McNair et al. (2000), an overall decrease in the risk of ACL injury was seen in the subjects in the experimental group, as they were given instruction while performing the exercises. A trend seen between the three participants of the experimental group reveals a reduction in score of the same five categories: “stance width,” “amount of lateral trunk flexion,” “amount of knee-flexion displacement,” “total displacement in the sagittal plane,” and “overall impression.” These results correlate positively with the technical cues that were given throughout the program as the participants were instructed before and during each exercise throughout the four-week program.

Myer et al. (2006) conducted a study comparing the results of two different single-component programs: plyometric versus balance. The current study included similar exercises in both the plyometric and balance programs that Myer et al. (2006) studied, resulting in similar results between the two studies. In congruence to the results found by Myer et al. (2006), an increase in knee flexion was displayed after the participants completed the prevention programs in both studies. Myer et al. (2006) discovered that the implementation of a plyometric program elicits such results. Therefore, it can be concluded that the increase in the amount of knee-flexion displacement observed in the current study resulted from the implementation of the several plyometric exercises in the program. The improvements of lower extremity valgus in both the hip and the ankle

observed by Myer et al. (2006) in both the plyometric and balance programs correlated with the results seen in the current study. However, the current study did not include resistance training. This factor could have impacted the comparison of the results.

The ACL injury prevention programs analyzed in the literature review of Alentorn-Geli et al. (2009) present decreases in the athlete’s risk of ACL injury due to improved coactivation of muscles; increased strength and stability of the knee and hip; reduced valgus, varus, internal rotation; and adduction of the knee and hip. The current study includes a program lasting four weeks, with the hypothesis that a decrease in ACL injury risk factors would still be observed. Improvement was observed in the participants’ Modified LESS scores in the current study, specifically in knee flexion when landing from a jump. Of the studies focusing on improvement on landing biomechanics by implementing programs including plyometric, stabilization, and strengthening exercises reviewed by Alentorn-Geli et al. (2009) similar results were observed. However, it is important to note that the Modified LESS was not the primary analysis tool used in each of these studies. In comparison, the studies reviewed by Alentorn-Geli (2009) lasted six to nine weeks as opposed to the four-week implementation of the prevention program of the current study. Therefore, it can be concluded that ACL injury risk associated with landing biomechanics can be reduced in four weeks.

The limitations of this study include the number of participants. Although five participants began the program in the experimental group, two dropped out due to not having the time to participate in the program three times a week for one hour. This may have limited my ability to observe accurate results in the Modified LESS scores of the participants. Because of the size of the experimental group, I suggest replicating this study with more participants. Due to the limited time frame of which the participants were evaluated for risk, as well as the participants being in the much less rigorous off-season, there was a 0% incidence of ACL injury observed. In comparison, most studies of the ACL injury prevention programs, ACL injuries are often observed while the study is undergoing. Confirmation bias may have impacted the results as well, due to the researcher not being blinded as to which participants participated in the injury prevention program and those who did not.

I suggest that this study be replicated while the participants are in-season as opposed to being in the off-season in order to determine if the 0% incidence of ACL injury found in this study was due to the diminished stress of the off-season. As no studies were found to be limited to a four-week prevention program, conducting studies with different variables over a four-week time span is suggested. The variables can include separate programs that consists of a single exercise component (plyometric, stabilizing, strengthening) in order to observe which constituent is most effective at reducing the Modified LESS score. The women’s varsity soccer team at the university where the study was conducted does not participate in resistance training while in season or in their off season. Therefore, we recommend comparing the athletes from this team to another university’s soccer team who participates in resistance training to compare the effects of resistance training paired with an ACL injury prevention program.

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APPENDIX A: TABLES

TABLE 1

Protocol for Experimental Group: Progression 1

Exercise	Sets	Reps	Time, s
Ankle jumps	3	-	10
Lateral jumps	3	-	10
Lunges	3	10 (each leg)	-
Duck walks with TB (R)	2	15 yards	-
Jump to SL- balance on foam pad	3	10 (each leg)	-
Bridges	3	20	-
Clams with TB (R)	3	10 (each leg)	-
Russian hamstrings	3	10	-
Planks	3	-	45

Note. Reps = repetitions; TB = TheraBand; (R) = red; s = seconds.

TABLE 2

Protocol for Experimental Group: Progression 2

Exercise	Sets	Reps	Time, s
Ankle jumps to squats	3	10 (squats) 20 (ankle jumps)	-
Lateral jumps	3	-	20
Switch jumps	3	-	20
Duck walks with TB (G)	2	15 yards	-
SL balance and volley on foam pad	3	10 (each leg)	-
Bridges with TB (R)	3	-	20
Clams with TB (G)	3	10 (each leg)	-
Russian Hamstrings	3	15	-
Planks	3	-	45

Note. Reps = repetitions; TB = TheraBand; (R) = red; (G) = green;
SL = single leg; s = seconds

TABLE 3

Protocol for Experimental Group: Progression 3

Exercise	Sets	Reps	Time, s
Ankle jumps to squat jumps	3	-	40, 20 each
Lateral jumps	3	-	30
Prisoner switch jumps	3	-	30
Duck walks with TB (B)	2	15 yards	-
Jump to SL balance with volley on foam pad	3	10 (each leg)	-
SL bridge	3	10	-
Clams with TB (B)	3	10 (each leg)	-
Russian hamstrings	3	20	-
Planks	3	-	75

Note. Reps = repetitions; TB = TheraBand; (B) = blue;
SL = single leg; s = seconds.



Weed and Crop Discrimination Through an Offline Computer Vision Algorithm

Phillip J. Putney

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ABSTRACT

With the recent global interest in organic farming and cultivation, many people are turning away from chemical-based herbicides and moving towards alternate methods to extirpate weeds living amongst their crops. Of the methods proposed, robotic weed detection and removal is the most promising because of its possibility to be completely autonomous. Several robust, fully-autonomous robots have been developed, although none have been approved for commercial use. This paper proposes a weed and crop discrimination algorithm that utilizes an excessive green filter paired with principal component analysis to detect specific spatial frequencies within an image corresponding to different types of weeds and crops. This method also works to reduce dimensions in data by representing an image as a small set of values obtained from a projection. This technique optimizes performance while allowing for simpler calculations. These calculations were used to develop thresholds for weeds, crops, and soil for discrimination purposes. The algorithm resulted in an overall classification rate of 77%. 46% of all crops were identified correctly; 78% of all weeds were identified correctly; and 91% of all soil was identified correctly. The low rate of correct crop classification was due to poor edge detection by the algorithm but could be improved in future research by applying one or more edge-detection algorithms. This technique can be adapted in the future with other image-analysis techniques to be used on low-cost systems.

INTRODUCTION

Weeding is one of the simplest, yet most time-consuming tasks given to farmers and gardeners around the world. Chemically-induced weed control is the most popular approach for small and large-scale farms, with the U.S. using over one billion pounds of pesticide and herbicide annually, 5.6 billion worldwide [1]. Slaughter et al.'s review of robotic weed control noted that although herbicide-based weed control systems reduce financial cost drastically compared to traditional weeding methods, "it is not without environmental costs" [2]. Because of the increase in pesticide and herbicide usage over the past decades, it has become evident that using these chemicals can spread toxic debris to regions far beyond their original target area. Most commonly, these chemicals remain in the soil for years and are eventually moved by water runoff to local water sources, which can harm aquatic systems and pollute drinking water [3]. The USDA estimates that fifty million people living in the United States obtain their drinking water from sources that could be contaminated by chemicals such as pesticides and herbicides [1]. Additionally, twenty-five million agricultural workers around the world are unintentionally poisoned by chemicals each year, with some of these chemicals being related to cancer [3]. With global population on the rise and food production demand increasing with it, solutions must be proposed to halt this toxic epidemic.

Organic farming has recently gained popularity because of its lack of genetically modified seed and herbicide use. However, organic farmers have had to revert to primitive techniques in weed control which can be inefficient, inaccurate, and costly [4]. For most small-scale organic farmers, hand labor is the weed control method of

choice. Marty Gray of Gray Farms in Watseka, Illinois, claims that he hires multiple full-time seasonal employees for hand-weeding labor; however, this choice limits his farm in terms of net profit [5]. A study on weeding techniques for carrot farmers found that hand weeding yielded a net profit of \$740/hectare (approximately 2.5 acres) while applying an herbicide yielded \$1409/hectare in comparison [6]. For industrial-style farms that focus on maximizing production and minimizing cost, this job could be accomplished by a single machine that uniformly covers the plot with a weed-killing herbicide; however, hand weeding requires skilled laborers who can accurately identify the weeds and remove them completely without damaging the crop.

Robotics in Agriculture

In order to reduce the cost of labor in agriculture, the area of robotics has been proposed as a solution to not only weeding, but also various other farming-related tasks. Tasks such as site-specific herbicide application, mechanical intra row weed control, and individual seed planting have been tested because of the advancements in sensors, actuators, and electrical equipment over the past twenty years [7]. Although weeding sounds like a simple task, many variables must be taken into account in order to create a reliable weeding machine. Row guidance and control, GPS location, weed identification/classification, and weed removal are some of the major tasks that must be accomplished by a weeding robot.

The biggest challenge in developing a weeding robot is replicating human behavior. Humans have the capability of processing much information through the senses and making rational decisions based upon this information. In the context of weeding, humans can visualize the color of the plants, shape of the leaves, size of the leaves, and even crop spacing. However, this intuition must be learned by robotic systems in order to replicate the human process of manual weeding. A process like this can be learned by computer systems, but challenges still exist when trying to differentiate between minute details. For example, young broad-leaf weed sprouts can look very similar to young lettuce sprouts. A balanced robotic weeding system would have the ability to make fast-paced decisions based upon significant characteristics in data, but could also distinguish between minute details in similar-looking plants.

Many robots have already been developed and proposed with several agricultural functions ranging from weeding to soil sampling. Of the published works on weeding robots, Deepfield Robotics' 'Bonirob' is one of the most developed. It has the capability to perform soil measurement, plant phenotyping, precision pesticide application, and more [8]. Although there are no early estimates of its costs, the price of the machine will undoubtedly be steep because of its robust research applications and advanced technology.

A recent article discussed a group of engineers in India that have developed a robot called the Greenbot that uses simple computing techniques to identify weeds in a vineyard. The Greenbot is a solar-powered autonomous bot run by a Raspberry Pi computer that handles the calculations for weed detection [9]. Its program is designed for the robot to travel under grape vines while detecting green values, target where

those values are, and use a device to uproot the weeds. The Greenbot's calculations detect green values by using a segmentation technique that amplifies green values and creates a global threshold to binarize the image into plant and background [9]. The only issue with this system is that it is not able to discriminate between weeds and crops; it only detects plant life.

The scope of this project was to create an algorithm that will be able to discriminate between plant life in order to identify weeds in a crop field while using computationally efficient techniques. In the future, this system can be implemented on a small-scale robot that utilizes low cost materials like the Raspberry Pi (\$40) to offer a more affordable robotic weeding solution.

Computer Vision Systems

Typically, computer vision systems use one of two mechanisms to identify weeds: shape analysis or color analysis.

Shape Analysis: In general, algorithms using shape analyses tend to be more robust and require more computing power than other techniques [2]. Another important drawback to shape-based analysis is that it is hindered by occlusion of weed and plant leaves; if the leaves are overlapping, then a program can find it difficult to determine which leaf belongs to which plant [10]. To overcome this obstacle, most shape analysis occurs during the early stage of life for both crop and weed, so the leaves do not overlap each other like they do when fully grown. Accuracy rates for the shape analysis procedures found ranged from 75 to 100%, depending on occlusion and lighting [10] [11] [12] [13].

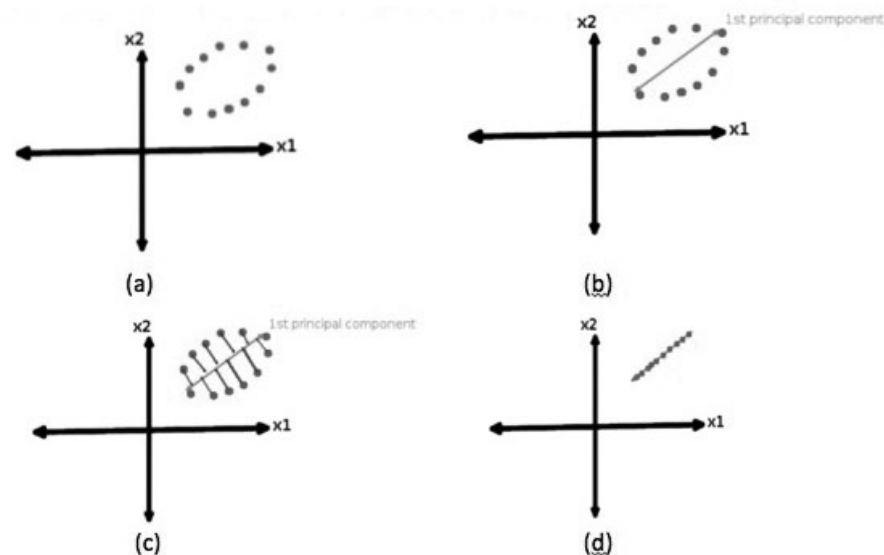


Figure 1: Two dimensional PCA. (a) represents the data plotted on an XY plane. (b) represents the first principal component line being draw through the data. (c) shows the variance of each point from that principal component. (d) Projects each data point onto the principal component [17].

Color Analysis: While the accuracy of shape-based analysis is difficult to match, an important part of this project was computational simplicity, and color-based analysis tends to be less taxing on computing systems [2]. In fact, in all of the research that has been reviewed, this is the only approach that has been used for segmentation between plant and soil. Sujaritha et al.'s Greenbot experiment mentioned earlier was done entirely with segmentation, since no weed/crop discrimination was needed. Through this process, Sujaritha et al. were able to segment the picture, removing 98% of the soil and leaving only the shape of the leaves [9].

Weed and crop discrimination can also be accomplished with the use of color analysis techniques as shown by Franz et al., Lamm, and Borregaard et al. with accuracies reaching 94% [14] [15] [16]. All of the data that is analyzed in these techniques is in the form of pictures that contain millions of pixels—the data points. In order to sort through these millions of data points in a computationally conservative manner, a matrix decomposition algorithm known as principal component analysis (PCA) was proposed. It is important to note that no one has previously applied matrix decomposition algorithms, such as PCA, to discriminate between weeds and crops in a ground level photograph.

Principal Component Analysis

PCA takes large groups of data and simplifies that data as a projected value onto a principal component of the data. In Figure 1a we see a set of data in which each data point consists of both an X and Y value. If we then draw a line through that data and project each point onto that line, we are left with our first principal component. By doing this, we are able to reduce dimensions of the data from an X and Y value, to only one value along our first principal component line. This type of mathematics is called dimensionality reduction.

This same method of dimensionality reduction can be applied to image analysis and has been used by facial recognition software since the early 90s to reduce large quantities of data. Whereas the example above shows data being projected onto a line that represents a principal component, image analysis uses a matrix of values developed using the principal components, and data can be projected onto that matrix. An example of how this is accomplished in facial recognition can be seen in **Figure 2**. In this example, each face can be reconstructed by taking a weighted sum of the principal component values. This process is used to represent the data as a variance of known values, which reduces dimensions.

In this application of PCA, we will be detecting the most significant patterns in spatial frequencies within each image. By using PCA, we will be able to represent these patterns as different principal components; each principal component representing different spectral features of the image. The most significant patterns in spatial frequencies will be represented by the first principal component and the variance of the data from that component. The results of our experimentation showed that PCA can be used to simplify data in image analysis while retaining accuracy. After principal component analysis was used in this study, the proposed algorithm achieved 77% overall accuracy in image classification.



Figure 2: PCA in facial recognition. (a) the original dataset of images and (b) the resulting principal components represented as matrices of data. Each face in the original dataset can be represented as a weighted sum of the principal component values [18].

METHODS

Photos of various crop fields were obtained from Gray Farms in Watseka, Illinois, to use as data sets [5]. All images were taken with a five megapixel autofocus camera and included several types of lettuces and weeds. These photos were separated into two sets to be used in training and testing the discrimination algorithm.

Image Pre-Processing

Images were pre-processed in order to prepare the spectra for matrix decomposition. Initial pre-processing used the application of an excessive green filter, like the one used by Jeon et al. in their study using artificial neural networks to segment crops and weeds [19]. The filter works by applying the equation below to each pixel in the original image; the resulting image can be seen in **Figure 3a**. This green filter was used in the same way that humans would detect different hues of green and use them to identify crops and weeds.

$$EXG = (2G - B - R) / (R + G + B) \quad (1)$$

Where,

EXG= excessive green normalized value

R= red pixel value

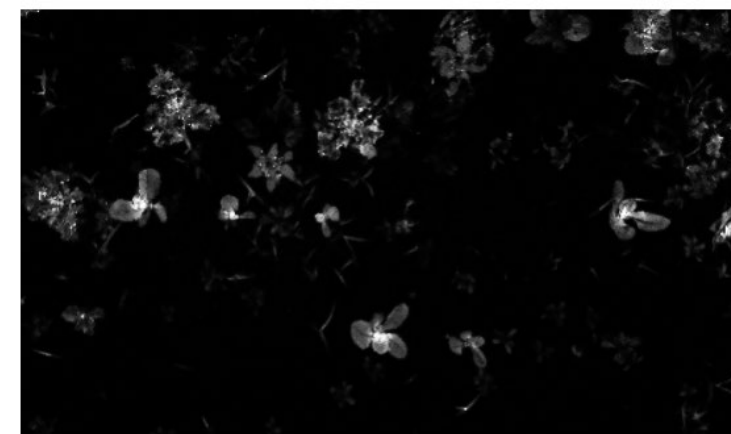
G= green pixel value

B= blue pixel value

Images were then separated into an overlapping grid of blocks and analyzed one block at a time. This block-based analysis approach has been used by other researchers who stress the importance of block size. If the block size is too small, there may not be enough information to use, and if it is too large, both crop and weed could exist in a block, skewing data [4]. The block size used in this experiment was 100 x 100 pixels with an 80% overlap, which was selected based upon average plant size and maximum computing power.



(a)



(b)

Figure 3: EXG filter. (a) is the original image and (b) is the resulting image after applying the excessive green image filter.

Once the image was divided into blocks, a fast-Fourier transform was applied to each block, transforming the data from a two-dimensional image space into a two-dimensional spatial frequency domain that can be used in the applications of PCA.

Principal Component Analysis

Principal component analysis was then used to derive a set of principal component values using the pre-processed spectra. Each matrix of principal component values can be remapped into the shape of the original image. By doing this, the values can visually represent different spectral features shown by crops, weeds, and soil. These values were taken from the matrices produced by the singular value decomposition function in MATLAB. After all matrices were collected, a projection matrix was created by using the code below:

```
[U, S, V] = svd(spectra);
Vectors = V;
Values = U*S;
Projection_Matrix = inv(Vectors');
```

The first three principal component value matrices were then derived by multiplying the original spectra by the projection matrix and remapped to represent the dimensions of the original image. These values are shown below in **Figure 4**.

As shown in Figure 4, PCV1 represents the most variance in its values, and thus represents the most information about what is in that image. Although the other two matrices do contain information, there is not enough visible variance to help in the discrimination between crops and weeds.

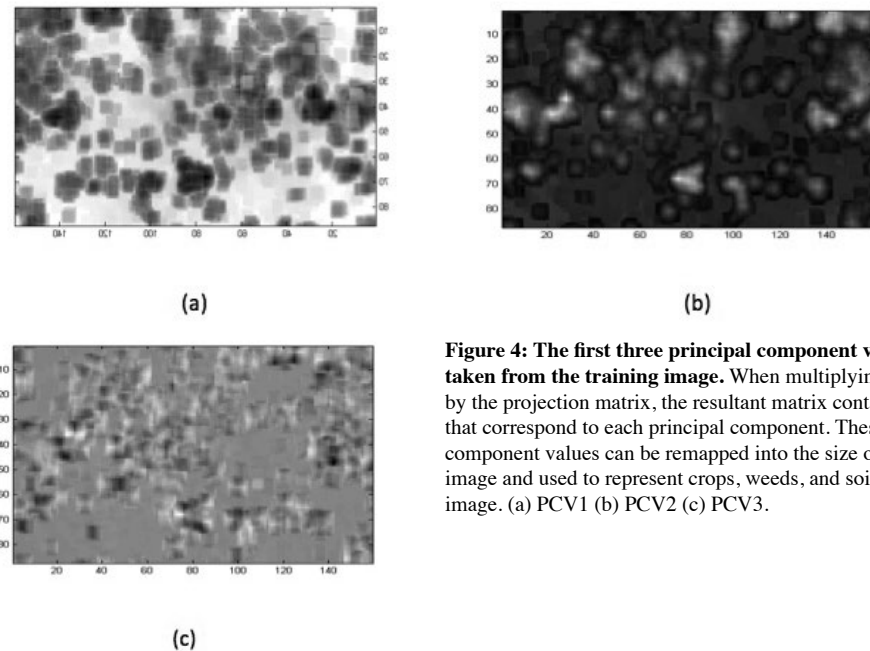


Figure 4: The first three principal component value matrices taken from the training image. When multiplying the spectra by the projection matrix, the resultant matrix contains values that correspond to each principal component. These principal component values can be remapped into the size of the original image and used to represent crops, weeds, and soil within the image. (a) PCV1 (b) PCV2 (c) PCV3.

Training Image

In order to train the algorithm, a duplicate image (**Figure 5**) that was manually marked with the locations of all weeds and crops was projected onto the first principal component value matrix. This duplicate image was divided into six different categories/ colors represented by three types of lettuce, two types of weeds, and soil.

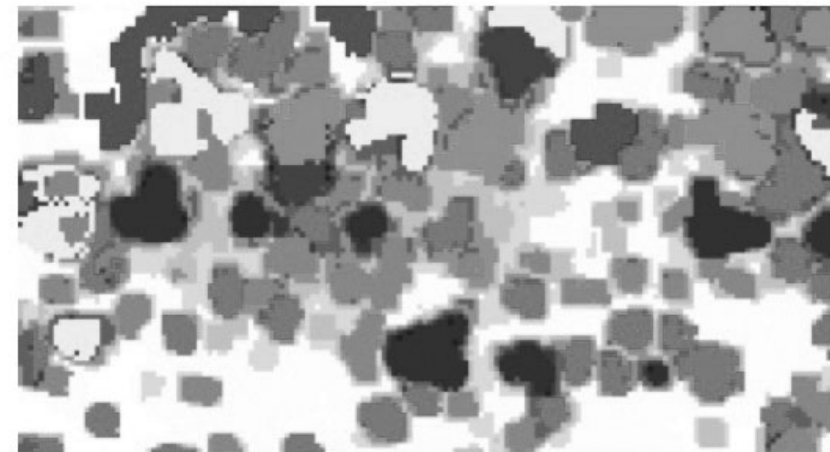


Figure 5: Training image mask. This image contains three different lettuces, two different weeds, and soil as the background. Each category was painted over with a different color to represent the location of weeds, crops, and soil in the image.

Once the mask was created, it was projected onto the first principal component image to associate each color with a range of principal component values. As shown in **Figure 6**, there is a clear region of values that can be represented as weeds, although the values separating each specific crop are not as clear. By separating the data into three categories, weed, crop, and soil, a clearer separation can be noticed in **Figure 7**. This information was used to develop two thresholds that represent each eigenvalue location as either weed, crop, or soil.

From the two thresholds developed by the data shown in the **Figure 7**, it can be assumed where values are < -0.063 there exists a lettuce crop, where values are < -0.035 but > -0.063 there exists a weed, and where values are > -0.035 exists the background/soil. These thresholds can be adjusted to hone the algorithm to be as general or as specific as needed in classification.

Classification

In order to classify an image, the same pre-processing steps were completed on every image to develop a matrix of spectra that can undergo calculation. Once the spectra were accumulated, the weighted principal component values were found by multiplying the spectra matrices by the projection matrix from the training image. Performing this image classification on our original training image should result in a figure similar to the mask that was created (**Figure 8**).

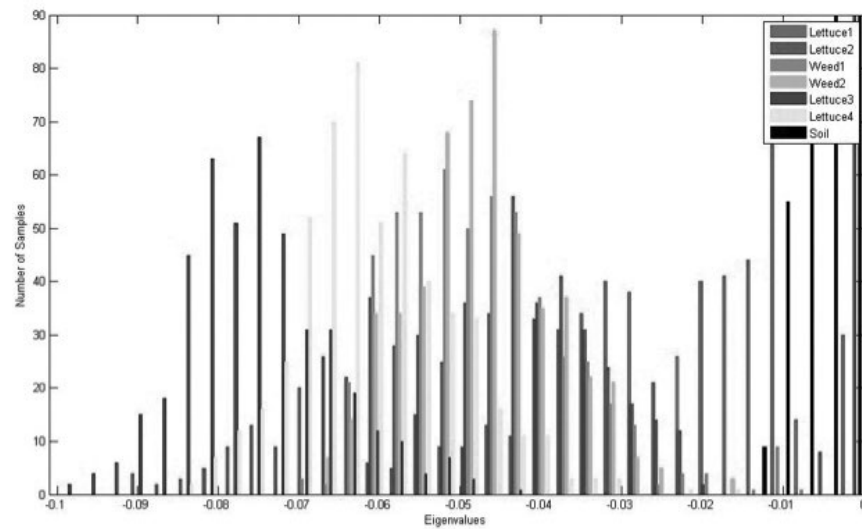


Figure 6: Categorical histogram of principal component values. Each category’s values were plotted against the number of samples found within that value range. This was used to find specific ranges for each category in the image.

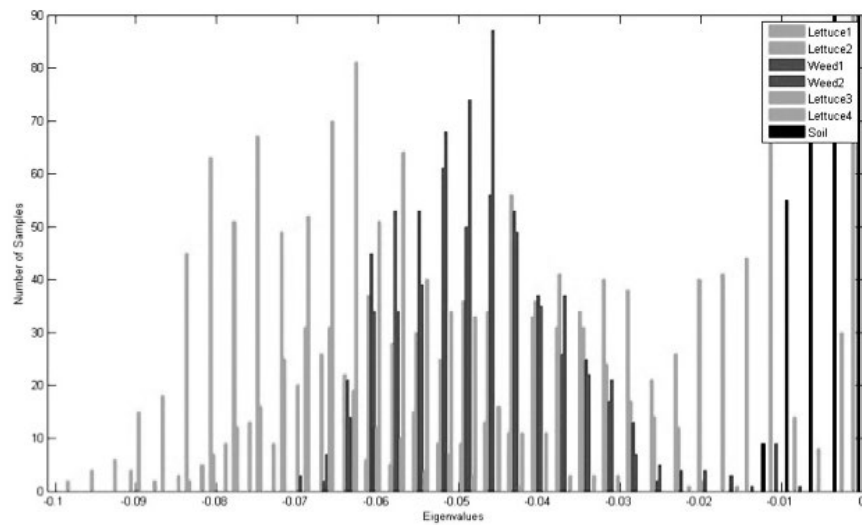


Figure 7: Segmented histogram of principal component values. By combining the categories into only three groups — weed, crop, and soil — a clear threshold of values was discovered.

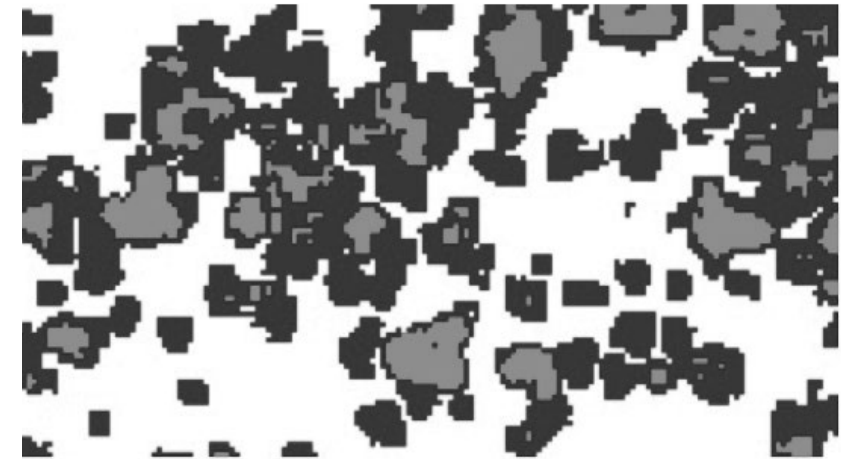


Figure 8: Training image classification. The classified representation of our original training image, where the light gray regions represent crops, the dark gray regions represent weeds, and the white regions represent soil. It should be noted that the purple cabbages were misclassified because of their lack of light gray values.

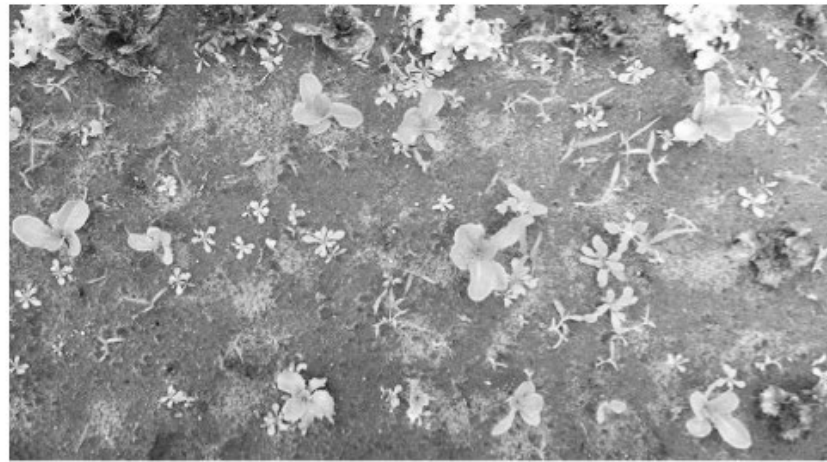
RESULTS

The results of the algorithm conducted on a new test image are shown in **Figure 9**. This image was also taken at Gray Farms but at a different section of the lettuce field. The classification image was obtained by performing the original pre-processing steps to obtain a matrix of spectra. The matrix of spectra was then multiplied by the projection matrix, which resulted in principal component values for the image corresponding to the values developed in the training image.

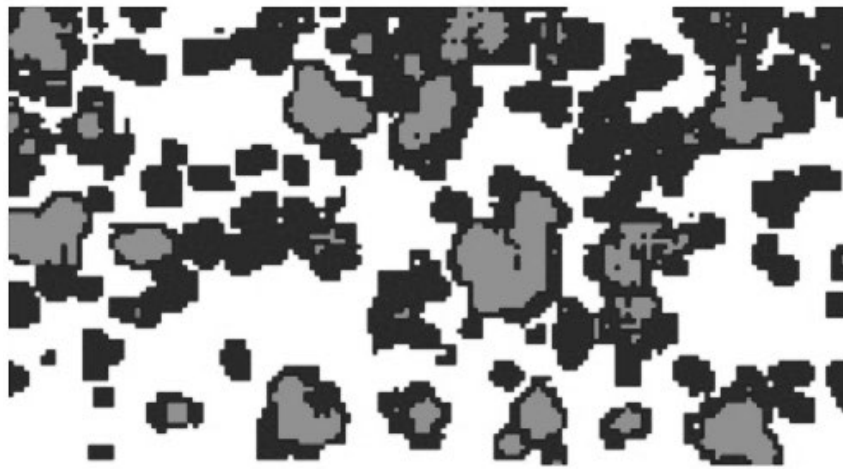
In order to test the results and accuracy of the classification image developed by the algorithm, a manual classification was created. To do this, the first principal component value matrix of the test image was opened in Microsoft Paint. Each region of the image was then manually classified by visual inspection and labeled as crop, weed, or soil. The resulting manual classification image was projected onto the algorithm’s classification image to determine correlating values.

The result of this classification image was an overall accuracy of 77%. The contingency table show below displays a more in-depth analysis of the data. 45.6% of all crops were correctly classified, 77.8% of all weeds were correctly classified, and 90.8% of all soil was correctly classified.

TABLE 1



(a)



(b)

Figure 9: Classification of plant type on a new image using the discrimination algorithm. The test image (a) and the resulting test classification image, where the light gray regions represent crops, the dark gray regions represent weeds, and the white regions represent soil (b). This represents an overall classification accuracy of 77%.

Classification Contingency Table

This table shows the number of blocks that were correctly classified by the algorithm (shown in light gray) versus the number of blocks that were misclassified by the algorithm (shown in dark gray).

		Predicted		
		Crop	Weed	Soil
Actual	Crop	1221	1085	373
	Weed	339	3936	787
	Soil	6	528	5269

The overall results of this experiment demonstrated that dimensionality reduction techniques can be paired with image processing to discriminate between weeds and crops in a ground level photograph. With a total accuracy of 77%, an analysis of misclassification must be made. The largest type of misclassification that occurred was crop/weed misclassification. To clarify, 1085 out of 2679 known crop regions were classified as weed instead of crop. This 40% misclassification rate is oddly high in comparison to other misclassifications of weed/crop at 7%, weed/soil at 15%, soil/weed at 9%, and soil/crop at 0.1%. To further analyze this high misclassification rate, the crop/weed misclassifications were binarized and plotted as shown in **Figure 10**. Nearly all of these misclassifications were found bordering sections of crops. By taking

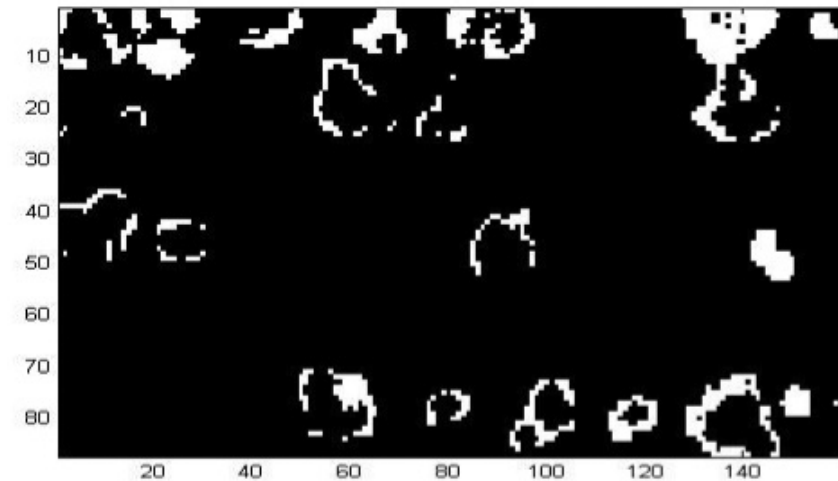


Figure 10: Crop/weed misclassifications due to insufficient edge detection. All instances of crop/weed misclassification, where the algorithm identified the block as a weed when it was actually a crop, are displayed in white. It can be noted that most of these misclassifications occurred at the edges of existing crop regions.

another look at the first principal component image, it can be noted that the values around the border of most crop regions were higher than the specific range of values that was used to identify crops. Many of these misclassifications could be eliminated by applying additional image-processing techniques in the future.

Edge detection is a very prominent sub-field of image processing and is used to define clear borders between objects in an image. Every image processing technique uses some form of edge detection to separate regions from one another and create borders. In this algorithm, the principal component values define a “fuzzy” edge that is not as useful for minute details but works well overall. In order to better discriminate between crops and weeds, an edge-detection algorithm could be applied during the pre-processing steps of our methods or applied directly to the principal component values to better separate regions and group them together as crops, weeds, or soil with strict borders.

Neighborhood and cluster-based approaches are two general ways to better classify edges within an image. The most notable techniques in image processing for edge detection are the Roberts Detection, Sobel Edge Detection, and Laplacian of Gaussian Detection [20]. However, because the proposed algorithm in this paper requires computationally efficient techniques, these methods would not be able to be used. Another approach to edge detection would be a fuzzy edge detection technique like the one described by Liang and Looney [21]. This type of edge detection classifies regions based upon gray level variation in multiple directions. When compared to other techniques, like the popular Canny edge detector, Liang and Looney’s algorithm produced similar results at a much faster rate [22].

Because of the use of the excessive green filter shown in Figure 3, it can be noted that most of the purple lettuces in each picture were faded into the background of the image and classified as soil. These purple lettuces would most likely fall within the value range of > -0.03 and < -0.015 . For the sake of accuracy, all purple lettuces were thrown out of our data and simply included in the value range for background. It can be concluded that this technique works most successfully with crops and weeds of a green color and that a separate image filter would need to be used in order to discriminate between crops and weeds of another color.

The overall accuracy reached by this algorithm was comparable to other research done by teams that used color analysis or shape analysis. Tian et al., Kiani et al., Cho et al., and Perez et al. were able to reach accuracies ranging from 75 to 100% using techniques like neural networks on cereal fields and tomato seedlings [10] [11] [12] [13]. Franz et al., Lamm, and Borregard et al. also had accuracy rates ranging from 75 to 100% while using spectral analysis of the images to analyze the colors of each plant [14] [15] [16]. Considering the lack of additional edge detection techniques, an overall accuracy rate of 77% is adequate when compared to similar research. If additional edge detection techniques were to be applied to this algorithm in future research, accuracy rates would be expected to rise.

Compared to other techniques currently being used, the results of this study showed

a similar classification rate. This proves that the data from images can be reduced in dimension while maintaining a relatively high classification rate. As image processing technology and techniques evolve, this method can be adapted and used to more accurately differentiate between crops and weeds in an efficient manner. The future of image processing in weed and crop discrimination can use this work to simplify data calculations and develop smarter systems that work on smaller, more affordable platforms.

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Empirical Correlates of Mental Health Stigma

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ABSTRACT

Background

Mental health stigma describes the prejudice and discrimination faced by those with mental health disorders. Existing literature has connected heightened levels of stigma to lower levels of mental health education and lower levels of interpersonal contact with those experiencing mental health issues. Research also suggests a possible link between high religious fundamentalism and stigma.

Methods

To assess relationships among these variables, a questionnaire was distributed online to 194 undergraduate students at a small religious university in the Midwest. The questionnaire included scales measuring fundamentalism and stigma, along with questions about mental health education levels and interpersonal contact with those experiencing mental health issues. Participants were recruited through professors known by the researcher, who e-mailed a link to the survey to their classes. Informed consent was given before participants continued to the rest of the survey.

Results

Data were analyzed using correlational tests and t-tests, and no statistically significant relationships were found between stigma and fundamentalism, contact, or education.

Conclusion

The lack of statistical significance suggests that the anticipated relationships did not exist in the sample surveyed. However, the scale used to assess stigma also has questionable validity, as demonstrated in the most recent research in which it has been used. Therefore, it is difficult to draw weighty conclusions from the study.

LITERATURE REVIEW

Empirical Correlates of Mental Health Stigma

Mental health stigma is the phenomenon of prejudice and discrimination placed on those who have experienced or are currently experiencing a psychological disorder (Phelan, Link, and Dovidio, 2008). This stigma impacts many areas of life for those experiencing mental health issues, as discussed in the meta-analysis of Sickel, Seacat, and Nabors (2014). Research has connected stigma to lower levels of self-esteem, discrimination in employment and housing, difficulties in interpersonal relationships, and negative physical health outcomes. These factors can contribute to increased mental health symptoms and reduced coping methods, while discouraging treatment seeking and compliance.

Several authors have developed theories on the origin and continuation of stigma. An early pioneer in the field was the sociologist Erving Goffman (1963), who wrote that stigma arises from incongruencies between a person's expected and actual attributes. His work provides the foundation on which all other stigma research has been built,

including the following modern theorizations of the term. Corrigan's conceptualization (2000) is based in attribution theory and is centered on the controllability and stability perceived within mental disorders. His research suggests that increased levels of perceived stability or controllability at either onset or offset of illness are related to increased levels of stigma. Link and Phelan (2001) propose a theory that focuses on a combination of labeling, ingroups and outgroups, and power differentials leading to prejudice and discriminatory actions. The combination of these theories, particularly Corrigan's and Link's, form a strong theoretical basis for understanding stigma and the backbone of much of modern research in the field.

Religious Fundamentalism

One variable worthy of analysis involves the role of religious beliefs in mental health stigma. Very little research has been devoted to this specific relationship, but there is a wealth of research connecting religious fundamentalism to other stereotypes and prejudices, with a specific focus on Christian fundamentalism due to the religion's prominence within the United States (Johnson et al., 2011; Rowatt, Kelly, LaMartina, McCullers, and McKinley, 2006). Through a careful analysis of prominent conceptualizations of stigma and prejudice, Phelan et al. (2008) established that the two concepts overlap greatly and are used to describe and study the same phenomenon. This allows researchers to form a conceptual link between existing theory on the prejudice associated with religiosity and stigma.

In order to understand this connection, the elements of religiosity that contribute to prejudice must first be established. Johnson et al. (2011) conducted a correlational study analyzing the relationships between racial and sexual orientation-based prejudices and religious authoritarianism and fundamentalism. A questionnaire was administered to 289 college students with scales of religious fundamentalism, authoritarianism, racial prejudice, and attitudes toward men and women in same-sex relationships. The results demonstrate that fundamentalism is associated with higher levels of value-violating prejudices, or prejudices against things that violate Christian values, such as those toward homosexuality ($r = 0.63, p < 0.001$). Authoritarianism is associated with higher levels of racial prejudice ($r = 0.27, p < 0.001$ for the aggression subscale). The authors suggest that this implicates religious fundamentalism, which they define as "a close-minded set of beliefs contingent upon one fundamental inerrant set of teachings about humanity and the deity" (p. 851), as a likely correlate of mental health stigma, as mental illness is categorized as value-violating as well.

Altemeyer (2003) surveyed 837 college students and 1,308 of their parents, finding a strong correlation between religious ethnocentrism and religious fundamentalism. Altemeyer states that this correlation suggests that individuals high in religious fundamentalism will express a greater tendency toward conceptualizing others as part of an outgroup on the grounds of religion, as other studies have shown those high in religious ethnocentrism to do. He argues that the emphasis that religious fundamentalism places on being a member of a religious organization provides the basis for an "us versus them" mindset. This allows individuals who differ in ways

other than religion to be placed more easily into outgroups and to consequently face prejudice. The often-religious perception of high onset and offset controllability of mental illness, along with this tendency toward outgroup formation, suggests that stigma is likely to follow.

The first to analyze the fundamentalism-stigma link outside of the context of pastoral ministry were Wesselmann and Graziana (2010). They conducted a correlational study on the connection between religiosity and mental health stigma. The study was conducted using a questionnaire with an informal scale to identify the prejudices commonly held among religious college students and previously validated scales to assess religious fundamentalism and orthodoxy. Fundamentalism was linked to more strongly held prejudices and negative beliefs about mental illness. Participants were also asked whether they have had exposure to individuals with mental disorders. Having close contact with an individual with a mental illness lessened the effects of fundamentalism on stigma. Essentially, the authors found that high religious fundamentalism was connected to a higher level of stigma.

Research in the connection between religious fundamentalism and mental health stigma is sparse but does suggest that such a connection does exist. This led to this study's Hypothesis 1, which improves on Wesselmann and Graziana (2010) by measuring stigma with a validated scale. H1: *Mental health stigma and religious fundamentalism will be positively correlated.*

Intergroup Contact Theory

According to the meta-analysis conducted by Pettigrew, Tropp, Wagener, and Christ (2011), intergroup contact theory states that contact between different groups results in lower levels of prejudice. The theory originated in the wake of the Civil Rights movements, when racial tensions were eased in individuals that had contact with others of a different race. Some research operates on the assumption that contact requires four positive features to effectively reduce prejudice: equal status between groups, common goals, intergroup cooperation, and the support of authority. Recent research and meta-analysis has supported the effect of these factors but demonstrated that they are not necessary for attitude change. With or without these optimal factors, the increase in knowledge and empathy for the group and the decrease in anxiety associated with intergroup contact contributes to a negative correlation between contact and prejudice.

In 2013, Aggarwall, Thompson, Falik, Shaw, O'Sullivan, and Lowenstein initiated and evaluated a mental health education program for first-year medical school students. The program consisted of a panel of four to six students sharing their personal experiences with mental illness for one hour, followed by a small group discussion for another hour. The students who participated in the program showed decreases in social distancing and increases in willingness to disclose personal struggles from pre-test to post-test ($p < 0.01$), suggesting a reduction in stigma.

Bizub and Davidson (2011) completed a qualitative study of the effects of the completion of a program called Compeer, in which individuals with mental illness are paired with community volunteers to foster friendship. The student participants, all senior psychology majors, were simply asked to describe their thoughts going into the program and their thoughts on the friendship that was formed. Major themes include anxiety about the program prior to its beginning, with roots in a sense of dangerousness and unpredictability of those with mental illnesses. Empathy and greater understanding were more prevalent at the completion of the program, stemming from the friendship that was formed.

Studies on contact often fail to assess the relationship between everyday, casual contact and stigma. To address this gap, this study assesses this kind of casual contact. This, along with the existing research on intergroup contact theory, leads to Hypothesis 2. H2: *Individuals with higher levels of contact with individuals experiencing mental health issues will report lower levels of mental health stigma than individuals with lower levels of contact.*

Education

Many researchers investigating mental health stigma are primarily concerned with stigma among those who work professionally with individuals experiencing these disorders. As such, a great deal of research has centered on the assessment and reduction of stigma among mental health professionals and other professionals who are likely to encounter mental health issues, such as medical professionals. This area of research has also been extended to students intending to enter these fields, in the hopes that intervention while in training can reduce potential harm while in practice.

Emul et al. (2011) conducted a quasi-experiment studying the stigmatization of suicide attempters among medical and non-medical students at a Turkish school. Students completed a questionnaire that measured prejudices. Most comparisons between medical and non-medical students were not statistically significant. Comparisons that were significant seem to demonstrate that the medical students hold lower levels of stigma than the non-medical students and that medical students in clinicals have lower levels of stigma than those that are in earlier stages of the program. These differences, however, were only demonstrated on select questionnaire items and were relatively small.

Zellmann, Madden, and Aguiniga (2014) conducted a study with a school's social work department, using a survey devised by the authors. They found that many students believed that social work in mental health is not rewarding. Using a cross-section of students in various class levels, those at higher class levels were more likely to believe that meaningful goals and successful careers are not accomplishable for individuals with mental illnesses. The results of the study are concerning but very limited. The scale was devised by the authors and has no tested reliability or validity, so the results may not reflect stigma itself but another related construct. Additionally, it may be true that mental health work can at times be unrewarding, but the authors were quite concerned about this belief among their students.

Smith and Cashwell (2010) used a questionnaire to gather data on and analyze the authoritarianism, benevolence, social restrictiveness, and community mental health ideology of 188 graduate students and professionals in the mental health field in comparison with those not in the mental health field. Results indicated that those in the mental health field have lower levels of authoritarianism and social restrictiveness with higher levels of benevolence and community mental health ideology (all p 's < 0.05). Essentially, in this study mental health workers and students did in fact display lower levels of stigma than non-mental health workers and students.

Research on the attitudes of students often focuses on one area of study, instead of assessing various areas or even comparing different groups entering the mental health field. Additionally, comparing students who have and have not taken mental health-related courses could assess their impact on stigma. This, along with the conclusion of most existing research, leads to this study's Hypothesis 3. H3: *Individuals with higher levels of education on mental health will report lower levels of mental health stigma than those with lower levels of education.*

METHODS

Participants

The participants included 194 undergraduate students at a small religious university in the Midwestern United States recruited through general education and social work courses. The average age of the students was just over twenty, and most students were between the ages of eighteen and twenty-two. Females accounted for 144 of the 194 responses. Of the participants, 84% identified as White, 3% identified as Black or African American, and 3.5% identified as Hispanic, Latino, or Spanish origin.

Sixteen students reported not knowing anyone experiencing mental health issues, thirty-four reported knowing someone but not well, eighty-seven reporting knowing someone well, and fifty-eight reported experiencing mental health issues themselves. Thirty-five participants were majoring in social work, thirty-one were majoring in psychology, and 128 were majoring in other areas. Most participants had taken none of mental health-related courses offered at the university, twenty-four had taken one of the courses, three had taken two of the courses, and two had taken three.

Materials

Stigma was assessed using the Perceived Devaluation-Discrimination Scale (Link, Cullen, Frank, and Wozniak, 1987). The scale includes twelve statements accompanied by Likert scales with four points ranging from strongly agree to strongly disagree. Link et al. (1987) began statements with the phrase "most people would . . ." to reduce social desirability in responses. This occurs when participants select responses based on a desire to appear likable or good; the use of "most people" allowed participants to express their own views in a depersonalized way. (See Appendix A.) This scale had high internal consistency, with $\alpha = 0.84$.

Religious fundamentalism was assessed using the Revised Religious Fundamentalism Scale (Altemeyer and Huntsberger, 2004). The scale included another twelve statements and accompanying Likert scales with eight points ranging from very strongly disagree to very strongly agree. (See Appendix B.) Internal consistency was high, with $\alpha = 0.89$.

Contact was assessed through the question, "Have you known anyone personally experiencing mental health issues?" Responses included "no," "yes but I do not know them well," "yes and I do know them well," and "I have experienced these issues myself." Education was assessed using college major and the question, "Which of the following courses have you taken (or are you currently taking)?" Options included mental health-related courses in the social work, psychology, nursing, and theology departments.

Procedures

Participants received an email from various professors briefly describing the study and requesting their participation. Participants then followed a link to an online survey, where they read an informed consent page then selected "continue" to complete the rest of the survey. Some participants entered their names to receive extra credit in a course, and many entered their name to be placed in a drawing for one of two \$25 gift cards that were awarded as survey incentives. All data were de-identified immediately after gift card winner selection and before beginning data analysis using SPSS. Hypotheses were tested using correlational tests (H1), t-tests (H3 – college major), and ANOVAs (H2 and H3 – number of courses taken).

RESULTS

Students' mean score on the Perceived Devaluation-Discrimination Scale (Link et al., 1987) was 19.8, with a standard deviation of 4.7. The Revised Religious Fundamentalism Scale (Altemeyer and Huntsberger, 2004), which could produce a negative score, had a mean score of 9.3 and a standard deviation of 18.7.

There was no relationship between religious fundamentalism and mental health stigma, $r(195) = 0.06$, $p = 0.42$. This is inconsistent with the prediction in H1. There was also no relationship between interpersonal contact and mental health stigma, $F(4, 192) = 0.67$, $\eta^2 = 0.01$, and $p = 0.62$, which is inconsistent with the prediction in H2. Additionally, no relationship was found between education and mental health stigma when education was measured as the number of courses taken, $F(3, 192) = 1.03$, $\eta^2 = 0.01$, and $p = 0.38$, and by college major, $t(192) = -1.37$, $\eta^2 = 0.21$, and $p = 0.17$. Therefore, results from both operational definitions are inconsistent with H3.

DISCUSSION

This study assessed stigma and its relationship with several other variables among university students, finding no significant relationships between stigma, religious fundamentalism, contact, and education. This contradicts the research hypotheses and appears to contradict the existing literature on these topics or at least suggests that

relationships between these variables are less meaningful than other research suggests. The notable exception to this is in the relationship between fundamentalism and stigma, which has not been researched enough for generalized conclusions to be drawn.

However, this study is hindered by several limitations. The sample may not be representative of the general undergraduate population, which could be remedied through random selection. All data were obtained through self-report, which may not accurately represent student attitudes. There may be a sampling bias due to the recruiting methods used to obtain participants (through professors known by the researcher).

Additionally, the scale used to assess stigma may not be valid in measuring this variable. Further literature review suggests that the scale is being used to assess self-stigma in recent research (Cathoor, Schrijvers, Hutsebaut, Feenstra, and Sabbe, 2015; Martinez-Zambrano, Pizzimenti, Barbeito, Vila-Badia, Comellas, Escandell, ... Ochoa, 2016). The “most people . . .” phrasing, originally used to reduce social desirability bias, is now being used to measure the way those with mental health issues believe others perceive them. As such, measurement of this study’s dependent variable may be invalid, making it more difficult to draw conclusions from the results. The hypothesized relationships, therefore, may truly not exist in this population, or they may have been identified using a different, valid scale to measure stigma.

Future research in this area would benefit from different methods of assessing stigma levels. A different self-report scale could be used to address the possible invalidity of the Perceived Devaluation-Discrimination Scale (Link et al., 1987). Assessment not based in self-report could also eliminate social desirability response bias and provide strong, valid measurements of both stigma and religious fundamentalism.

Given the strong and negative consequences of mental health stigma, research identifying causes and correlates could lead to better outcomes for those experiencing mental health issues. If the relationships between contact, education, and stigma are in fact nonexistent or weak, there are strong implications for social work and psychology education. Students in these programs will ideally hold lower levels of stigma, since they are more likely to enter the mental health field and any level of stigma could negatively impact clients. If these lower levels are not demonstrated, stigma reduction methods should be considered and integrated into coursework. Although this study did not provide conclusive results, the implications for mental health care and education should be considered.

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APPENDIX A

Perceived Devaluation-Discrimination Scale - Each question will be accompanied by a scale from “Strongly disagree” to “Strongly agree.”

1. Most people would accept a person who has been in a mental hospital as a close friend.*
2. Most people believe that someone who has been hospitalized for mental illness is dangerous.
3. Most people believe that a person who has been hospitalized for a mental illness is just as trustworthy as the average citizen.*
4. Most people would accept a person who has fully recovered from mental illness as a teacher of young children in a public school.*
5. Most employers will not hire a person who has been hospitalized for mental illness.
6. Most people think less of a person after he/she has been hospitalized for a mental illness.
7. Most people would be willing to marry someone who has been a patient in a mental hospital.*
8. Most employers will hire a person who has been hospitalized for mental illness if he or she is qualified for the job.*

9. Most people believe that entering a psychiatric hospital is a sign of personal failure.
10. Most people will not hire a person who has been hospitalized or serious mental illness to take care of their children, even if he or she had been known well for some time.
11. Most people in my community would treat a person who has been hospitalized for mental illness just as they would treat anyone.*
12. Most young people would be reluctant to date someone who has been hospitalized for a serious mental illness.

*Reverse coded

APPENDIX B

Revised Religious Fundamentalism Scale - Each question will be accompanied by a scale from “Very strongly disagree” to “Very strongly agree.” You may find that you sometimes have different reactions to different parts of a statement. For example, you might very strongly disagree with one idea in a statement, but slightly agree with another idea in the same item. When this happens, please combine your reactions, and indicate how you feel on balance.

1. God has given humanity a complete, unyielding guide to happiness and salvation, which must totally be followed.
2. No single book of religious teachings contains all the intrinsic, fundamental truths about life.*
3. The basic cause of evil in the world is Satan, who is still constantly and ferociously fighting against God.
4. It is more important to be a good person than to believe in God and the right religion.*
5. There is a particular set of religious teachings in this world that are so true, you can't go any “deeper” because they are the basic, bedrock message that God has given humanity.
6. When you get right down to it, there are basically only two kinds of people in the world, the righteous, who will be rewarded by God; and the rest, who will not.
7. Scriptures may contain general truths, but they should NOT be considered completely, literally true from beginning to end.*

8. To lead the best, most meaningful life, one must belong to the one, fundamentally true religion.
9. “Satan” is just the name people give to their own bad impulses. There really is no such thing as a diabolical “Prince of Darkness” who tempts us.*
10. Whenever science and sacred scripture conflict, science is probably right.*
11. The fundamentals of God’s religion should never be tampered with, or compromised with others’ beliefs.
12. All of the religions in the world have flaws and wrong teachings. There is no perfectly true, right religion.*

**Reverse coded*



Design and Evaluation of a 3D Printed Filar Micrometer

Emily M. Rull

One of my favorite parts of studying the night sky is that I know that my heritage in astronomy is full of men and women who have devoted themselves to the study of things that are just out of reach. It may seem futile to some, but as we know from our astronomical ancestors, you can learn a lot from what you cannot touch.

I would like to dedicate this thesis to everyone in my life who helps me to see my Creator as He is: much closer than the stars.

ACKNOWLEDGEMENTS

I would like to thank the Pence-Boyce Research Board for providing me the opportunity to pursue research in the summer of 2017. Funding from the Pence-Boyce Summer Research Opportunity made it possible for the foundation of this project to be completed at that time.

This project would have been impossible without Dr. Stephen Case. His mentorship, inspiration, and encouragement have been vital throughout every step of the process. Throughout the project, Professors Amanda Luby and Joseph Makarewicz have provided much-needed aid in programming and running the 3D printers used in this project. Advice from Dr. Beth Schurman and Dr. Dan Sharda has greatly improved the written quality of this thesis.

ABSTRACT

Background

Double stars are celestial objects that allow for calculating the mass of stars by assessing their orbits. Stellar mass affects every current model of stellar evolution, but the most accurate double star orbits can take decades to record. Due to the long-term nature of such observations and lack of groundbreaking research in double star studies, professional astronomers are no longer focused on making these measurements, so amateur astronomers can pick up where professionals have left off. Amateurs can only do this if they can get the equipment that they need at prices they can afford. A personally-manufactured filar micrometer could fill this need. Astronomers use a filar micrometer paired with a telescope to take visual double star measurements. Unfortunately, current commercial filar micrometers are cost prohibitive for most amateur astronomers. This project sought to design, 3D print, test, and calibrate a filar micrometer that amateur astronomers could produce cost effectively.

Methods

Creo Parametric 3.0 and a ProJet MJP 3600 Series 3D printer were used to design and print the filar micrometer. A Fowler 1-2" digital counter micrometer, 54-gauge Nichrome 80 wire, and a 6" Orion SkyQuest Dobsonian telescope were used with the printed filar micrometer to take measurements. The measurements were of the separation between components of an artificial double star created by flashlights reflected in a bearing ball. These measurements were used to calibrate the filar micrometer and find the preliminary accuracy of the filar micrometer.

Results

Twenty-one measurements were taken of three different arrangements of the artificial double star. The average calibration calculated from this data is $1.44\text{E-}4$ inches per arcsecond. Measurements of the 24 Coronae Borealis simulated star (with a separation of 20.14 arcseconds) produced a seventeen percent error with an average measured separation of 16.74 arcseconds.

Conclusion

Though there is no firm standard, according to multiple sources a professional-quality filar micrometer should be capable of precision to one tenth of an arcsecond when measuring close double stars or one arcsecond for wider double stars. The filar micrometer produced in this project is capable (by design) of precision to seven tenths of an arcsecond. This means that it cannot reach the accuracy of the very few professional filar micrometers available for resale when measuring close double stars. However, at a tenth of the cost of the professionally-produced version, it is an affordable amateur filar micrometer. Since these are preliminary values for the calibration and accuracy of the produced filar micrometer, future data collection in these areas will lead to a better view of the true calibration value and accuracy possible for this filar micrometer.

Keywords: filar micrometer, double stars, 3D printing, amateur astronomy

INTRODUCTION

Double Stars

Astronomers search for sources of data in their investigation of the cosmos. The study of exoplanets supports theories about solar systems; studying gravitational waves is changing the way astronomers search for dark matter; and double stars offer a direct means for gathering data about the mass of stars.

Double stars are systems of two or more stars that are gravitationally bound and orbit each other. Many observable double stars have measurable orbits around the system's center of gravity (Argyle, 2012, p. 5). The mass of the component stars and their distance from each other are factors that affect the orbit, which can be calculated by measuring the separation and position angle between the stars over the period of their orbit. The period of a double star can vary anywhere between a few hours and several millennia (Argyle, 2012, p. 8). Plotting the orbit of double stars from the measurements collected makes it possible to calculate the masses of the stars. Using the orbits of gravitationally bound double stars remains the only direct method of calculating the mass of stars (Mullaney, 2005, p. 25). Since mass is the largest contributing factor to the life and death of a star, this direct data on stellar masses increases the reliability of models of stellar evolution, structure, and movements.

In order to accurately document double star orbits, careful and frequent measurements must be made of the position angle and separation of the pair. When observing a double star, the position angle (θ) is measured as the angle in degrees between the north point in the telescope's field of view and the line observed which connects the primary star to the fainter companion star (**Figure 1**). The separation (q) is the observed separation between the primary star and its companion measured in arcseconds (Worley, 1961, p. 74; Argyle, 2012, p. 2).

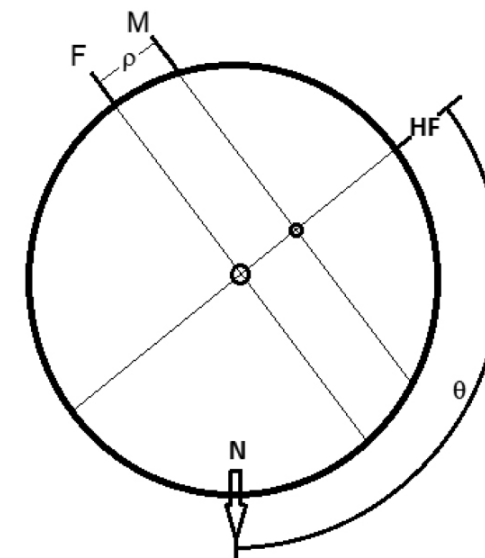


Figure 1: Measuring double stars with a filar micrometer. HF is the horizontal fixed wire of the filar micrometer. F is the vertical fixed wire. M is the vertical moveable wire. N is north in the field of view. θ represents the position angle of the double star. q represents the separation of the double star. The primary star is at the intersection of F and HF. The component star is at the intersection of M and HF.

Star Catalogs

There are many star catalogs that focus on specific kinds of stars. The Washington Double Star Catalog (WDS) is a resource that can be used to find the location and expected separation and position angle of specific double star pairs. The WDS is maintained by the United States Naval Observatory and is updated nightly. A list within the WDS consists of neglected double stars, which are characterized as double star pairs that have not been measured in twenty years and have a separation greater than three arcseconds (Washington Double Star Catalog, 2013). Neglected double stars offer the opportunity to collect data on double stars that have very few recorded observations and can provide new data to stellar mass calculations. Since these stars often have very few recorded measurements, they are targets that amateur astronomers can focus on observing in order to expand orbit and mass catalogs to include data on these neglected stars.

Filar Micrometer

Tracking the orbits of double stars to strengthen the reliability of stellar mass calculations is an important aspect of astronomy that is currently receiving little attention. Since stellar evolution relies more heavily on stellar mass than any other characteristic, stellar mass data collections are some of the most important catalogs of astronomical parameters to maintain. Using instruments like the filar micrometer can allow amateurs to contribute to the study of double stars if the instruments are accessible to those who would use them.

In the early years of telescopic astronomy, there was very little interest in double stars. In order to investigate stellar parallax, William Herschel began observing double stars. Before this time, astronomers paid little attention to the most striking double stars in the sky because they did not understand what the celestial objects revealed (Argyle, 1986, p. 1). In 1803, Herschel published his paper outlining the existence, motion, and measurements of double stars which brought the significance of the double stars in the sky to the attention of astronomers (p. 2).

The filar micrometer has historically been the most commonly used instrument for visually determining position angle and separation by the observational astronomers studying double stars. The filar micrometer was designed and initially used in the early seventeenth century (Argyle, 2012, p. 169). John Herschel noted that the filar micrometer can also be used to make “micrometric measures of planetary diameters, solar spots, distance or elongations of satellites, lunar topography” and more (as cited in Case, 2014, p. 366). Since the early 1800s when William Herschel used his homemade filar micrometer to measure these newly-discovered double stars, many other astronomers followed his example using similar methods (Argyle, 1986, p.2).

Eventually, the most common use for the instrument became measuring double stars in order to plot their orbits (Argyle, 2012, p. 169). However, with the advent of photographic methods, making direct observations with filar micrometers fell out of use. A double star expert noted that in 1986, there were only three professional astronomers who continued to carry out visual observations of double stars with filar micrometers (p. 6). In the years since, research does not indicate any new professionals taking on

visual measurements. This means that the number of professionals engaged in these measurements and the professional need for filar micrometers is dwindling. Yet this does not mean there is not still important data to be gleaned from double stars, and this neglect on the part of professional astronomers is actually an opportunity for amateur observers to once again make important contributions in astronomy. For this work to occur, there must be affordable filar micrometers available for amateurs to use.

A filar micrometer fits into a telescope behind the eyepiece. The instrument consists of three wires. Positioned vertically across the field of view are two wires: one is fixed and the other able to slide across the field of view. The third wire is fixed in place perpendicular to the two vertical wires (Byrne, Beesley, and Dunsby, p. 181). The crosshair created by the fixed wires provides a target for one of the double star components. The other component is positioned on the cross created by the moving vertical and fixed horizontal wires. **Figure 1** illustrates this positioning. The measurement made with the filar micrometer must be converted from inches read by the micrometer to arcseconds using the calibration value calculated for the filar micrometer and telescope (Greaney, 2012, p. 350). In this project, the calibration value of the filar micrometer has been calculated as $1.44\text{E-}4$ inches per arcsecond of separation. Once the separation is recorded, the angle between the stars can be read from a protractor at the base of the instrument.

Filar micrometers declined in use in the 1900s as astronomers moved from observing and taking measurements while standing behind a telescope to using photography and computer software to make the same measurements from digital images. Double stars with a separation larger than approximately ten arcseconds can be easily measured using photographic methods, if the observer has access to the high precision cameras, mounts, and software necessary to measure in this way. In addition, interest in the observation and measurement of double stars has waned due to the long span of time, sometimes extending to decades or centuries, required to follow the complete orbits of many pairs. Since filar micrometers became less popular in the twentieth century, they are difficult to find and expensive to manufacture, which is evident in the fact that Argyle referenced only two manufacturers in 2012 (p. 181). Before closing in 2013, Van Slyke Instruments in Colorado charged between \$2,500 and \$3,000 for their instruments (p. 406). As of 2018, there are no current manufacturers of filar micrometers.

Despite their rarity, these instruments remain useful for taking measurements of resolvable star pairs with close orbits or stars that have very unequal magnitudes. For this type of pair, photographs and computers have difficulty determining the separation because of the way starlight from each component interferes and makes the images unclear (Buchheim, 2007, p. 254). Measuring close doubles remains of interest to amateur astronomers because their tight orbits likely have extremely short periods (decades instead of centuries) and thus present targets the orbits of which could be observed over entire revolutions (Mullaney, 2005, p. 8). Most double stars take many years to travel through their orbit and thus require just as long to produce a reliable mass calculation from measurements taken at regular intervals throughout the orbit.

Since other areas of astronomy take much less time to make advances, this is an area of astronomy that has been almost entirely neglected by professionals. This means that amateurs can make significant contributions to the index of stellar masses if they have reasonable access to equipment such as a filar micrometer.

Amateur Astronomers and Filar Micrometers

As commercial filar micrometers are often cost-prohibitive for amateur astronomers looking to study double stars, some have turned to their own resources to produce their own filar micrometers. Each of the amateurs discussed below designed and manufactured their instruments solely for their own use rather than for distribution. Amateur manufacturers Polman (1977) and Robertson (1985) have worked with machined metals and the traditional micrometer screws to produce instruments whose manufacturing cost is far less than half of their retail value. The Robertson filar micrometer was completed at a cost of \$46 since he used scrap aluminum and did not have to pay for labor because he completed the work himself (Robertson, 1985, p. 359). An amateur astronomer in California built a filar micrometer that employed a vernier micrometer, similar to the one used in this project, as the primary measuring tool (Byrne, Beesley, and Dunsby, 1984, p. 182). A fourth amateur carried out a project very similar to this in 1999, but with aluminum workings in combination with a vernier micrometer (De Villier, 1999, p.164). The materials and method of measurement chosen by each of these amateurs has been outlined in Table 1 alongside the cost and accuracy of the instruments.

This project is unique when compared to these previous designs in that it made use of 3D printers to produce the primary components of the filar micrometer. This allows the micrometer components to be easily reproduced in the future by any other interested party with access to a 3D printer. Since its conception in the 1980s, 3D printing is most commonly used for small scale production of prototypes in the design process (Lipson, 2010, p.30). In 2012, almost half of the 3D printers sold were purchased for consumer rather than commercial use (p. 34). This means that over the last ten years, 3D printers have become more available to the general public than ever before.

Despite the low cost of personally-produced filar micrometers, these improvised instruments are a valuable tool for gaining experience using the equipment and understanding the measurement of double stars. Authors Polman and Robertson acknowledge the imperfect nature of their “homemade” filar micrometers by reporting margins of error in their measurements of approximately ten percent (translating to 0.1 arcseconds when measuring the closest double stars), which is equal to the error allowed in professional measurements (Polman, 1977, p. 396; Robertson, 1985, p. 360). Neither claims to have a professional quality filar micrometer from their personal design and manufacturing process, but they have made instruments similar to the expensive equipment of professionals accessible to the amateur community.

TABLE 1
Comparative Information on Amateur Filar Micrometers

<i>Astronomer</i>	Polman	Robertson	Byrne, Beesley, and Dunsby	De Villier	Rull
<i>Year</i>	1977	1985	1984	1999	2017
<i>Material</i>	Aluminum and Brass	Brass	*	Aluminum	Plastic
<i>Cost</i>	*	\$46	*	*	\$235
<i>Percent Error</i>	10%	*	<10%	2%	17%
<i>Measuring Tool</i>	Micrometer Screw	Micrometer Screw	Vernier Micrometer	Vernier Micrometer	Vernier Micrometer

* Data not reported

Professionally Produced Filar Micrometers

As with any measuring tool, filar micrometers have a tolerance by professional standards when it comes to an acceptable margin of error in measurements. Tanguay, in his article for *Sky & Telescope* in 1999, outlines acceptable margins of error:

For pairs in the 1.0-arcsecond separation range, measurements of separation should not differ more than about ± 10 percent and position angle not more than about $\pm 5.0^\circ$ from the published values. For wider pairs that span around 100 arcseconds, your separation measurements should not vary more than about ± 1 percent and position-angle measurements not more than about $\pm 0.5^\circ$ from the WDS [Washington Double Star Catalog] values (p.120).

This means that in order for the filar micrometer to be of a professional standard specific to close double stars, it must be accurate to 0.1 arcseconds. In order for it to be of a professional standard specific to wider stars, it must be accurate to one arcsecond.

The Fowler 1-2” Digital Counter Micrometer, the primary measuring component in this design, is precise to the ten thousandth of an inch, which (according to the calibration value found in this project) is equal to 0.7 arcseconds in separation with respect to this filar micrometer setup. This value will change if there is a change in telescope, filar micrometer, or eyepiece. This means that the filar micrometer produced in this project is not quite up to professional standards with respect to the closest double stars but is capable (in principle) of professional data collection for wider double stars. The accuracy of the filar micrometer also depends heavily on the accuracy made possible by the skill of the operator. Since a precision of 0.7 arcseconds is possible by design of the filar micrometer, it is the limiting factor in terms of the accuracy of the filar micrometer. The amateur astronomers mentioned above all reported margins of error much lower than those reported in this project. One of the likely contributors to that discrepancy is the fact that the amateurs referenced above were all visual observers of double stars before they began work with their filar micrometer, whereas I had minimal experience working with telescopes and observing double stars before the project began.

Calibration of a Filar Micrometer

Proper calibration of a filar micrometer is vital to making the most accurate measurements possible. The separation observed with the telescope is measured in tiny fractions of an inch in the filar micrometer. In order for the linear measurements in inches to translate properly to the subtended angle of separation between the stars in arcseconds, an accurate calibration value must be calculated. To determine this calibration, a known subtended angle of separation must be measured to find the equivalent linear measurement in inches (as measured by the Fowler micrometer).

Paul Couteau describes a method of practicing double star observations utilizing an artificial double star that offers a simple method of calibration (p. 89). Using two flashlights reflected on a bearing ball more than one hundred meters away, the astronomer can create an artificial double star with a predetermined separation of components (**Figure 2**). A telescope can then be used to observe the artificial double and take measurements with the filar micrometer to calibrate the instrument. This simulation is useful because the astronomer can carry out these observations during daylight or cloudy weather and does not need to be concerned with the diurnal motion of the stars. This method of observation does not allow for simulations of position angle.

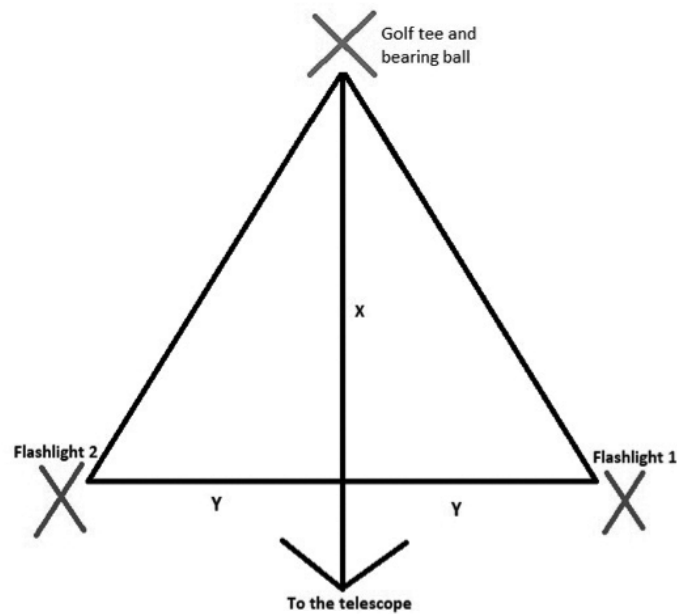


Figure 2: Artificial double star arrangement to calibrate the filar micrometer. The x and y parameters are changed depending on the desired separation. The flashlights used are directed toward the bearing ball in this arrangement. The bearing ball then reflects the light from the flashlights to the observer at the telescope.

This project sought to design and 3D print a filar micrometer that amateur astronomers could produce cost effectively. To evaluate the effectiveness of the filar micrometer, data was collected for calibration and analysis of accuracy. Paul Couteau's method of calibration was selected because of the season and climate in which the measurements would need to take place.

METHODS

The 3D printed components of the filar micrometer were designed using Creo Parametric 3.0. **Figure 3** shows the complete CAD model in an assembly file. The 3D-modeled parts of the filar micrometer are the front plate, slide, slide casing, and position angle circle. The front plate, slide, and position angle circle are all approximately one tenth of an inch thick, and the slide casing's thickness is nearly two tenths of an inch. All four components have a hole for the sightline between the telescope's mirrors and the eyepiece. The designed components were printed with a ProJet MJP 3600 Series 3D printer out of Visijet M3 plastic. Drawings of each component can be found in the appendix. A Fowler 1-2" Digital Counter micrometer, which is a type of vernier micrometer, was selected to work with the filar micrometer design. To be sure that the wires were thin enough to be observed through the eyepiece, 54-gauge Nichrome 80 wire was selected. In order for the wires to be visible in the field of view of the telescope, an LED circuit was assembled and fixed to the front plate of the filar micrometer. The positioning of the LED illuminated the wires to make them visible against the dark field of view.

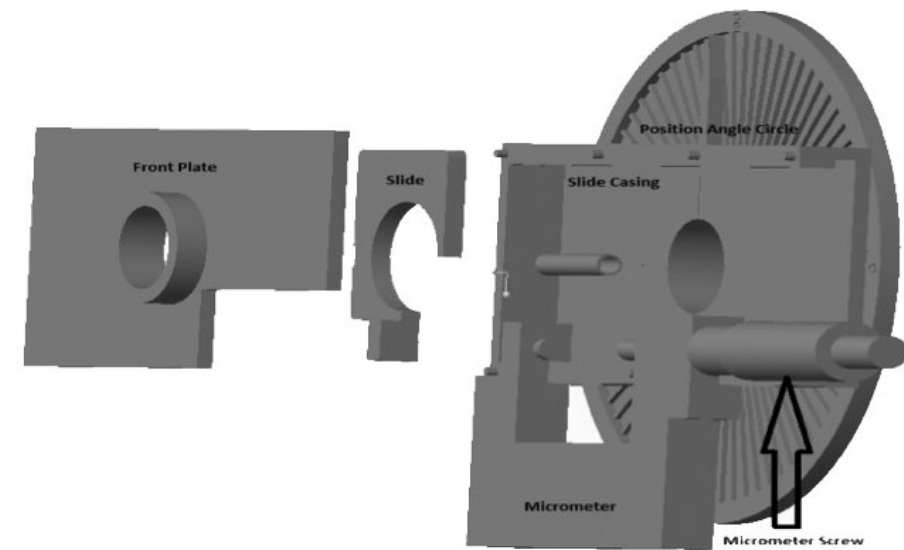


Figure 3: Assembly file of the filar micrometer produced in Creo Parametric 3.0. The front plate carries the eyepiece and the LED circuit. The slide carries the moving wire and is moved by the micrometer spindle. The micrometer is a Fowler 1-2" digital counter micrometer. The slide casing has a tube for the spring, grooves for the fixed wires, and gaps for the slide and the micrometer. The position angle is the reference used to measure the position angle of the double star when the horizontal fixed thread has been placed along the line between the primary and component stars.

Since eyepieces generally enclose their own focal plane, a 16.3-millimeter Criterion eyepiece was disassembled and fitted specifically to this filar micrometer to achieve the necessary placement of the wires within the telescope's focal plane. Disassembly of the eyepiece decreased its diameter to 0.96 inches, so the front plate of the filar micrometer was designed with a 0.97-inch hole so that the Criterion eyepiece would fit tightly. The six-inch Dobsonian-mounted reflecting telescope used with this project is an Orion Skyquest telescope.

A measurement made with the filar micrometer required that the horizontal fixed wire be positioned so that it bisected both stars. The moveable wire is moved on the slide using the spindle of the Fowler micrometer (as labeled in **Figure 4**) to position the wire first on the primary star, then on the secondary star, making note of each position on the Fowler micrometer. The difference between these positions is equal to the separation between the stars in inches. The Fowler micrometer used in this project has been designed to measure distances in inches, so the raw measurements are in inches.

The calibration of the filar micrometer was accomplished using Paul Couteau's artificial double star to simulate double stars of known separation. Figure 2 displays the layout of the bearing ball and flashlights used to find the separation simulated by the artificial star. Calibration of the filar micrometer in this research utilized simulations of three wider double star pairs: 16 Cygni (39.75 arcseconds), 24 Coronae Borealis (20.14 arcseconds) and Delta Orionis (52.42 arcseconds). According to the professional standards referenced above, we would hope for an error of ten percent or less. These stars were selected from a list of calibration pairs that have not changed in separation in recent history. This selection makes it possible to observe the simulated pairs for measurement before turning the telescope to the real pairs for comparison.

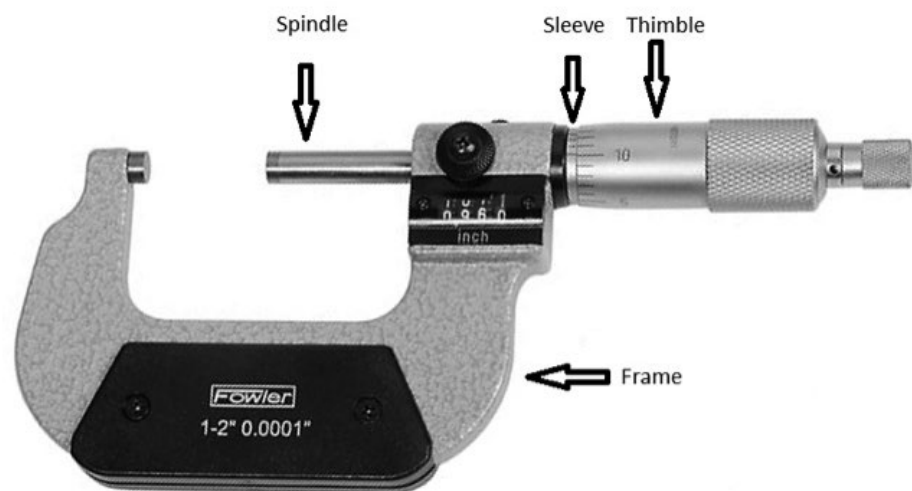


Figure 4: The Fowler 1-2" digital micrometer. The spindle remains in contact with the slide and enables measurement of the location of the moveable wire. The sleeve increases the precision of the micrometer to the ten thousandth of an inch. The thimble is rotated to move the spindle linearly. The frame fits into the slide casing which keeps it in a consistent location.

The equation used to find the simulated separation is shown below, and the distances used in these simulations are provided in Table 2. To collect the artificial double star measurements, a double star was set up and the filar micrometer was used to measure the separation between the stars. The calibration value determined from this data is essentially a conversion factor that makes it possible to compare the measurements made in inches using the filar micrometer to the actual separation of a double star in arcseconds. The bearing ball and flashlights were positioned according to Figure 2 with the bearing ball located on the bearing ball stand. Measurements of position angle were not made because Couteau's artificial double star setup does not simulate position angle. Table 2 represents object distances used to calculate the separation created by the artificial double star set-up using the following equation:

$$s = RL/(hD) \times 10^5$$

where

s is the double star separation in arcseconds

R is the radius of the bearing ball in meters

L is the distance between the flashlights in meters determined by solving for L given all other values

h is the distance from the flashlights to the bearing ball in meters

D is the distance from the telescope's objective lens to the bearing ball in meters

All values were selected with respect to advice from Couteau. For example, he recommends appropriate bearing ball sizes, an acceptable distance from the flashlights to the bearing ball, and a distance from the telescope to the bearing ball. Since the bearing ball was made to precise measurements, we can be confident in all provided decimal values. The distances L , h , and D can be assumed accurate to six thousandths of a meter (approximately half a centimeter). Using significant figure rules, we find that the separation between the simulated stars can realistically only be precise to two significant figures, or to the arcsecond. This is important to consider when comparing this first approximation of calibration and accuracy values to those reported by other amateur filar micrometer producers.

TABLE 2
Values Used In Determination Of Artificial Double Star Separation

Variable	Delta Orionis	16 Cygni	24 Coronae Bernices
s	52.42 [arcseconds]	39.75 [arcseconds]	20.14 [arcseconds]
R	00.0127 [m]	00.0127 [m]	00.0127 [m]
L	01.07 [m]	00.81 [m]	00.41 [m]
h	00.50 [m]	00.50 [m]	00.50 [m]
D	51.80 [m]	51.80 [m]	51.80 [m]

Since the separation of the artificial double star is known (at a particular confidence level), once the micrometer measures the apparent distance between the component stars, the linear separation in inches can be connected to the angular separation in arcseconds. Dividing the distance in inches by the known separation in arcseconds provides the calibration data for the micrometer-telescope-eyepiece apparatus. This calibration data is specific to the telescope, filar micrometer, and eyepiece combination of this project and can be used to interpret measurements of separations in the focal plane of the telescope. Two of the artificial double stars were used to calibrate the device, but the measurements of the third artificial double star were used to develop preliminary data on the accuracy and precision of the tool (Table 3). This method of calibration and accuracy evaluation was selected so that the accuracy data found by evaluating the simulated 24 Coronae Borealis would be independent of its “personal” calibration value.

RESULTS

A total of twenty-one measurements of three artificial double stars were analyzed to produce a conversion factor. 16 Cygni, 24 Coronae Borealis, and Delta Orionis were selected from a list of calibration pairs that do not have any recorded changes in separation. This means that the simulated double star should produce an image very similar to observations of the real stars when observed for measurement. The preliminary calibration found is that one arcsecond of separation measured by the micrometer is equal to $1.44\text{E-}4$ inches. The average calibrations and percent error reported in Table 3 represent the difference between the location of the primary star and the location of the companion star when observing the artificial double star. The calibration values found for 16 Cygni ($1.466\text{E-}4$ inches per arcsecond) and Delta Orionis ($1.64\text{E-}4$ inches per arcsecond) were averaged and applied to the measurements of 24 Coronae Borealis. This process produced the converted measurements that were taken of 24 Coronae Borealis. Comparing these measurements to the true separation of the simulated double star results in a percent error of 16.88 for measurements of the simulated 24 Coronae Borealis.

TABLE 3
Average Calibration and Accuracy

Star and Type of Data	Value	Standard Deviation
16 Cygni Average Calibration	$1.466\text{E-}4$ inches	$1.199\text{E-}4$
Delta Orionis Average Calibration	$1.640\text{E-}4$ inches	$1.970\text{E-}4$
24 Coronae Borealis Percent Error	16.88 percent	$8.263\text{E-}4$

DISCUSSION

Proof of Concept

This project was a proof-of-concept design of an affordable and easy-to-replicate filar micrometer for amateur astronomers. This filar micrometer can be used to contribute to an area of astronomy that has become neglected by professionals. This 3D printable prototype lowers the barrier of expense and makes the instrument accessible to amateurs. The plans provided in the Appendix make it possible for anyone to build their own copy of the filar micrometer to take double star measurements.

In most cases, 3D printing is less expensive than metal working, and 3D printers are becoming more accessible to the general population through personal purchase and public access locations such as libraries (Lisbon, 2010, p.30). 3D printing this project in plastic was chosen because this method is the most accessible for public use by amateur astronomers who may print the device in the future. In this project, the final cost of the designed filar micrometer and the necessary accessories came to \$235, not including a fee to use the printer. Van Slyke Instruments charged between \$2500 and \$3000 for the same instrument made from aluminum. With such a dramatic difference in price, an amateur astronomer could purchase a 3D printer (\$1140), purchase a telescope (\$600), print the filar micrometer, and outfit the instrument (\$235) for the same cost.

In addition to being inexpensive, the design could be adjusted to another telescope and printed within about a week. Changes to the design could be completed by a user with basic CAD experience in just a few hours, and the printing and preparation for use would take a few more days. Since the design has already been made and needs only minor adjustments to be used with a different telescope, the filar micrometer presented here offers broad application for the amateur astronomer. A new user must only make adjustments to the size of the eyepiece tube and the distance created between the eyepiece and the micrometer wires. These simple changes make this design flexible and easy-to-adapt to the individual needs of a user.

A difficulty that results from using an artificial arrangement such as that described by Paul Couteau is that the calibration value is dependent on the measurements made between a telescope, two flashlights, and a bearing ball. This forces a much larger uncertainty value to be considered with respect to the calibration and accuracy. This challenge is another likely cause of the lower reported accuracy when compared with other amateur filar micrometers. The components of the artificial double star are subject to certainty of only about a quarter of an inch. The lowered level of certainty here decreases the certainty with which we can quote the set separation between the components in the simulated double star. This could result in a higher margin of error as a result of an imprecise simulated separation. This challenge can be overcome by taking measurements of real double stars whose separations are well recorded in order to calculate the necessary calibration value. Since this was not the method selected for this project, the calibration and accuracy values can be considered a first approximation of their values given more extensive use.

The seventeen percent preliminary accuracy and 0.7 arcseconds precision limits the double star separation that an amateur can measure with this filar micrometer. For example, it may provide sufficient measurements of α Bootis at 12.6 arcseconds of separation, but it will do even better measuring 90 Leonis at 63.1 arcseconds of separation because a smaller percentage of the error could be attributed to the filar micrometer itself. The precision of the measurements made of α Bootis would be equal to five percent of the distance being measured compared to 90 Leonis, where the precision is only one percent. So although the closest double stars have the shortest periods, this filar micrometer (at its current precision) may be best used on wider double stars when making measurements to professional standards. I would recommend not using this filar micrometer, as is, to measure stars closer than ten arcseconds if the goal is to make professional-quality measurements.

Design Comparisons and Advantages

It has been calculated that the filar micrometer designed here is capable of precision to 0.7 arcseconds, which results in imprecision in principle but not inherent inaccuracy. This precision could be increased by choosing another method of making the linear measurements such as a more precise vernier micrometer or a micrometer screw. This imprecision is a result of the limitations of the Fowler micrometer selected for this project. Consequently, the designed filar micrometer does not quite meet the professional standard of precision to 0.1 arcseconds (for close double stars) expected from a filar micrometer costing a customer nearly \$3000.

Another design for taking measurements with a filar micrometer is to use a micrometer screw rather than a vernier micrometer. The micrometer screw carries out the same function as the micrometer used in this project but without the advantage of a built in numerical display for easier measurement. In order to make a measurement with the micrometer screw, the operator must know how many partial turns of the screw produce the separation between the stars. Micrometer screws have been the prominent tool used in filar micrometers since their invention (Argyle, 1986, p.29). With either of these methods, Becker cites the advantage of using screw mechanisms in micrometers as producing and measuring a small linear displacement using a large rotational displacement which is seen by turning the thimble of the micrometer (as seen in Figure 4) (p. 52). This theory applies similarly to vernier micrometers.

A Fowler micrometer was selected for this project in order to simplify the measurement process and decrease the prerequisite knowledge and skill necessary to make meaningful measurements. It is widely acknowledged that micrometric measurements of a new observer follow a pattern of increasing accuracy for their first few years of observation. Although this research is specific to observers using a micrometer screw filar micrometer, there is no reason to expect observers using a vernier micrometer to experience anything different.

The data obtained by calibration through observation of an artificial double star makes it possible to apply this method not only to practical use as an amateur astronomer but also to pedagogical use in an astronomy lab. In fact, this design has already been shared by personal communication at a recent conference, and will be used by Frank Florian at the TELUS World of Science in Edmonton, Alberta, Canada (F. Florian, personal communication, October 31, 2017).

Future research should continue to enhance the precision of the instrument by either choosing a more precise micrometer that has smaller units of measurement or choosing a different method of measurement that will produce a higher precision than the filar micrometer is capable of at this time. Reverting to a micrometer screw method of determining measurements could be utilized for experimentation to compare measurements from the vernier micrometer and the micrometer screw. Study of these different methods could additionally be useful to amateurs in the future. Collecting additional observational data by measuring either artificial double or real double stars would also increase the observer's accuracy when working with the filar micrometer. These increases in accuracy are realized with continuous use of the filar micrometer by increasing the skill of the operator and the reliability of the measurements.

The filar micrometer designed in this project can be 3D printed for individual amateur astronomers and outfitted for \$235. Paul Couteau's method of artificial double stars provided targets of a specified separation to calibrate the filar micrometer and find the conversion value from measured inches to arcseconds of separation. Amateur astronomers will be able to use this design to make micrometric measurements of double stars which can be used to record their orbit and calculate their mass. This important data makes it possible for dedicated amateur astronomer observers to contribute to astrophysical research.

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APPENDIX
Engineering Drawings of 3D Printed Filar Micrometer Parts

Figure A.1
Drawing of final
Front Plate design.

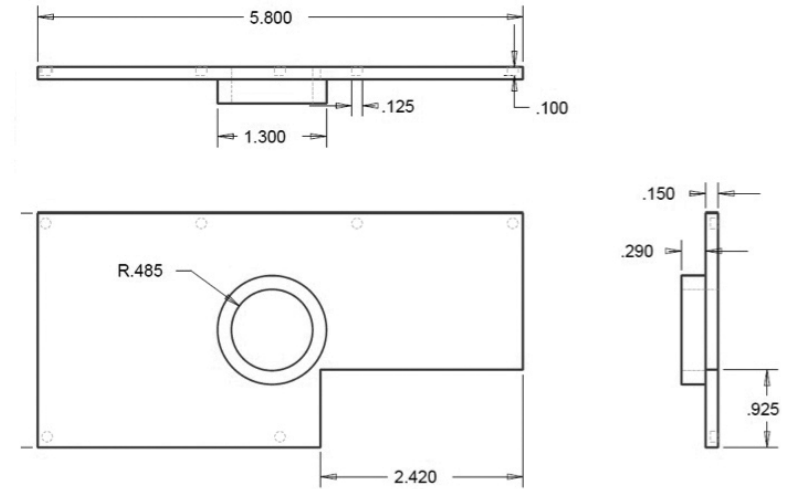
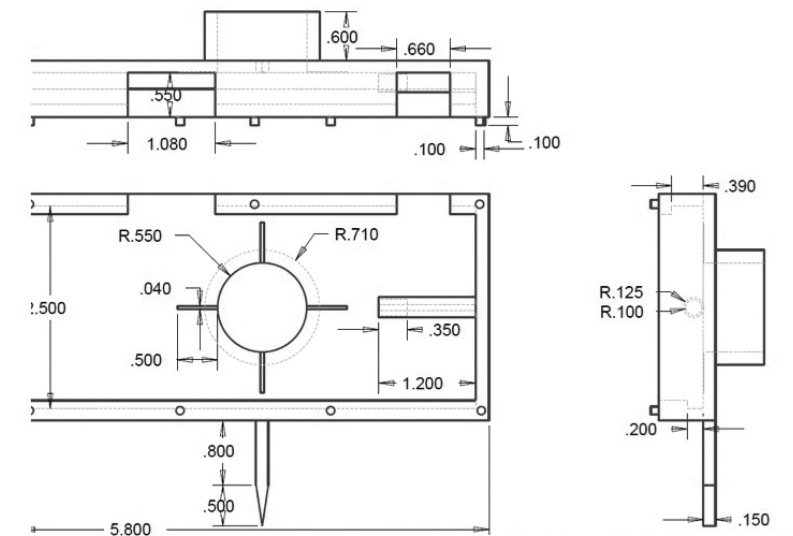


Figure A.2
Drawing of final
Slidecasing design.



APPENDIX
Engineering Drawings of 3D Printed Filar Micrometer Parts

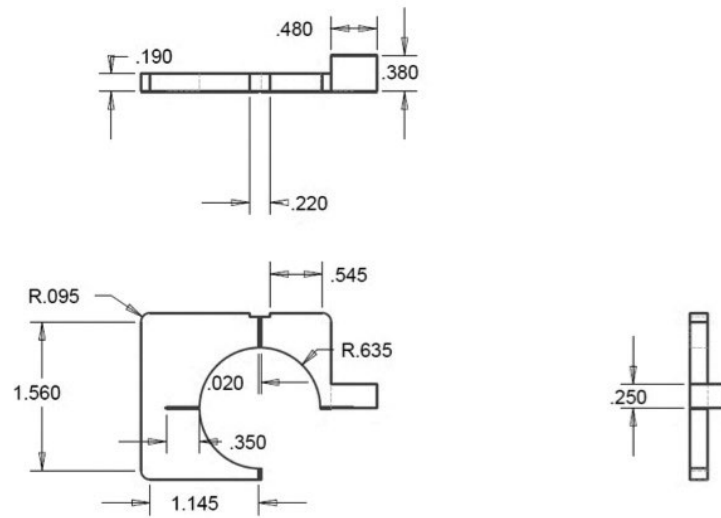


Figure A.3
Drawing of final
Slide design.

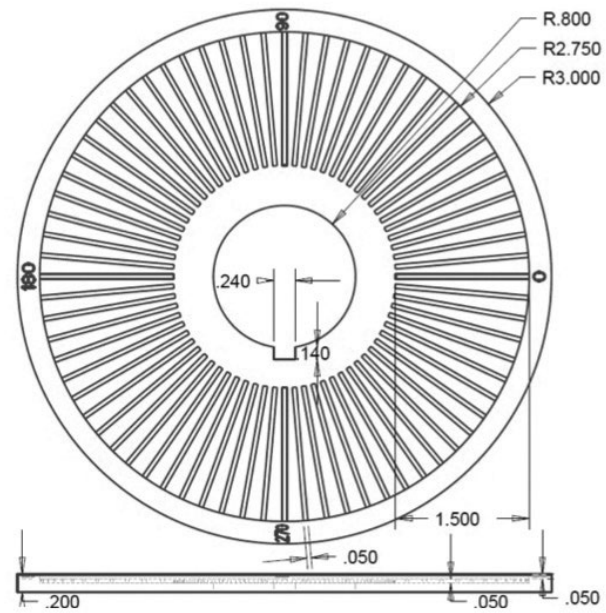


Figure A.4
Drawing of final
Position Angle
Circle design.



**Stigma as a Predictor of Parental Willingness
to Seek Mental Health Services for Their Children
in Rural America**

Reed M. Smith

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ABSTRACT

Stigma exists in some capacity towards mental illness. This stigma is a barrier to mental health services for some people. Rural populations are known to have more stigma than their urban counterparts. This is on top of already lacking access to mental health services. This especially affects children. Polaha and Williams (2015) found stigma to be negatively correlated with willingness to seek help in rural parents. This study sought to explore this relationship in a more generalized sample. I posted a survey on Amazon Mechanical Turk that screened for rural parents of children under the age of eighteen. It included a two-factor measure of self and public stigma and a one item assessment of willingness to seek mental health services for their children. Eighty-one responses were used to analyze for correlational relationships between variables including age, gender, perceived public stigma, perceived self-stigma, and willingness to seek mental health services for their children. Analysis revealed a significant positive correlation between public and self-stigma. It revealed a significant negative correlation between self-stigma and age. It revealed a significant negative correlation between self-stigma and expressed willingness to seek help from a mental health professional for children. The finding that more perceived self-stigma makes someone less likely to seek mental health services supports findings from previous studies. However, it is curious that only self-stigma and not public stigma was found to be a predictor. More studies would have to be done to establish a cause and effect relationship.

Keywords: stigma, self-stigma, public stigma, mental illness stigma, mental health, rural, rural America, help-seeking

INTRODUCTION

Stigma has been a common topic of research in last the thirty years. Researchers are interested in where it is present, why it is present, the effects of its presence, what types are present, and how it can be reduced, among other things. The number of studies with stigma as a component began to dramatically increase in the latter half of the twentieth century (Link and Phelan, 2001). However, there was not a common consensus in the academic community on what stigma is. Link and Phelan set out to create a better understanding of all aspects of stigma. They identified the components of stigma as labeling, stereotyping, separation, status loss, and discrimination. Since then, there have been scores of studies on stigma, many of which have been on its relationship with mental illness.

It is well established that stigma exists in some capacity towards mental illness (Hinshaw, 2005). It is a topic of much importance and interest because research shows it to be a barrier to mental health services for some people (Boydell et al., 2006; Gulliver, Griffiths, and Christensen, 2010). In 1999, the Surgeon General declared mental illness stigma as the primary obstacle in fighting mental illness (United States Public Health Service, Office of the Surgeon General, 1999). If people are aware that they will be looked down upon for having a mental illness, they may decide not to seek help that would potentially lead to a diagnosis. Thus, researchers want to find what populations

have this stigma. Once it has been demonstrated that a certain population has stigma of mental illness, researchers often attempt to create and/or implement interventions aimed at reducing the stigma.

Stigma of mental health services is especially prevalent in rural populations (Hammer, Vogel, and Heimerdinger-Edwards, 2013; Starr, Campbell, and Herrick, 2013; Stewart, Jameson, and Curtin, 2015), sometimes acting as a barrier to the services (Boydell et al., 2006; Polaha, Williams, Heflinger, and Studts, 2015). Studies show that rural communities have more mental illness stigma than their urban counterparts (Hammer et al., 2013; Stewart et al., 2015). This is possibly because the rural value system emphasizes solving one's own problems and seeking help as weakness (Stewart et al., 2015; Smith, Buckwalter, and DeCroix, 1997). Another contributor is the small community size in rural areas. People feel a lack anonymity and worry that everyone will know that they have problems if they seek mental health services (Boydell et al., 2006). This is in addition to other documented barriers to mental health services, including lack of access, lack of awareness of services, and financial difficulties (Boydell et al., 2006; Gulliver et al., 2010).

Parents are faced with tough decisions when it comes to seeking help for their children. Up to ten percent of children aged five to sixteen have a diagnosed mental disorder, not including conduct disorders or hyperkinetic disorders (Green, McGinnity, Meltzer, Ford, and Goodman, 2004). The percentage of those with diagnosable disorders is higher. Unfortunately, only about one-third of children receive help for these issues (Sayal, 2006). Stigma is a factor in parental help-seeking behavior. Parents of children with mental health disorders often feel like they are not good parents (Eaton, Ohan, Stritzke, and Corrigan, 2016).

Researchers have addressed this subject in both rural and urban areas. A study on low-income, urban African-Americans found self-stigma as a predictor of help seeking (Dempster, Davis, Jones, Keating, and Wildman, 2015). Polaha and Williams conducted the first study to focus on rural parent's stigma of help seeking for their children in 2015. The researchers approached parents in eight different primary clinics in rural Appalachia and administered a survey to willing participants. They also called eligible parents and sent the survey by mail to those who were willing to participate. The first part of the study used a measure to identify parents of children with borderline and clinical psychosocial concerns. The researchers then assessed perceived stigma for if they were to seek mental health services for their children. They found that parents reported low perception of public stigma, which was inconsistent with previous research done with rural people (Starr et al., 2013). The results also showed that the higher levels of stigma perceived, the less likely the parents were to seek help. This study used the Parents' Perceived Stigma of Service Seeking (PPSSS) measure, developed and tested for validity and reliability in a previous study (Williams and Polaha, 2014). This measure contains two factors, public and self-stigma, to measure a parent's level of perceived stigma of seeking mental health services for their children. Public stigma is the stigma felt from other people, and self-stigma is the stigma felt from one's self. I use this measure to assess just that in rural American parents.

In my study, I assess the relationship between perception of stigma and parents' intentions of seeking mental health services for their children in rural America. It is similar to Williams and Polaha's 2015 study. It is different in that I collected a more geographically diverse sample and the participants were not required to have a child with borderline and/or clinical psychosocial concerns. Thus, my study should be more generalizable.

METHOD

I obtained a voluntary sample of 111 participants via Amazon Mechanical Turk. I chose Amazon Mechanical Turk as a means of sampling for its ability to quickly collect responses from participants all over the United States. Participants answered preliminary demographic questions that determined if they are a parent with a child under the age of eighteen and living in rural America and screened out those that are not. The remaining participants were issued the two-factor PPSSS measure to assess perceived public and self-stigma in response to statements about taking his or her child to a mental health professional. Participants could select an answer ranging from "Strongly Disagree" to "Strongly Agree." Each statement started with "If I were to take my child to a mental health professional for them to receive services . . ." The self-stigma scale included items like, "It would make me feel strange," "It would make me feel embarrassed," and "It would make me feel like a bad parent." The public stigma scale included items like, "Some people might say bad things behind my back," "Some people would treat me with less respect," and "Some people would avoid me."

There was an additional item asking, "How willing are you to seek mental health services for your child?" The responses were assigned numerical value for analysis, ranging from 1 (Strongly Disagree) to 5 (Strongly Agree). Each participant who finished this section was paid fifty cents. There were three quality control items. One safeguard item simply instructed the participant to select a specific response (i.e. Neither Agree nor Disagree), another asked for their zip code, and another asked if they were being honest. If the wrong response was selected, if the zip code was that of an urban area, if they indicated that they had not been honest, or if the responses were incomplete, then the responses were not included. Thirty responses were removed due to these safeguards. Data collection occurred from February 23 to February 25 of 2017. I analyzed the remaining eighty-one responses using two-tailed t-tests.

RESULTS

The sample was composed of forty-eight females and thirty-three males. Seventy-three percent were white, 15% Asian, and 6% American Indian or Alaskan native. Eighty-six percent had at least some college education, and 68% were married. The geographical distribution of participants is shown in **Figure 1**.



Figure 1.A Geographical distribution of participants in the lower 48 states.

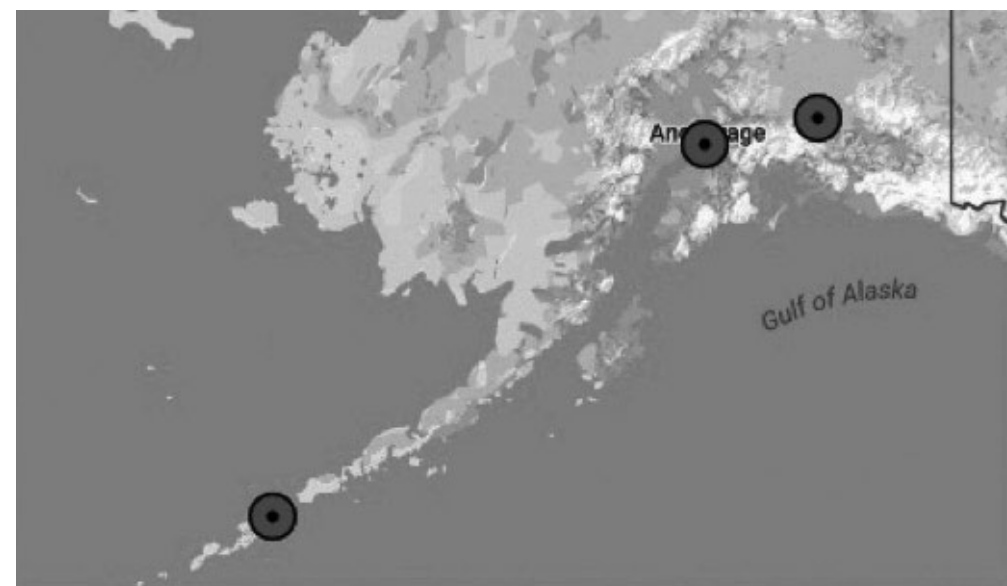


Figure 1.B Geographical distribution of participants in Alaska.

Both the public stigma and self-stigma factors had excellent internal consistency, with Cronbach's alphas of .94 and .95 respectively. Self-stigma scores varied across the entire possible range of 6 to 30. The average self-stigma score was 15.1, just below an average response of "Neither Agree nor Disagree." The standard deviation was 7.4. Public stigma scores also varied across the entire possible range of 11 to 55 and did range from 11 to 55. The average public stigma score was 31.3, also just below an average answer of "Neither Agree nor Disagree." The standard deviation was 11.2. Two-tailed correlational analyses were run on perceived self-stigma, perceived public stigma, a person's willingness to take their child to see a mental health professional if they saw the need, gender, and age. A significant, negative correlation was found between age and perceived self-stigma ($r = -.281$, $p = .013$). Another significant, negative correlation was found between a person's willingness to take their child to see a mental health professional if he or she saw the need and perceived self-stigma ($r = -.231$, $p = .042$). A significant, positive correlation was found between perceived self-stigma and perceived public stigma ($r = .784$, $p = .000$). There were no other statistically significant relationships found. The correlational data are shown in Table 1.

TABLE 1
Correlations

		Self-stigma	Public Stigma	Willingness to take child to mental health professional	What is your gender?	What is your age?
Self-stigma	Pearson Correlation	1	.784**	-.231*	-.020	-.281*
	Sig. (2-tailed)		.000	.042	.864	.013
	N	78	76	78	78	78
Public Stigma	Pearson Correlation	.784**	1	-.127	.026	-.199
	Sig. (2-tailed)	.000		.268	.824	.081
	N	76	78	78	78	78
Willingness to take child to mental health professional	Pearson Correlation	-.231*	-.127	1	-.136	.110
	Sig. (2-tailed)	.042	.268		.227	.328
	N	78	78	81	81	81
What is your gender?	Pearson Correlation	-.020	.026	-.136	1	-.062
	Sig. (2-tailed)	.864	.824	.227		.585
	N	78	78	81	81	81
What is your age?	Pearson Correlation	-.281*	-.199	.110	-.062	1
	Sig. (2-tailed)	.013	.081	.328	.585	
	N	78	78	81	81	81

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

Perceived Self-Stigma as Predictor

Perceived self-stigma of seeking mental health services for a child was negatively correlated with willingness to seek mental health services for a child. This was similar to the findings of Dempster et-al (2015), who found that self-stigma was negatively correlated with help seeking in an urban sample. This was also consistent with the findings of Polaha and Williams (2015) that stigma is negatively correlated with help seeking in rural parents with children who are diagnosable with a mental disorder. These consistent findings point to the importance of considering stigma to be an issue for parents when it comes to seeking care for their children. Although we cannot conclude that self-stigma causes parents to avoid seeking mental health services for their children, it remains a likely candidate for identification as a factor that influences parents' decisions in such situations. Moreover, since we know that mental illness stigma is more prevalent in rural populations when compared with urban populations (Hammer et al., 2013; Stewart et al., 2015), mental health care providers in rural America should be especially aware of this. If the providers are aware of this relationship, they can properly prepare care models and maybe even conduct outreach to parents with self-stigma.

Stigma Levels

The average response to the measure with statements that indicated stigma was just below Neither Agree nor Disagree, closer to Disagree than Agree. Thus, participants did not agree they had public or self-stigma on average. The finding of relatively low levels of perceived public stigma was consistent with the findings of Polaha and Williams (2015). This challenges much research that suggests there is heavy mental illness stigma in rural America, though it does not necessarily challenge research that shows it to be more prevalent in rural than in urban populations, because the latter research does not demonstrate magnitude. Polaha and Williams (2015) were the most recent researchers to conduct such a study until this study, which corroborates their findings. Thus, it is possible that rural America's level of stigma is on a reversal. Parcesepe and Cabassa (2012) conducted a literature review and found that the American public in general has a positive attitude about mental health help seeking and the positivity is increasing over time. Further studies with a focus on rural America would be needed to draw any conclusions.

Perceived Self-stigma vs. Public Stigma

Another noteworthy finding was that perceived self-stigma is a predictor of help seeking, while perceived public stigma is not. This is especially interesting considering the known barrier of a perceived lack of anonymity. Also, although the strongest relationship was between public stigma and self-stigma, public stigma was not significantly related to any of the variables to which self-stigma was related. Perhaps this is because the participants are valuing what they think about themselves more than what the public will think of them, despite lacking anonymity. Public stigma, when present, is dangerous, as it can lead to discrimination and restricted autonomy

(Parcesepe and Cabassa, 2012). Thus, although public stigma might not predict help seeking, its presence should not be dismissed.

Perceived Self-stigma and Age

It was an unexpected finding that as age increases, perceived self-stigma decreases. Perhaps this is a reflection of maturity. This is consistent with previous findings. Sirey et al. (2001) found that younger patients with depression perceived more stigma than older patients with depression. One recent study found that people were more likely to distance themselves socially from people with mental illnesses as their age increased, but this varied with different mental illnesses (Schomerus, Van der Auwera, Matschinger, Baumeister, and Angermeyer, 2015). However, the stigma examined in this study was not self-stigma. There is not much research on self-stigma and age, and I believe that my finding is novel.

Limitations

Because of Amazon Mechanical Turk being used to administer the survey, there are some limitations. It is possible that some users lied about their rurality because they knew there would be a monetary incentive. One user indicated that he or she was not truthful and the results should not be used. Another limitation is that the measure I used was tested for reliability and validity when being used with parents with children with borderline and/or clinical psychosocial concerns, while this was not a requirement to participate in my study. Furthermore, although there was a statistically significant relationship between age and perceived self-stigma, the item surveying age was accidentally administered as a discreet range rather than a continuous variable. A continuous variable would have yielded more analyzable, meaningful results. Moreover, rurality has been operationalized in many various ways throughout other studies. The distinction in this study between who does and does not live in a rural America is somewhat arbitrary. This study showed only correlations and no causation. I suggest further research that involves an intervention that targets self-stigma to better understand if self-stigma is a barrier for rural parents seeking help for their children.

CONCLUSION

Mental illness stigma has been a hot topic of research. It is a barrier to services for some people and is recognized as a major problem in rural populations. There is not much research on the relationship of stigma and rural parental willingness to seek mental health services for children. The extant research was conducted in a limited geographical region. I found that parents with higher perceived self-stigma of taking their children to see a mental health professional were less likely to express willingness to do so. This self-stigma was lower when age increased. Lastly, public stigma and self-stigma were heavily positively correlated. My study does not imply causation, and further research will be needed to establish whether stigma is a real barrier to seeking mental health services itself for rural parents or not.

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APPENDIX

Stigma Measure (Williams and Polaha, 2014)

If I were to take my child to a mental health professional for them to receive services, . . .	
Self-stigma Factor	Public Stigma Factor
it would make me feel strange.	some people might treat me unfairly.
it would make me feel embarrassed.	some people might look down on me.
it would make me feel like a bad parent.	some people might say bad things about me behind my back.
my view of myself would be less.	some people would treat me with less respect.
it would make me feel that I am weak.	some people would avoid me.
it would make me feel like there is something wrong with me.	my child might be labeled at school.
	people in my church might frown on my decision.
	my child's teacher would treat him or her unfairly.
	I would be worried that people in town would find out.
	I would try to hide that I was getting counseling for my child.





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