Concord McNair Scholars Research Journal

Volume 21 (2018)

Table of Contents

106

The Microbiome of a Pierced Belly Button Compared to a Non-Pierced Belly Button

Allison Garrett
Mentor: Thomas Ford, Ph.D.
Biology – Concord University

115

Perception of Music Education

Kristen Knight
Mentor: Timothy Mainland, Ph.D.
Music Education – Concord University

123

Effects of Bisphenol A (BPA) and Two BPA Derivatives on Radish (*Raphanus raphanistrum* subsp. *sativus*) and Bean (*Phaseolus vulgaris*) Seed Germination and Growth

Nicole Matthews
Mentor: Dr. Darla Wise
Biology – Concord University

142

Utilizing a GI transit Assay in Zebra Fish Larvae to screen for Autism Spectrum Disorder Related Genes

Brady Shrader with Syed. M. Ali
Mentor: Dr. James Walters
Applied Sciences – Bluefield State College

156

Gender, Age, and Sports Affiliation as Predictors of Body Dysmorphia

Keira Wickline
Mentor: Karen Griffee, Ph.D.
Psychology – Concord University

Survey and Interpretation of Deformation Features in the Footwall Syncline of the St. Clair Thrust Fault

Dustin Anderson

Mentor: Joseph Allen, Professor of Geology

Concord University, Athens, WV

Abstract

A survey and mapping project during Concord University's 2018 field camp in Bluefield,WV, is the source of planar data of a regime of sub-vertical (~90°) faults in the Glen Lyn Syncline – the footwall of the thrust-fold structure of the St. Clair thrust. This paper summarizes the field data and descriptions of the formation, as well as literature related to structures of this kind in order to offer possible models for how these structures formed. Formerly interpreted as post-orogenic deformation features, it is more likely that these faults formed in a back-thrust geometry or in buckle-fold related geometry and were later rotated into their current orientation.

Introduction

A road-cut exposure of overturned Paleozoic strata in the Glen Lyn syncline in Bluefield,WV, related to a bridge construction site off of Route 460, was the subject of a mapping project for Concord University's 2018 Field Camp. This Appalachian syncline in the foot wall of the St. Clair thrust fault was host to smaller scale deformation features including fault-related folding, axial-planar cleavages, and – of most interest here – faults in sub-vertical orientation (on the scale of several meters). There were also faults which crosscut these sub-vertical faults, potentially having propagated at a different time. Because of the amount of directional force necessary for a fault to propagate, faulting at a near vertical orientation is not structurally likely (van der Pluijm and Marshak 1997), ipso facto the original formation of these sub-vertical faults and their original orientations became one of the Field Camp class' research questions. To offer aproposed timeline for when these faults propagated and in what orientation they propagated in

would require modeling the kinematic data in the deformational features, the aim of this paper

is to synthesize the available research related both specifically and generally to the features available at the road-cut, and to discuss the possible models for how these sub-vertical faults in the footwall of the St. Clair thrust fault may have formed. Surveying literature related to this topic, because the specific kinematics of the thrusting and the smaller scale structures that exist on the footwall syncline are unique, not only gives context to these specific local structures, regional tectonics and structure, and geo-historical events that these features are the result of, butelucidates the implications of the sort of deformation going on here, according to generally observed definitions and models in structural geology and texts related specifically to thrust-faultgeometry.

There is limited available research on the specific formations discussed here, however thegoal of this paper is to describe the potential models by which the sub-vertical faults formed, andthese structures are not unique to this syncline, and much of the literature review consists of indirectly related resources on similar structures and fault regimes. The literature reviewed here includes the following: second-hand/textbook sources, which are used as general reference; journal articles on either directly related to the Glen Lyn syncline or the St. Clair thrust fault, or structurally similar formations that are not directly related, but serve as a useful parallel to the topic; and a field guide in the region where the outcrop is exposed.

The sections of this paper are broken down into three sections: the Field Survey Data andObservations section, which describes the data collection methods and field observations made in the 2018 Field Camp research; the Discussion, which is broken up into subsections connectedto the possible models for how the sub-vertical faults were formed, with cases made by relevant

literature; and the Conclusion which summarizes the potential models for how the faults formed and points out the need for further research.

Field Survey Data and Observations

Concord University Field Camp began a structural mapping project in Bluefield, WV on 18 May 2018. Below is a map with cross section (Figure 1) showing the general structure of the mapped strata, which are part of the St Clair thrust fault, which is one of the large regional thrust faults associated with the Appalachian thrust front. In southern Mercer County WV (within the western Valley and Ridge Province), off route 460 in Bluefield, an exposure of both the thrust sheet and the overturned footwall (the Glen Lyn syncline) are exposed near-perpendicular to strike At the exposure (which was exposed by earthmoving related to bridge construction), the thrust sheet is represented by the Ordovician Beekmantown dolomite (in pink) and the footwall is made up of Devonian shales, turbidites and sandstone units, as well as Silurian units overturned below the younger units and exposed farther from the fold axis (in blues and purples). The system has a complex low-angle, thin-skinned thrust fault geometry (McDowell and Shultz 1990).

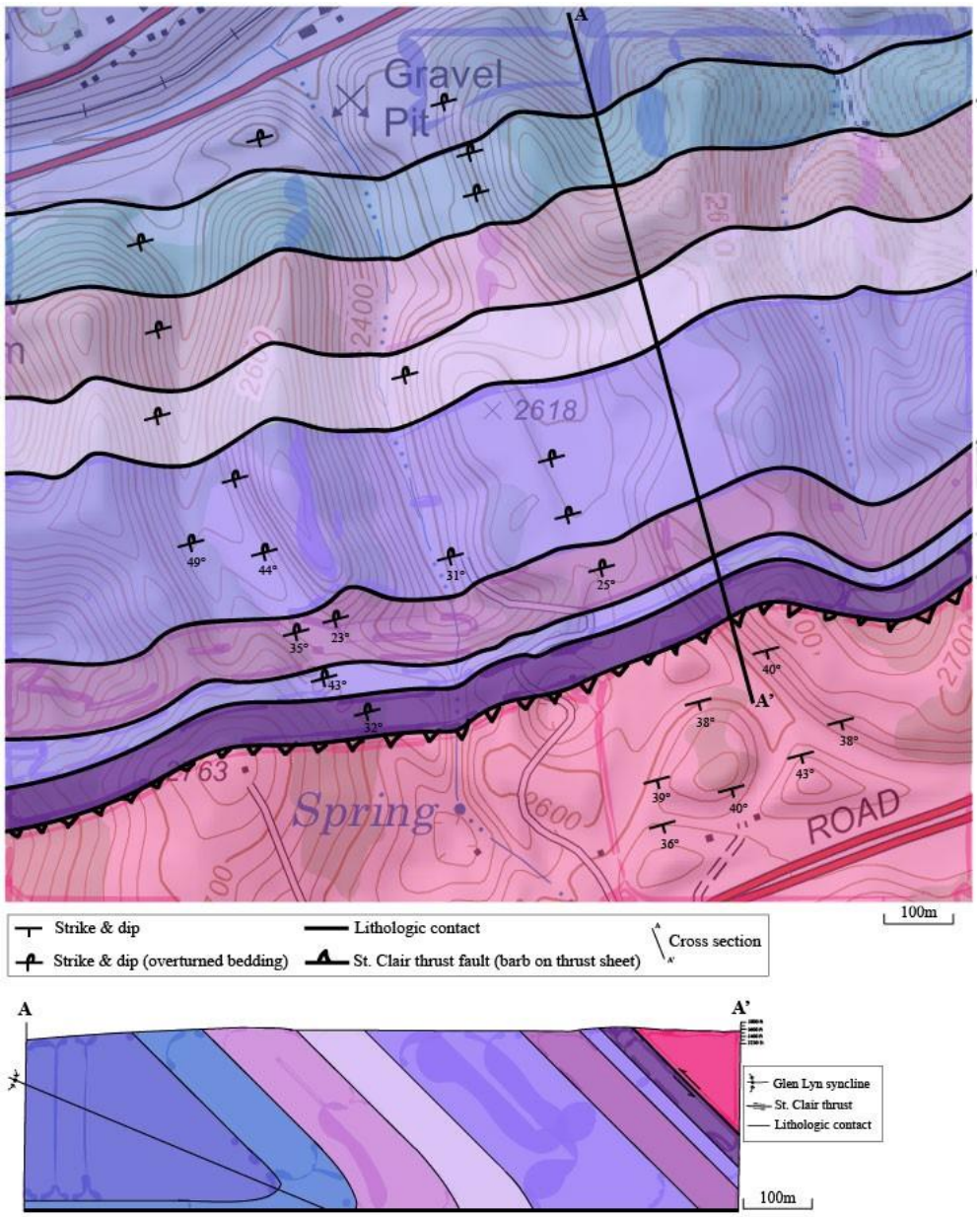


Figure 1 – Structural stratigraphic map of outcrop of St Clair Thrust in Bluefield WV. Pink: Ordovician Beekmantown dolomite; blues, purples: Devonian shales, turbidites and sandstone units, as well as Silurian unit. Mapped in collaboration with Joseph Allen, Stephen Kuehn, Tyler Green and Jonathan Hardman.

Surveying and mapping were done with common field methods, utilizing basic tools such as Jacob's staffs and Brunton compasses to plot our planar attitudes and lithographic contacts and bed thicknesses. To examine the rocks themselves and the smaller scale structure, tools such as Brunton compasses, hand-lenses and hydrochloric acid vials were utilizd. All of this took

about 7 days and the mapping project concluded on the 25th of May. Collected data was used in concordance with an adjacent data collection and mapping project in Bluefield, VA, near Lotito park, and also reconnaissance along a previously mapped exposure along Route 460 (Dennison 1996) to create the maps.

At the bridge cut outcrop, there are stark examples of previously defined outcrop scale structure types within the footwall syncline of the St Clair thrust, which have been observed in the surrounding area. There are different generations of faults, a regime of which are in a near vertical orientation (measured with between 78-90° dips, some with dip directions the opposite of the general trend, but in such cases only by a few degrees), and these and other faults of varying dips (ranging from 20-90°) have crosscutting relationships which gives indication of which groups of faults faulted first. Given correlations of trends of fault orientations, the generations are distinct, and likely faulted in response to different regional stresses. The fault regime which is host to the sub-vertical faults are one of the older of the fault regimes, in some instances the faults themselves are displaces along cross-cutting faults.

The sub-verticality in faults observed here is not consistent with natural rock mechanics, which prefer sheer fracture at 30 or 60° orientations (van der Pluijm and Marshak 1997). The reason for this is the nature of shear and normal forces as they relate to each other when a force is applied at an angle to a plane, this gives another line of evidence as to the age relations of fault groups are disparate, and it also implies that if the fault were to form within a natural range of orientations, it likely formed at some point during orogenesis, later being overturned into its current position, it may even have formed as a back-thrust in response the thrusting stress.

Dennison (1996) also did work near Lindside WV, and this area was also subject to a reconnaissance trip, because of its relationship with general structures mapped in Bluefield, in

order to get a picture of the outcrop scale deformation features (which are the focus of this project) in another comparable area within the regional structure. Dennison's field guide also makes note of the sub-vertical faults present in the outcrop mapped in the Concord University study.

Below is a drone photo (Figure 2) of a portion of the footwall at the outcrop, the subvertical fault regime prominent in the photo, and the largest through going member of that regime is marked in white; this fault has the most displacement of those measured (~0.66 m).

Figure 2 – A drone photomosaic taken by William Allen, and edited by Joseph Allen showing a portion of the roadcut outcrop in Bluefield WV.

Below is a stereonet (figure 3) made with Rick Allmendinger's Stereonet 11. It depicts the planar orientation (strike and dip) of two crosscutting groups of faults (A and B), of which many were examined. In this instance, four meter-scale subvertical normal faults on the first shelf of the roadcut (As) are juxtaposed with four smaller faults (Bs). This is one good sample of what much of this data looks like in this fashion, localized to avoid overcrowding the map. One

of the subvertical faults (A'') dips at 89° and is displaced slightly along B'. Some of the smaller faults (which only have displacement from 2-8 cm) go extinct into the larger faults, and are not represented on the other side of the larger fault plane.

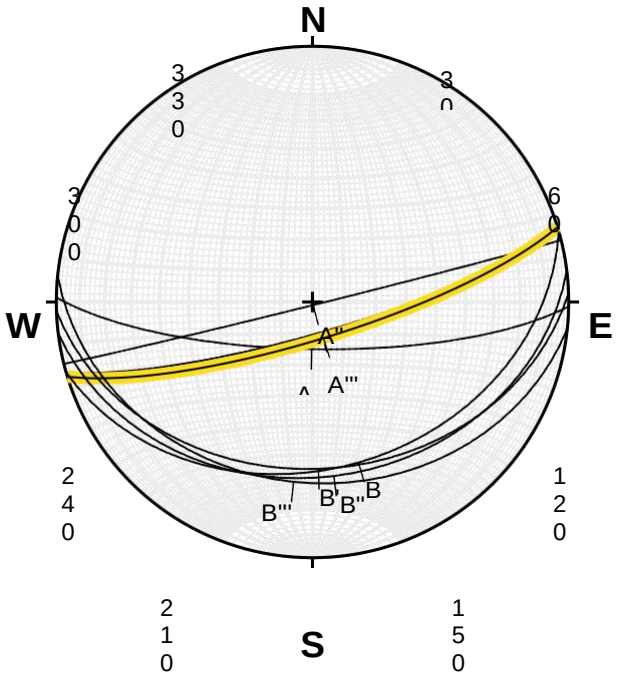


Figure 3 – A stereonet with eight small faults juxtaposed; A faults being a meter scale regime of near vertical faults, B faultsbeing centimeter scale faults with ~35° dip. Made with Stereonet 11 for Mac OS from Rick Allmendinger (http://www.geo.cornell.edu/geology/faculty/RWA/programs/stereonet.html)

The deformation of the larger subvertical faults along the smaller, lower-angle faults does seem to imply that the lower-angle faults formed later, but this does not necessarily bolster any one interpretation for the formation of the sub-vertical faults. This cross-cutting relationship does imply that the regional force that caused the second, lower-angle fault regime was applied at a later time.

Discussion

When and in response to what stress did this regime of subvertical faults rupture? Dennison's 1996 Field Guide, which has a depiction of the subvertical faults (Figure 50, page

111), was a piece of reference material for a part of Concord University 2018 Field camp, it

notes the curious near vertical fault structures. However, the first model for how these may have formed, which is the only one that is directly reflected in the literature is that the faults formed in their current orientation. In this model, the general structure of the regional synclinorium-anticlinorium is already in place, as is mapped in Figure 1, and then a regional force causes the fault formation (Mehlhop, et al., 2002). Crosscutting relationships are found at the outcrop scale and at the hand sample scale in fractures. Arrays found in the sandstone units within the Devonian shales near the Glen Lyn fold axis have many joints, some of which have been reactivated to exhibit displacement, which crosscut other joints/micro-faults. These are some of the examined by Mehlhop, et al. (2002). Their model for the extensional crosscutting faults is essentially a small-scale conjugate fault-block model. The trouble with this model is the very fact that some of the faults present in these arrays are in subvertical orientation (~90°), normal faults do not tend to form in this way. The basic relationship of normal stress and shear stress are distributed for different values of angle of a plane, given a principle force, is such that as the angle increases to 90°, normal stress decreased to 0. Shear stress will increase along with the angle, but peaks at 45° and then decreases to 0 as the angle goes to 90°. Because of this relationship, sheer fractures are most likely to occur when the angle between the normal and sheer stresses is between 30 and 60° (Pluijm and Marshak 1997).

The Appalachian orogen was a complex system of collisions that took place in the Paleozoic era, which caused regional forces that brought about a series of zippering thrust fault geometries (Hatcher 2010) The St Clair thrust fault is one of the large regional thrust faults associated with the Appalachian thrust front. The system has a complex low-angle, thin-skinned thrust fault geometry (McDowell and Shultz 1990). Given that the sub-vertical faults in question are on the overturned limb of the footwall normal synclinorium of this geometry, it is possible

that the faults formed as a sort of fold-accommodating fault regime, many of which Mitra (2002) categorized and a few of which may make sense in this setting, taking orientation into account. It could feasibly have been any of these forms of faults, such that they could slip and then later be overturned into their current orientation. In fold-accommodating faults, the different forms depend on the scale of the fold and the angle of the hanging wall (in this case the thrust sheet) (Mitra 2002). In this case, given the low angle (thrust) geometry, a back-thrust would be the most likely to have formed before the thrust sheet detached, and the walls were further folded into their current orientation. Back-thrusting can happen in response to strain in the hanging-wall of such geometries, and sometimes at the same rate as the main thrust fault propagates (Mitra 2002).

This kind of back-thrusting fault formation (which is coupled with the greater geometry) can also be the source of a regime of faults which can re-rupture in less optimal orientations and form a "domino" or "bookshelf" style of faulting normal faulting continues (Scholz 2002), meaning that the displacement may not have occurred all at once either, which is more likely in a complex system such as a fault system. Instead of the compartmentalized sequencing (step 1, then step 2, so on) way of interpreting these structures and their orientations offered by a conjugate fault-block model, this model would allow for a more dynamic interpretation of the initial thrusting, further reorientation in response to folding, and even further rupturing of these faults in less optimal orientations. Fault regimes that exist are also a control on the accommodation of further fault regimes as bed orientations and regional stresses change (Scholz 2002), and this is a more feasible explanation than the conjugate fault-block model for the cross-cutting fault regimes.

Wojital (1985) suggests that the statistical homogeneity of small-scale faults and deformation features in low angle thrust fault geometries suggest that the strain profiles are similar and that the regional stresses and the angles they are applied are similar. The footwall syncline of the St Clair has thinned beds and a unique deformation profile in its competent strata, but the general structure of the system is similar to other footwall synclines in the area which are a part of the Appalachian basin (McDowell and Schultz 1990). Couzens and Dunne (1993) prove that the kinematic role of different forms of displacement in the Saltville thrust, and show that they formed as fold-accommodating faults. They also posit that this implies more than 20 km of displacement of a similar kind within the St Clair thrust (Couzens and Dunne 1993), which is one of many of these low angle, almost flat-lying beds in the valley and ridge province (Evans 2016).

Given the necessity of a mechanism of displacement existing on the St. Clair fault, and the nature of most of the structures examined in other regional structures, it is more likely that the latter model including a combination of fold-accommodating faulting, and further deformation of faults created by such processes to be a plausible deformational model for the sub-vertical faults than the conjugate fault-block model. Although newer research into thrust fault structures may suggest that interpreting faults in the footwall of thrust faults in this way can be convenient but erroneous. Butler, et al. (2021) show that a thinned and non-viscous footwall can respond to imposing thrust related harmonic forces in a disharmonic way, and that this can create deformational system with buckle-fold related faults, which can even form in regular intervals (regimes). This is a possible model for the formation of the subvertical faults present at the inspected outcrop and, similar to the ramp related faulting, provides a more dynamic and physically probable history for the formation of the subvertical faults.

Conclusion

Shown below is spliced and correlated image of a segment of the regime of subvertical faults which where examined in the Bluefield WV road-cut of the St. Clair footwall syncline.

Figure 4 shows a portion of the outcrop (figure 2 – bottom right corner) with stratigraphy correlated along faults.

The orientation of the beds is uncommon, and the faults are large and frequent enough to be a substantial feature in understanding the general structure of the Glen Lyn syncline. The field study detailed here only mapped the local structure and attitude of the beds and these faults and other deformational features. There is a need for further study involving retro-deformative, kinematic modeling in order to posit that any particular model for how these structures formed, but according to the literature surrounding thrust fault geometries and deformation, it is more feasible that these subvertical faults formed in a dynamic response to the stress imposed by the St. Clair thrust (whether as a back thrust or in a buckle-fold related geometry) than the previously posited post-orogenic interpretation of these features.

Bibliography

Butler, R., Bond, C., et al., 2021, Fold-thrust structures – where have all the buckles gone? Lyell Collection, Fold-thrust Research Group, School of Geosciences, University of Aberdeen, Aberdeen, U.K.

Couzens, B. and Dunne, W., 1993, Displacement Transfer at Thrust Terminations: the Saltville Thrust and Sinking Creek Anticline, Virginia, U.S.A., Journal of Structural Geology, Volume 16, University of Tennessee, Knoxville.

Dennison, J., 1996, Geologic Field Guide to Hydrocarbon Stratigraphy of Southeastern WestVirginia and Adjacent Virginia, Appalachian Geological Society, Charleston, WV.

Hatcher, R., 2010, The Appalachian Orogen: A Brief Summary, The Geological Society ofAmerica, Univ. of Tennessee, Knoxville.

McDowell, R. and Schultz, M., 1990, Structural and Stratigraphic Framework of the Giles County Area, a Part of the Appalachian Basin of Virginia and West Virginia, UnitedStates Geologic Survey Bulletin.

Mehlhop, A, Dennison, J, and Stewart, K. (2002), Extensional Structures Along the AlleghenyFront in Virginia and West Virginia Near the Giles County Seismic Zone.

Mitra, S., (2002), Fold-accommodation Faults, AAPG Bulletin, v. 86, no. 4.

Scholz, C.H., 2002, The Mechanics of Earthquakes and Faulting, Second Edition, Cambridge University Press.

van der Pluijm, B. and Marshak, S., 1997, Earth Structure: An Introduction to Structural Geologyand Tectonics, Chapter 18 (p. 444-474), WCB/McGraw-Hill.

Wojtal, S., 1985, Deformation Within Foreland Thrust Sheets by Populations of Minor Faults,Journal of Structural Geology, Volume 8, Oberlin College.

"A World in a Grain of Sand": An Exploration of the Immeasurable Blakean Influence on Modern Times

Jordan Blankenship

Dr. Michelle Gompf

McNair Research

14 January 2019

"A World in a Grain of Sand": An Exploration of the Immeasurable Blakean Influence on Modern Times

William Blake's common reputation as a great Romantic poet is a grand understatement of the full effect of his works; however, it would be entirely accurate to describe him as a phenomenal poet on an completely alternate intellectual platform from his contemporaries altogether, standing under the same vast umbrella of Romanticism. Blake's works have been scrutinized for centuries in an attempt to make sense of his mythology, make connections to reality, and find the hidden meanings in his dense text. These are all equally difficult tasks, however, as Blake has skillfully managed to camouflage his meanings behind his ability to force the reader to question themselves and their purpose. His myths and poems entice the reader to look within and assess who they are, hoping they will see a contrast between themselves and the negative aspects Blake condemns in his work, or at least encourage them to correct these faults. One of the most notable overlying themes of Blake's texts is that perception is the true controlling factor of one's life: his characters are limited to what they perceive they are capable of or where they belong, and he makes clear connections to these limitations in humans. Many of his characters also feel a sense of loss, hopelessness, and lack of identity. This particular aspect of Blake's soul-searching has influenced his readers across time and around the world, many finding that the true purpose of literature and art is to embrace this loss as freedom of the spirit while others seem keen on warning their readers to not lose themselves too far. Since Blake's literary revival in the 1960s, a time when most of the population of America sought to escape reality or to find a greater purpose, writers and readers have turned to Blake's mythology and ultimate reasoning in an attempt to find an answer. Many have replicated his messages, either utilizing Blake's captivating themes or by mentioning and referencing his works directly to enhance their own plot with Blake's dense text and meaning. In addition to the classic literary applications of Blake's ideologies, his bold imagery and thematic influence are favorable assets that more recent writers have applied to add cinematic value or lyrical content. Regardless of how his themes and influence are used, Blake's works live on in various forms of written and visual works which themselves will remain through time as well.

William Blake was perhaps a man born before his time: his own circumstances create a scenario of the American dream, regardless of the fact that he is not American and may not have been the biggest fan of America. Blake was born poor and lived poor, dedicating his life to his work, even though it did not bring him fortune. This kind of unrequited dedication and passion is the foundation of the "American dream," simply doing whatever it is that makes one happy. This type of philosophy of life also goes along with the hippy vibes of the 60s and 70s: dreams of mind-altering drugs and groovy music, Woodstock and Jimi Hendrix, never relinquishing the freedom of one's own spirit. His revolutionary ideas, especially those illustrated in *America: A Prophecy*, are similar to and inspire the revolutionary thrusts of the 1960s and continue through the 1990s. This is the time period where most of the direct links to Blake's works can be found in American music and film. Perhaps it was a culmination of all these facets of Blake's works and his life that made him a cult favorite in America during a time when Americans began to demand change – also, one can only imagine how Blake's poetry would read under the influence of an altered reality from popular 60s psychedelic drugs. Furthermore, Blake's adaptation of Romantic themes seems to surpass the actual Romantics in application: Blake focuses more on separation from the previous ways of society in exchange for a refreshing new way of life. In this way, the forward-looking, quasi-pessimistic Romantic take on reality is very much in touch with the modernist movement of the early 20th century. These similarities in intellectual needs and interests bring the thoughts of William Blake closer to modernists and post-modernists than his own contemporary Romantics: his words give their ideas direction. The contraries and balancing contradictions of Blake's poetry and mythology drive these decades of transitional thought into a time when balance is valued, as Blake believed it should be.

Blake's Legacies

It appears that there are two main aspects of Blake's poetry that has held influence over time: his imagery and his empowering themes have withstood the test of time when his form and use of language could not due to the natural evolution of literary form. Therefore, these elements of Blake's works that have survived him have experienced an evolution of their own. His poetry focuses more on the revolution

of society and the human race to a greater depth than his contemporaries, "For man has closed himself up, till he sees all things thro' narrow/chinks of his cavern" (*The Marriage of Heaven and Hell*, Blake 39). Essentially, because humans have blinded themselves to truth/Truth, we are unable to see things as they are – rather, we see them through filters in small views. Many people of various professions who have been influenced by Blake agree with this sentiment, and this ideal can stand as the foundation of Blakean mythology. The previous line states, "If the doors of perception were cleansed everything would appear/to man as it is: infinite": perhaps his most popular singular statement, and most of those who show singularity with Blakean ideology can trace their connection with Blake's views back to this sentence, or at least to his general idea that perception is limited in society's expectations (Blake 39). His most iconic image is arguably that of "the fall": the character Los falls from the Eternal dimension – Blake's version of paradise – and is bound in human form, an affront to his spiritually unmitigated freedom, losing himself in the damnation of materialization. "The Fall," as I will refer to this specific image, which is full of self-doubt and self-loathing, questioning existence and meaning, directionless wandering, is the foundation of many subsequent characters' stories. These are the two main aspects of Blake's works that will drive the remainder of this study on his influence on modern society; however, because these aspects are so intertwined, it will be difficult to completely separate the two, so there will be some overlap of topics between the two sections.

"The Fall"

John Milton's *Paradise Lost* is the most influential work in British Literature, from which Blake directly replicates the image of "the fall" of Los. In *Paradise Lost*, Lucifer is "hurled headlong flaming from th' ethereal sky […] down to the bottomless perdition" of fiery Hell (Milton 45-7). Although the description of his fall is not detailed at length, the biblical image of Lucifer's fall from grace, both literally and figuratively, is a well-known Christian tale which condemns disobedience. Blake takes this fall and turns it into a complex and relatable account of consequence and accountability. Los and Urizen, Blake's derivatives of imagination and reason, are both bound in human form, creating barriers to their

power and giving material limitations to their abilities. The fall and the subsequent creation of human flesh to end the falling gives the "fall from grace" idea more substance and complexity, creating a deeper torment to pull from to inspire future generations. Countless authors and screenwriters have utilized this image in their own works, most of which tie more directly with Los than with Lucifer. Of these writers, there are several that stand out significantly.

In Lewis Carroll's *Alice's Adventures in Wonderland*, there are many parallels between Alice and the characters she encounters and those of Blake's mythology. Much of Blake's poetry even seems to serve as a precursor or preface to Alice's story, warning of the dangers of relying on human perception: "Mans desires are limited by his perceptions," Blake tells his reader (*There is No Natural Religion*, Blake 2). Perhaps Carroll purposefully constructs Alice's desires to be less limited, allowing her to perceive more of the world around her and question herself more deeply. The first scene that connects with Blake's works is Alice's fall down the rabbit hole; Alice's fall is significantly Blakean, as it separates her entirely from the world she has always known and hurtles her into a world of unknown, a land that she fears, connecting her more deeply with Los' emotional torment of his fall. Alice follows the White Rabbit without a second thought down the hole, "never once considering" what repercussions or dangers she may encounter, or even how she would escape the hole, not knowing its depth (Carroll 2). Her impulsiveness and curiosity are both characteristics Blake encourages, broadening the horizon of the imagination. "Down, down down. Would the fall never come to an end!" Alice exclaims to herself after falling for what feels to her as an eternity (Carroll 3). Carroll uses Alice as a character with multiple purposes: she can warn young children to mind their curiosity so as not to get lost, but on the contrary, she serves as an encouragement for adults to do that exact thing, get lost in one's curiosity. These opposing purposes depend on the reader's perception of Alice's character; if they find her journey through Wonderland worth the risk of being lost, they are perhaps more inclined to see the latter purpose which would enrich their own sense of adventure and curiosity. Alternately, these opposing views further reinforce Blake's philosophy of balance and opposition coexisting in society as a necessity rather than a risk.

The second overlap between Carroll's scene and Blake's work is very literal, two characters experiencing a "fall" from their reality and their comfort zone. Blake's character Los experiences a fall from the Eternal dimension, Blake's portrayal of ultimate perfection and spiritual nirvana. Los's fall is also described with repetition similar to Alice's: "Falling, falling! Los fell & fell […] down down/Times on times," Blake opens Los's fall from grace, perhaps influencing Carroll with these very lines (Blake 27-8). Los and Alice both "fall" away from what they have always known: Los from the Eternals, Alice from her family and home. Alice's adventures following her trip down the rabbit hole open her imagination, allowing her to find herself and identify the person she wishes to become, but also incorporating the fear of a young girl in a realm of the unfamiliar. Los's fall is more complicated, inciting fear and lamentation of his new human form. The embodiment of a spiritual form is a negative limitation of perception in Blake's works; therefore, Los being bound in flesh is insulting and degrading, causing him to lose sight of himself. With this binding and splitting, the "opposites, contraries," and divisions of each fallen character become hostile toward each other, fighting for dominance "and they are at war" with themselves, attempting to find their identity in the rubble (Gleckner 374). The idea of perception is also evident in both accounts. Alice's perception of time and space is clouded by her wondering mind, causing the duration of her fall to be unclear to both her and the reader. The time lapse in Los's fall seems to be more literally eons, but Los still undergoes self-doubt during his fall with "contemplative thoughts" (Blake 40). The perception of reality and of the world are seen in both very similarly as well: Los creates the physical world along with his own form, which ends his fall, and Carroll's narrator describes Alice as coming "down upon a heap of sticks and dry leaves, and the fall was over" (Carroll 5). Although Alice did not create the physical world in Carroll's story, there are strong similarities between the two encounters with the earth. Alice is hit with these natural elements, pulling her out of her daydream as she falls and into the realm of exploration. Los's fall and creation could be viewed in the same manner; his being pulled from his reality and into a new world.

T. M. Coetzee uses "the fall" as a figurative fall from grace in his novel, *Disgrace*. The main character, David Laurie, experiences a very drastic fall after having an affair with a student at the university where, coincidentally, he teaches courses on the Romantics. He attempts to use a quote from Blake to excuse his behaviors and motivations, but it serves more to backfire on him: "'Do you remember Blake?' he says. 'Sooner murder an infant in its cradle than nurse unacted desires?' […] 'Unacted desires can turn as ugly in the old as in the young'" (Coetzee 131). Throughout the novel, especially the first half that details the affair, Laurie seems to be romanticizing his affair with the student, which is not a reciprocated engagement, in order to rationalize it to himself. As he spends time with the student, Melanie, he begins forcing his interests onto her, trying to wrangle passion from her, and forcing passion between them that simply does not exist: they watch a film together and "he wills the girl to be captivated" by the images as he is, but she shows almost complete disinterest (Coetzee 37). David lies with her and the narrator describes it as, "Not rape, not quite that, but undesired nevertheless" (Coetzee 53). Melanie plays on David's desire for her, coaxing him to help her when she needs it, but clearly never feels any desire for him sexually. Blake's characters Los and Enitharmon have a similar disposition. Los, driven by inspiration, imagination, and creative persuasions, and Enitharmon, who values the creation of "her Woman's World with its false religion of chastity and vengeance," are seemingly two opposing characters who would presumably have nothing in common – and that would more or less be an accurate assumption (Damon 125). However, the two come together to create Orc, Blake's representation of rebellion, a necessary aspect to balance the two extreme characters. The same concept can apply to David and Melanie: although they do not produce a child, David matures and grows into a more modest person as the result of the consequences of his affair. David and Melanie, two vastly opposing ends of the spectrum of societal behaviors, somehow produce a desire for balance and allow the reader to see the need for each characters' addition to this conversation.

However, despite how good of a read Alice's adventures and Coetzee's novel may be, the most effective use of "the fall" is on screen. To list all of the movies and television series that present a

"falling" scene which relates to Blake's fall of Los would require an entire study of its own. The most applicable and popular examples will be listed here somewhat briefly. First, Joel Schumacher's 1987 cult classic *The Lost Boys* applies "the fall" to a very effective end. Michael experiences a fall at a turning point in the film: his reality has shifted drastically with his realization that, not only do vampires exist, but also that he has become one. Michael follows the infamous vampire cult over the side of a railroad bridge and subsequently falls off the bridge. This scene replicates Los' fall in the most modernized, late 1980s way possible: Michael slips off the railing and is immediately filled with fear and regret, screaming as he falls unknown depths. The length of time he is falling is entirely unclear, mostly due to the use of fog, which denies any background imagery, and slow-motion effects, much like Los' "falling day & night without end" (Blake 33). His fall is then indirectly broken by a landing on his bed the next morning, connecting to Los' "day and night" comment, lengthening the amount of time that the audience assumes Michael may have been falling. This interruption in logical sequencing between Michael falling off the bridge and somehow ending up in his own room further distorts the existence of time and place. Although it is not as direct a reference to Los' encounter with material earth, it is still a fall which separates Michael from his previously known reality, throwing him into a new world of unexplored possibilities of being a vampire. Michael's experience differs from most examples of "the fall" in that Michael is not the villain of *The Lost Boys*; in fact, he is represented as the hero in most aspects. Although Los does not share this quality with Michael, Blake does not necessarily represent Los as the villain either, rather as a necessary element of human existence, a balancing factor of life.

In the 2001 film *Lara Croft: Tomb Raider*, Lara and her enemy, Powell, race to retrieve the power to control time, a race that Powell loses before falling into an abyss. Powell's fall shares similarities with Los', but it is not the focus of the scene. Lara uses the power of time to visit her deceased father. This particular discussion of why it is wrong to control time and use it to one's advantage is a true representation of Blake's philosophy of balance and contraries. Lara's selfishness and self-centered complaining of how "time was stolen" from her and her father is a lesson on perspective: her father's

reply is the reasoning and acceptance of the natural order, "But you have stolen time itself, and you must give it back" (West 2001). Lara's father holds Lara directly accountable for her own actions, instead of allowing her to wallow in anger of the unfairness of life and death. Los experiences similar consequences after his "many ages of groans" of despair and regret, until he finally "became element" and took on the human form (Blake 43-50). This scene and circumstance are also a fantastic visual representation of Blake's "spiritual divide between life and death," the known and the unknown, comfort and exploration (Lobdell 53). Lara breaches the boundaries of physical existence, contacting the spiritual remain of her father, and reaching beyond the limitations of time and space all in one fell swoop.

A topic that may be unexpected in this particular study – but that I have found fits most of the criteria for "the fall" – is the discussion of the typical Disney villain. In every Disney movie, there is a villain that is written for the audience to despise yet sympathize with, mostly because sympathy is a necessary characteristic for society and an important lesson for young children. However, there is perhaps a deeper agenda with the fall of Disney's most disliked villains. Some of these movies take on more biblical implications, such as *The Hunchback of Notre Dame*, *Hercules*, and even *Cinderella* – the cat's name is Lucifer, if any other indication was needed for an underlying religious agenda. Other films simply mirror aspects of Los' peril.

Claude Frollo from *The Hunchback of Notre Dame* is the image of every corrupt church/clergy member: he is spiteful, cruel, unforgiving, and sexually oppressive. When he meets his final fall, he quotes the Bible, saying, "And he shall smite the wicked and plunge them into the fiery pit," moments before he is literally plunged into a fiery pit (Hahn 1996). Not only is this ironically satisfying, it makes similar statements about religion that Blake attempts to convey: that religious organizations should not only be limited in the power they hold over the people, but that religion is often corrupted by power-hungry fools such as Frollo. *Cinderella* holds a similar message without demonizing a power-driven maniac. Instead, this story relies more on irony and imagery. Lucifer the cat terrorizes, taunts, and mocks the entire cast of characters, human and animal alike, throughout the entire movie until he receives his

just end at the hand of his most tortured subject, Bruno the dog. Even Cinderella comments on the desire to seek revenge and the quality of vengefulness: "Did you catch him this time?" she asks Bruno after he's had a dream of chasing Lucifer (Geronimi 1950). "That's bad," she continues, "Just learn to like cats. […] There must be something good about him" (Geronimi 1950). Although Lucifer continues with his tricks and mischief, and the audience is heartbroken to see Bruno manipulated so, when Lucifer is finally chased out the window, we all feel a bit of relief for Bruno and the rest of the non-wicked household. However, does Bruno essentially murdering Lucifer not make him evil in turn? Blake's comment "a dead body revenges not injuries" comes directly into play: not only does this crime make Bruno less of an innocent, but it also accomplishes nothing in the end (Blake 16). *Sleeping Beauty* is quite different and in some ways much more Blakean. The villain, Maleficent, in her dragon form, meets her end at the sword of Prince Philip. She falls off a cliff and when the screen shows her at the bottom of the cavern, all that is left are the remnants of her cape and the sword. This takes Los' creation of material self and reverses it, as she seemingly dissipated into nothingness. So how does this compare to Blake's comments on the human form? Maleficent was never truly human – so does evil intent not truly exist, or does it not exist in human form? After Los creates the human form, "Light first [began]" (Blake 10). Thus, after the material form of evil is destroyed, perhaps that is where the darkness lies. Although, in Blakean mythology, nothing is as black and white as good and evil, dark and light, and right and wrong. Blake thrives in the grey area, the in-between, the space of contradiction between opposing forces. However, this concept of coexistence would not have made for a successful industry of children's films. Regardless, these Disney movies hold more in common with Blake's mythology than one would assume at first glance.

Perception, Identity, and Perspective

Many of these examples of thematic similarities directly cite Blake as an influence, openly talk about their appreciation for Blake, or even more obviously name their own work after or similarly to Blake's own titles. This makes the comparison of certain artists more direct, but it also opens the possibilities of a snowball effect: for example, Jim Morrison was very blunt about his appreciation for

Blake's mythology, therefore his influence infiltrates the songs of his band, The Doors, and thus infiltrates the works of any subsequent artist that draws inspiration from The Doors. In this sequence, not only has Blake survived, but he has thrived throughout society and his ideals touch countless works and even political spectrums. However, because of this widespread influence, it would be impossible to ever really end the research following Blake's extensive touch. Thus, I will draw first from the works that have been mentioned in the previous section, then I will spiral very briefly into works that have been influenced by these.

Touching back on *Alice's Adventures*, both Blake and Carroll lead their readers to blame the characters for their circumstances. Alice is narrated as being careless, impulsive, and silly while Los is described as bitter and angry, each characteristic contributing to their reason for "falling" into these situations which they cannot seem to find an escape from. Similarly, Jim Morrison of the Doors wrote "You're Lost Little Girl" and "Unhappy Girl," which describe a girl with similar problems: she is lost without a sense of identity and has put herself into the mess she is in, but perhaps she knows the way to get out of her situation. In "Unhappy Girl," Morrison describes a lonely girl, "locked in a prison of [her] own devise," which is similar to both Los and Alice (Morrison 5-6). These "prisons" they are in are each of their own making; Alice because she was impatient and curious, Los because he was also impatient and vengeful, and Morrison's girl because she has built a wall between herself and the world, "playing warden to [her] soul" (Morrison 4). All these commonalities lead to Blake's argument of taking responsibility and freeing the soul beyond human perception. Morrison's approach is to bluntly snap the girl back to reality, telling her it is, in fact, her fault, but this prison of hers is not the end. Carroll is more forgiving, allowing Alice to find her own path out of her mistakes with the help of friends she meets along the way. Blake, of course, is the harshest of all, allowing Los to continue the cycle of binding by giving Urizen a human form, locking him in chains, and leaving him in solitude.

Another Blakean theme seen in Alice is her tendency to weep instead of act. After shrinking and growing depending on what she eats or drinks, she feels as if she will never be able to undo her mistakes

and return home. She begins trying to cope with the possibility of never being her true height again by personifying her feet and other such nonsense. Carroll uses Alice's dramatic monologue to insinuate that she is still a silly, young child trying to reach some level of maturity, but the bizarre events that take place keep her from maturing. She even admits that she "ought to be ashamed of [her]self" for crying so much, but then continues to cry again (Carroll 12). The narration is even dramatic, citing that she cries gallons enough to create a pool around her, creating such an irrational image that the reader is forced to see how irrational the act of crying is itself. This redundancy and self-pity is seen, and reprimanded, in Blake's works several times. Ahania is one of Blake's more irrational characters, continuously weeping and indulging herself in pleasures without real reason or meaning. In "The Book of Ahania," she weeps, formless in the realm of Eternals, for her lost Urizen quite dramatically: "I weep on the verge/Of Non-entity; […] Why didst thou despise Ahania/To cast me from thy bright presence/Into the World of Loneness" she cries (Blake 53-64). However, her lament reaches a few paradoxes; first, if she is in the "World of Loneness" then no one is around her to hear her torment, thus she is reveling in self-pity and has no shame about it, and secondly, if she is of "non-entity" and lack of importance, why then would Urizen, the embodiment of reason, love her? Ahania's lack of action and indulgence in self-pity is what Blake would have his readers believe led her to this place of indecision and self-torture. Lara Croft reaches a similar level of self-pity during the time breach with her father. She does not necessarily wallow in her anger like Ahania and Alice, but she does seem to revert to a childishness in seeing her father again. "Why can't we use the power just this once?" she asks of her father, "Time was stolen from us, and it's not fair" (West 2001). This gives her father an opportunity to scold Lara, reminding her that she is an adult and she has responsibilities, one being to relinquish the power of time before she suffers the consequences of messing with natural order. Even David Laurie suffers from a harsh case of self-wallowing during his trial at the university. He refuses to hear any of the items he is formally charged with, but instead he simply pleads guilty. "I do not wish to read Ms Isaac's statement. […] There are more important things in life than being prudent," he asserts repeatedly, shying away from the details of his crimes against the young student (Coetzee 95). However, this refusal to acknowledge one's faults is a

characteristic Blake could not tolerate: David's stubbornness is an example of Blake's condemnation of "man clos[ing] himself up, till he sees all things thro' narrow chinks" (*The Marriage of Heaven and Hell*, Blake 39). In David's case, the "chinks" are the details of his crimes he will tolerate being spoken aloud.

Alice's self-pity takes a similarly dramatic turn when she disassociates from herself further stating, "Who am I then? […] if I like being that person, I'll come up," when thinking of her reply if someone were to come looking for her (Carroll 16). This could be interpreted as the stubbornness of a little girl, or it could be understood as her true loss of self and struggle to understand who she is and what she is capable of. Morrison's "You're Lost Little Girl" uses the same themes of self-questioning and doubt. The speaker asking the girl "who are you?" is what Ahania and Alice both are experiencing internally. Ahania experiences the same self-doubt, "wander[ing] on the rocks/With hard necessity" of finding herself and her purpose (Blake 72-3). However, this too seems absurd considering Ahania does not have a physical form: perhaps Blake's purpose in contradicting himself here is for the reader to realize how selfish and wasteful it is to be "in search of oneself" when they can instead channel their energy into releasing and unbinding the soul. Even Ahania soon realizes her mistake in the last stanza of her book, saying, "selfish fear!/Self-destroying: how can delight/Renew in these chains […] Where bones […] are buried/Before they see the light" (Blake 41-7). Her exclamation of regret mirrors Blake's belief that the human form never truly allows our souls to be free as they are meant to be, and self-indulgent tendencies keep the souls further from achieving that harmony.

Carroll uses perception as a tool to create a more complex dialogue between Alice and his characters, while many others follow Blake in exploring the depths of human perception and how far the mind will allow humans to explore. Carroll's caterpillar character is perhaps the most perceptual character in Alice's travels. He questions Alice, asking her to explain her self-doubt when she replies that she does not know who she is. "Who are *you*?" he continues to ask her time and time again, receiving the same replies (Carroll 45). The surface-level content of this simple question is an inquiry of her name, asking what to call her. Alice, however, answers with more depth, saying, "I hardly know, sir […] I think I must

have changed several times since [this morning]" (Carroll 45). Her increasing depth of perception and self-awareness as her journey progresses pushes her toward the boundary of maturity. Much like in Blake's *Songs of Innocence and Experience,* the caterpillar is the experienced adult, encouraging the young innocent Alice to begin questioning herself within rather than accepting the world as it seems. The band Jefferson Airplane takes direct influence from Alice's story, specifically her encounter with the caterpillar, in their song "White Rabbit." The lyrics modernize many of the elements of Alice's story: the food Alice eats to change sizes is related to pills – presumably medication prescribed for mental illness that are ineffective – then "the men on the chessboard" represent societal expectations, and, of course, there is a reference to "some kind of mushroom" that could not be confused as anything other than magic mushrooms (Slick 1967). Slick uses Alice's story to comment on larger perspective as well: "When logic and proportion have fallen sloppy dead […] remember what the dormouse said, feed your head" (Slick 1967). This last line particularly encourages further learning, drive to seek more information and more life, regardless of how illogical society may see one's motivations or actions in furthering their own perspective. Although Grace Slick may not have directly taken this thirst for life from Blake's own works, they certainly transferred from Alice's story rather accurately.

Numerous musical artists apply these themes of perception and seeking more from life, especially during the same time period as Jefferson Airplane and the Doors. Led Zeppelin uses these themes in "Achilles' Last Stand" in a more cryptic tone along similar lines as Blake's "Echoing Green." "Days went by when you and I bathed in eternal summer's glow," the song chimes, reminiscent of everlasting youth and confidence (Page 1976). The song progresses into an acknowledgement that everyone has responsibilities, and no one can live their days in the summer sun forever: "What place to rest the search, the might arms of Atlas hold the heavens from the earth," a powerful image of responsibility and perspective (Page 1976). Bob Dylan, a well-known lover of Blake's works, modeled a large number of his songs after the ideas of Blake. In "Every Grain of Sand," Dylan comments on perception and perspective from the inspiration of Blake's "Auguries of Innocence." Blake's poem begins, "To see a

world in a grain of sand/And a heaven in a wild flower,/Hold infinity in the palm of your hand/And eternity in an hour": this poem opens with strong imagery of perspective and space, comparing things which are incredibly large to small, miniscule objects of seemingly no importance to everyday life (Blake 1-4). A comparison on this scale seems impossible to comprehend, but Dylan finds a way to put this concept into more approachable terms: "I gaze into the doorway of temptation's angry flame […] then onward in my journey I come to understand that every hair is numbered like every grain of sand" (Dylan 1981). The basis of both the poem and Dylan's song is basically that life is a miracle no matter what you do with it, but that it seems a waste to not enjoy what life has to offer to its fullest extent.

In Blake's *Songs of Innocence and Experience*, the world is described in two stages, which make the title obvious. Two poems within these songs, "The Little Girl Lost" and "The Little Girl Found," parallel many of the themes in both *Alice's Adventures* and most of the songs that have been detailed here. The overall concept of changing perception and broadening horizons throughout life are also evident in these texts. One major connection in "Lost" and Alice is the appreciation of and comfort in their surroundings; each character seeks shelter in her environment, embracing the natural elements for safety. For "experienced" adults, this idea seems ludicrous; trusting a tiger to keep one safe through the night. For the "innocent" however, this is her only option and makes as much sense as her being alone in the wilderness. Alice shares a similar appreciation for her surroundings, allowing them to distract her from the reality of her situation to avoid having to cope. Various elements of Alice's environment challenge her, despite her avoidance mechanisms. The door she has to fit through to get to the garden is mere inches tall and forces her to adjust her size for entry and freedom from the room she is in. Blake's "Marriage of Heaven and Hell" mirrors this image perfectly: "If the doors of perception were cleansed everything would appear to man as it is: infinite" (Blake 39). Through Alice's change in self, she is permitted to enter the outside world through the door. Effectively, her perception of herself has shifted so that she has opened her mind to a new expanse of possibilities, which is a concept many of these artists apply either to

the works listed in this study or in others. In general, the idea of expanding one's perception and perspective is a shared opinion of all Blake's fans.

Conclusion

One could argue that the door Alice encounters has nothing in common with Blake's "door of perception," and that Morrison did not keep Blake's messages in mind when he wrote his songs. It could also be argued that Blake's influence is so wide-spread that his message can be seen in almost any work of literature or certain visual representations in films, even if his work did not directly influence that work. Blake's idea of how limited perception can cloud one's judgement and distance them from reality is a very Romantic ideal that has evolved into the modern era with more Blakean flare than ever, but is distanced from some readers through his extensive mythology. Blake plays on this theme of perception and perspective to tell the reader not to limit themselves within the confines of their human forms when the spiritual form holds much more potential and freedom. However, his dense theory and often confusing character lineage can deter the reader from seeing this underlying theme. There is a stark contrast between the "'accepted' meaning" and the meaning the reader chooses to accept, based on the reader's perception of the work they are involved with (Siemens 298). Whether the audience of Blake's own works, works influenced by him, or films that have adapted these images and ideas chooses to identify with the surface content of Blake's message to not be closed-minded, or furthers delves into what it means to be self-aware and conscientiously unbound to the limitations of the human form, completely determines how Blake's ideas are spread further. If one watches *Tomb Raider* and accepts that Powell simply loses a battle and falls to his death, that person has overlooked the opportunity to explore the concept of the desire for power within all humans and assess how deep that desire runs within their self. Regardless of how one chooses to perceive Blake's mythology and poetry, his ideology, style, rebelliousness, and creativity ring strong through time and space without bounds and continues to push the human race to evolve, even if just a little.

Works Cited

Carroll, Lewis. *Alice's Adventures in Wonderland*. UK: Penguin Random House UK, 2015. Print.

Damon, S. Foster, and Morris Eaves. *A Blake Dictionary: the Ideas and Symbols of William Blake*. Dartmouth College Press, 2013.

Doors, The, comp. and perf. *Strange Days.* Sunset Sounds Records, 1967. Google Play App.

Dylan, Bob. "Every Grain of Sand." *Shot of Love*. 1981.

Erdman, David V., ed. *The Complete Poetry and Prose of William Blake*. New York: Anchor Books, 1988. Print.

- *The Book of Ahania,* 84-9.
- *The Marriage of Heaven and Hell,* 33-44.
- *There is No Natural Religion,* 2.
- *Songs of Innocence and Esperience,* 7-31.

Gleckner, Robert F. "William Blake and the Human Abstract." *PMLA* vol. 76, no. 4 (Sept. 1961): 373-9. *JSTOR*. Web. 24 April 2017.

Milton, John. *Paradise Lost*. Ed. Greenblatt, Stephen, et al. *The Norton Anthology of English Literature*. W.W. Norton, 2018.

Page, Jimmy. "Achilles' Last Stand." *Presence*. Led Zeppelin. 1976.

Rovira, James. *Rock and Romanticism: Blake, Wordsworth, and Rock from Dylan to U2*. Lexington Books, 2018.

Siemens, William L. "Mirrors and Metamorphosis: Lewis Carroll's Presence in Tres Tristes Tigres." *Hispania* vol. 62, no. 3 (May 1979): 297-303. *JSTOR*. Web. 24 April 2017.

Slick, Grace. "White Rabbit." *Surrealistic Pillow*. Jefferson Airplane. 1967.

Trousdale, Gary et. al. *The Hunchback of Notre Dame*. Buena Vista Pictures. 1996.

West, Simon. *Lara Croft: Tomb Raider*. Paramount. 2001.

To Create and Kill a Misogynistic Society

Hannah Bollin

Amberyl Malkovich, Ph.D.

To Create and Kill a Misogynistic Society

Harper Lee's *To Kill A Mockingbird* follows a brother and sister duo as they grow up in the deep south, being narrated by the sister years later as an adult. This work confronts social issues such as racism, domestic violence, mental illness, sexual assault, and general sexism in her the 1960s world - the most prominent being young Scout's fight against patriarchal norms and generalized sexism. Scout is a little girl growing up in a very male-dominated environment and takes over the majority of the work's plot - while there are a few other female characters who come in and out of the spotlight, Scout is the only female who consistently contributes to the plot overall. Southern morals weighed down heavily on women during this time period and these sexist principles limited everything women did. These morals had high expectations for young females and our main character Scout is a victim of this – but she spends the majority of the work rebelling from them. These sexist ethics only encourage her to act out *more* against her own culture, and as time progresses she develops her own prejudice against both men and women. Scout's entire upbringing – meaning all intentional and unintentional gendered teachings - shaped her gender expression and overall person. In this paper we will explore how Scout's lack of a consistent and positive female role model, her exposure to primarily men in her adolescent years, and the repercussions that she faced as a result of her gender expression led to Scout's internalized sexism and gender ranking.

One of the first background details that we learn about Scout is that her mother died many years ago of a sudden heart attack. This being one of the first things that the audience is told by Scout herself signifies that she, even at an older age when she writes this, thinks her mother's death was an important factor of her childhood. Despite its obvious importance, Scout makes a conscious effort to make it seem to the audience as if her mother's death and overall absence was not a big deal to her. This forced disregard for her absent mother makes it very apparent that she is trying to make, not only the audience but herself, believe that it didn't affect her – but her subconscious says otherwise. Scout

introduces the idea of her dead mother to the audience by writing "Our mother died when I was two, so I never felt her absence" (6). In this statement, Scout immediately disregards not only the death of her mother but the entire existence of her mother on the audience - once she brings her deceased mother up, she immediately feels the need to let the audience know that she was fine without her, Scout is not one to beg for empathy.

Scout is a character who prides herself on her independence, although she grew up clinging onto her brother Jem and emulating his every move. Her admiration for her brother is obvious, Scout even goes as far as to say exaggerated things like "Jem was a born hero" (44) after he does something as simple as assuring Scout that their scary neighbor Boo Radley is dead and can't hurt her. This is just one of the many examples of Scout making a point to admire Jem's courage – which parallels the third dimension of Deborah David and Robert Brannon's research. In their work, this pair breaks down the different characteristics one must have to accurately fit the definition of *masculine,* these being "'no sissy stuff,' the rejection of femininity; (2) the 'big wheel,' ambition and the pursuit of success, fame, and wealth; (3) 'the sturdy oak,' confidence, competence, stoicism, and toughness; and (4) 'give 'em hell,' the machismo element." (26). Jem fits every one of David and Brannon's key points and represents the "ideal male" in the text, but he it is Jem's confidence that young Scout often presses on.

Despite this admiration, when Scout talks about the difficult situation of her mother's passing, she makes a point to compare herself to Jem. Scout describes her emotions, compared to his, by saying "I did not miss her, but I think Jem did" (6). Jem shows a lot of emotion about their mother's passing, but Scout insists that she doesn't. Society has stigmatized men to not show emotion, therefore when Scout sees her emulated brother grieve, she feels empowered by her disregard for the event. Scout is only six years old, but she already has these gendered stereotypes implemented into her mindset, which Judith Lorder (1994) says is a normal process that takes for all young people. In her work "The Social Construction of Gender" she writes that "gender is so pervasive that in our society we assume it is bred

into our genes [and that] gender is so constantly created and re-created out of human interaction, out of social life and is the texture and order of that social life" (141). From these gendered patterns in society Scout learned that women are more emotional than men, so by turning the tables with her brother she feels empowered.

Scout sees herself crossing that gendered line and she likes it, but by doing this she is rebelling against the modern institutions that are meant to put women in their place. Susan Shaw and Janet Lee (2015) say that these institutions are "social organizations that involve established patterns of behavior organized by particular purposes" (63), examples of institutions in American society are the "ideal" family, marriage, the economy, government and criminal justice systems, religion, education, science, health and medicine, and mass media. It is institutions such as these that teach children the social constructs of gender mentioned earlier – meaning they redundantly show gendered patterns to children indicating that there is a right and a wrong way to "do" gender. Scout is integrated into multiple institutions which are connected to one another. These being that she is the daughter of a respected lawyer, a sister, a student, and from a rural area. Institutions such as these are interconnected in society and work to support and maintain one another in a Scout's life, meaning they enforce certain morals even more – like when Atticus tells the children "[they] must try to behave like the little lady and gentleman that [they] are" (207) so that Jem and Scout can grow up to become civilized adults.

According to Judith Gardiner, the author of "A Wake for Mother: The Maternal Deathbed in Women's Fiction," "the daughter must pattern herself after her mother, acquire her gender identity, learn her roles, and at the same time differentiate herself as a person" (147) – meaning that a mother figure in a young girl's life provides basic guardianship to the child as well as the teaching of a woman's role in society. Scout's father, Atticus, recognizes her lack of a mother figure early on and tries to force different forms of female guidance into Scout's life, which only causes an adverse reaction from the impressionable young girl. Scout fails to bond with any of the mother-figures placed in front of her and

pushes them all away. Shaw and Lee write that there are "three basic types of childcare associated with mothering: activities to meet children's basic physical needs; work that attends to children's emotional, cognitive, and recreational needs; and activities for maintaining children's general well-being" (450) and, conveniently enough, Scout has three mother figures in her life – but not a single one of them cover all three of these bases. Scout's mother may have been able to cover all of these childcare needs – but she exists only as the role of her mother. Nobody could ever fill the mother's metaphorical shoes as *the* mother – but she never *does* anything for Scout emotionally or physically. To fill this hole in Scout's life and try to give her a gender-appropriate role model, Atticus introduces the two characters of Calpurnia and Aunt Alexandria into Scout's life.

Calpurnia, the Finch family's live-in African American cook, looked after Scout and her family – making sure that everyone was fed and clean. The character of Calpurnia represents the majority of African American women after the rise of industrialization who worked in domestic services. Hesse-Biber and Carter (1999) report that the percentage of African American household workers increased from 38.5 percent in 1910 to 59.9 percent in 1940 [and] for the next three decades, African American women remained the single largest group in domestic service" (511). Despite Calpurnia working for the Finch family she is, in terms of the three basic types of childcare mentioned earlier, the closest thing Scout has to a mother figure in her life. Scout talks about her like a daughter might write about her mother when narrating "Our battles were epic and one-sided. Calpurnia always won, mainly because Atticus always took her side. She had been with us ever since Jem was born, and I had felt her tyrannical presence as long as I could remember" (6). Calpurnia is Scout's basic caregiver but the two of them have a relationship that fails to surpass housework and dinner time – although there are a few occasions where Calpurnia tries to teach Scout a life lesson or two. An example of this being when Scout yells at Walter Cunningham after he pours syrup on his meat and potatoes, Calpurnia requested Scout into the kitchen and then said "anybody sets foot in this house's yo' comp'ny, and don't you let me catch you

remarkin' on their ways like you was so high and mighty! Yo' folks might be better'n the Cunningham's but it don't count for nothin' the way you're disgracin' em" (40). If Calpurnia taught the children anything, it was manners. It's later in the work when Atticus decides that having Calpurnia as a female role model for Scout is not acceptable. This being not only because of the cook's working-class status, but also because she is not an effeminate type of woman.

Aunt Alexandra moves into the home in the latter part of the work, with her only goal being to give Scout some "feminine influence" (145). Aunt Alexandra fits the very definition of the classic southern belle - meaning that she is a woman of her time and represents what is to be strived for in terms of femininity. Shaw and Lee list that the ideal women is "beautiful, thin, athletic, and sexy; yet also loving, sensitive, competent domestically, and emotionally healthy" (133). The entire goal behind Aunt Alexandra's character is to lead Scout by example and mold her into the ideal woman, too. Upon Alexandra's arrival, she warns Scout "It won't be many years, Jean Louise, before you become interested in clothes and boys" (145). This remark portrays the idea that all young girls are fixated on heterosexual relationships and material objects, which is generalized sexism. This misogynistic statement may seem surprising because it is a female character saying it, but that just shows the level of suppression that women faced in both this region and time. Emma Goldman touches on this heteronormative expectation of women in her work "Marriage and Love" (1910) with "from infancy, almost, the average girl is told that marriage is her ultimate goal; therefore her training and education must be directed towards the end" (452). This training and education is provided by Aunt Alexandria, who redundantly pushes Scout to be *more* feminine so that she will be *more* fit for a heterosexual relationship in the future.

In this quote about boys and clothes, we see Aunt Alexandria pulling what femininity she can out of Scout by referring to her as her given name of Jean Louise – and therefore deleting the androgynous name of Scout and all that this name stands for. Scout never tells her audience the story

behind her nickname, but the closest she ever gets to doing so is when she tells her teacher that "Jem says my name's really Jean Louis Bullfinch, that I got swapped when I was born" (72). This is really just a crazy story that Jem told Scout while trying to convince her that she's adopted – but it proves that Scout looks at her identity as Jean Louis the same way that she looks at the last name Bullfinch compared to her real last name of Finch, meaning these names belong to two very different people. The word *scout* has a masculine undertone to it, it infers searching and investigating. This name is fitting for Scout because she seeks out adventure and spends the entire first half of this novel investigating the story behind her mysterious neighbor. It's when Scout doesn't fit this adventurous and thrill-seeking personae that she's oftentimes referred to as her given name, an example of this being when Scout is forced to wear a dress and attend a formal lunch party, to which one of Aunt Alexandria's friends say "You're mighty dressed up, Miss Jean Louise. […] Where are your britches today?" (360).

Scout, being a little girl with many androgynous traits, rebels against this narrow-minded training – it is as if young Scout has such a radical mindset that she knows it's asinine to think that her hobbies should be determined by her gender. Scout sarcastically explains these stereotypical expectations by writing "Aunt Alexandra's vision of my deportment involved playing with small stoves, tea sets, and wearing the Add-A-Pearl necklace she gave me when I was born; furthermore, I should be a ray of sunshine in my father's lonely life" (92). This line is Scout's satirical outlook and breakdown of femininity and the social construct of gender stereotypes – which seems fitting since her response to her aunt's request is "I suggested that one could be a ray of sunshine in pants just as well" (92). This scene is a solid representation of Scout as a gender bending character.

With this lack of a mother to follow Gardiner's terms of being both a female role model and a caregiver, Scout becomes a rebellion to society by simply being a tomboy. This is most likely due to the fact that she lives a life surrounded by men. The two most prominent men in her life are primarily positive influences, with her father being a progressive lawyer and her brother being her courageous

mentor. She found more than just Jem's boyish and adventurous personality inspiring, she also looked up to her father's high level of intelligence as well. During this era it wasn't common for a woman to receive an education. Miewald and McCann assert that women in Appalachia don't receive an education because there are "few places for women to work outside of the home, the need to care for children and other family members, and social pressure to follow gender norms [meaning] that there were few alternatives to the traditional role for women" (qtd. in Swank, Fahs, and Haywood 127). Atticus is described by Scout with "It was times like these when I thought my father, who hated guns and had never been to any wars, was the bravest man who ever lived" (115) which parallels her description of Jem with "I suppose he loved honor more than his head" (14). These quotes about the men in her life prove that in the eyes of Scout, being masculine did not always mean being the most brawn. She sees her father as brave because of his high level of education, which only men typically had the privilege of during this time, and the way that he stands up for those in need – like he did for Tom Robinson. Then Scout sees her brother as her courageous leader and selfless savior during their many adventures. With these positive male models in her life, it makes sense that Scout would see a correlation between masculine traits and success in life. It is through these male role models that Scout begins to internalize gender ranking, that being where women and effeminate people are of less value, in her mind, than men and masculine people. Shaw and Lee discuss how internalized sexism such as gender ranking can happen to anyone, even characters like Scout, with:

> the social system here that discriminates and privileges on the basis of gender is sexism, although any one person experiencing entitlements or obstacles associated with sexism may also experience entitlements and/or obstacles associated with other intersecting differences or identities. Sexism works by viewing the differences between women and men as important for determining access to social, economic, and political resources (134)

This mirrors Scout's situation almost perfectly – that being that she idolizes the outcomes that her role models and peers receive due to their masculine traits, therefore she idolizes masculinity while devaluing femininity. Scout watches intelligent and courageous men succeed all around her, so she subconsciously aspires to act like them hoping to be just as successful - and therefore as masculine - as they are. She doesn't get the same outcome as her counterparts do, though – instead she faces constant ridicule for her attempts at presenting in a masculine way.

While Atticus is putting Scout through this mock gender-training, Scout still faces repercussions when she shows any sign of femininity during an inconvenient time for the male characters around her – they only want her to "act like a girl" when it's to their advantage. Many of these comments on Scout's societal wrongdoings come from Jem. Upon Scout being afraid of going near the Radley house, Jem says "Scout, I'm tellin' you for the last time, you shut your trap or go home - I declare to the Lord you're getting more like a girl everyday" (58). He is using the term "girl" as if it is a bad thing to be, feeding more into young Scout's gender ranking. While this dispute may just be an innocent sibling rivalry, the underlying problem with this statement is that Jem's rebuttal is fueled by ignorant sexism. Scout, who normally acts fearless "like a boy," is showing signs of weakness, and Jem automatically blames her gender for this weakness – as if it makes her and her gender identity "less than." Society has taught young men like Jem that showing emotion is effeminate, therefore it is bad. Even if Scout did match that "ideal woman" mentioned earlier, she *still* wouldn't be good enough. According to Nancy Chodorow, the author of *Reproduction of Mothering*, men "repressing their emotions and relational needs is functional in preparing men to participate in alienated work" (qtd. in Gardiner 610). Jem doesn't even want Scout to be near him when she acts in a "weak" way, as if she is easier to deal with when she acts like a tomboy. This kind of reaction from a loved one only motivates Scout to ignore her emotions, just like Jem does, as she follows his sexist remark by only responding "I had no option but to join them" (58).

While Atticus is not aggressive and particularly direct with his sexist remarks towards Scout, he does press the idea of societal gender roles on her – much like any patriarchal family setting would. When the children are confronted by a rude neighbor Atticus tells Jem "You just hold your head high and be a gentleman. Whatever she says to you, it's your job to not let her make you mad" (115). Here Atticus is expressing his high expectations that he has for Jem – because, as a male, he is to maintain a level of calmness and must always act mannerly. This mirrors Kimmel and Sommer's in "What's Up with Boys?" (2013), which says "that young men and boys are constantly and relentlessly policed by other guys, and pressured to conform to a very narrow definition of masculinity" (157). Although it's after Jem vandalizes this neighbor's property in a fit of rage that Atticus tells the completely innocent Scout "I never thought Jem'd be the one to lose his head over this - thought I'd have more trouble with you" (120). This statement is completely uncalled for and shows Atticus' low expectations that he has for Scout as a woman. Atticus expects for Scout to act emotionally and for Jem to act courtly, the motivation behind these expectations for his children are motivated by misogynistic social stigmas. When Atticus' sexist assumptions are proven wrong, he has no shame in telling Scout about the misogynistic mindset that he has – even when Scout doesn't do *anything* wrong she still faces repercussion for her gender. It is no surprise that Scout lives between the gender binaries, she is likely confused by all the mixed messages that she has received.

This misogynistic society, which made her stand out so much, constantly told Scout that all of her means of gender expression were wrong – and as a result, she internalized this misogynistic mindset. While Scout literally fights sexism - like when she shoves Walter Cunningham's face into the dirt after he "disrespect[s] her" (67) - and breaks every misogynistic claim made about woman in *To Kill A* Mockingbird, the paradox in her character is that she has not only internalized sexism but that she openly expresses it as well. The lack of a mother and the overpowering male presence in Scout's life

created her toxic gender ranking, which is best summarized by Shaw and Lee in their claim that "experiences of gender are very much shaped by the gender composition of family members" (444).

At the beginning of this work Scout writes favorably about how well Atticus played as their only living parent with "Jem and I found our father satisfactory: he played with us, read to us, and treated us with courteous detachment" (6). While Scout did think that Atticus was a good parent, she still subconsciously placed gender stereotypes on him. Scout did not expect her father to be her caretaker, she thought that was not what society had taught men to do for his children – so she had very low expectations for Atticus as a parent in terms of caretaking and emotional gratification. When Scout talks about his "courteous detachment" (142) she is talking about the time that he was away at work, providing for the family, and the emotional distance that he kept from his children - that is what was expected of fathers. Scout's expectations of her father fits Shaw and Lee's claim that "the traditional marriage contract assumes the husband will be the head of the household with responsibilities to provide a family wage and the wife will take primary responsibility for the home and the raising of children" (445) – meaning that they are following traditional marriage roles, therefore Calpurnia and Aunt Alexandria are taking up the role as the wife and mother. Later when Atticus does try to reach out on an emotional level with Scout, she taunts his attempt by writing "I know now what he was trying to do, but Atticus was only a man. It takes a woman to do that kind of work" (153). Here we are seeing yet another connection between emotions and gender, men must lack them while women have too many. Since Scout is a prime example of breaking gender roles, it is unsettling to see her spout such sexist ideas – but this shows that she has internalized gender stereotypes only at the age of six. One would expect for her to lead by example and stay open-minded, but she is not one to keep her biased thoughts to herself.

Scout encounters many ramifications for her gender - therefore she formed sexist ideologies against both men and women, while the society that she grew up in only pressured these beliefs more.

To Kill a Mockingbird may just seem like a coming-of-age story with a southern twist, but at a deeper level it is more so the telling of the strict gender rules of this time. Scout is the direct product of her upbringing – meaning that because she lacked a positive and stable female role model and then faced repercussion later on for mirroring the men in her life, that Scout then formed her own unique inclinations of gender at a very young age. Scout is a paradoxical character who breaks sexist stereotypes against women while simultaneously feeding into sexist ideologies herself. Society made Scout this wonderful gender bending character, and the backlash that she received for expressing her interpretation of gender is what caused Scout to resent what she once admired and valued so much.

Works Cited

Gardiner, Judith Kegan. "A Wake for Mother: The Maternal Deathbed in Women's Fiction." *Feminist Studies*, vol. 4, no. 2, (1978): 146–165. *JSTOR.* Web. 22 Apr. 2017.

Gardiner, Judith Kegan. "Female Masculinity and Phallic Women— Unruly Concepts." *Feminist Studies,* vol. 38, no. 3, (2012): 597-624. *JSTOR*. Web. 23 Apr. 2017.

Deborah S. David and Robert Brannon, eds., *The Forty-Nine Percent Majority: The Male Sex Role (*Reading MA; Addison-Wesley, 1976), pp. 13-35.

Goldman, Emma. "Marriage and Love." Women's Voice Feminist Visions, no. 6, 1910, pp. 452–454.

Hesse-Biber, Sharlene, and Gregg Lee Carter. "A Brief History of Working Women." Women's Voice Feminist Visions, no. 6, 1999, pp. 503–515.

Kimmel, Michael, and Christina Hoff Sommers. "What's Up with Boys?" Women's Voice Feminist Visions, no. 6, 2013, pp. 156–159.

Lee, Harper. *To Kill A Mockingbird*. New York: Harper Collins Publishers, 1995. Print.

Lorder, Judith. "The Social Construction of Gender." *Women's Voice Feminist Visions*, no. 6, pp. 141–144.

Shaw, Susan, and Janet Lee. Women's Voices Feminist Visions. 6th ed., McGraw-Hill Education, 2015.

Swank, Eric, Breanne Fahs, and Holly N. Haywood. "Evaluating Appalachian Distinctiveness for Gender Expectations, Sexual Violence, and Rape Myths." *Journal of Appalachian Studies,* vol. 17, no. ½, (2011): 123-43. *JSTOR*. Web. 22 Apr. 2017.

Cytokine Production Profile in CD4+ T Cells of Stressed Mice during *Chlamydia muridarum* Genital Infection

Gezelle Brown

McNair Final Paper

Bluefield State College

Abstract:

Cold-induced stress is known to influence the dynamics of cytokine production during chlamydia genital tract infection, but the balance between Th1 and Th2 cytokines of CD4+ T cells or CD+8 T cells in stressed and chlamydia-infected mice is unknown. We hypothesized that cold-induced stress results in elevated gene expression and production of IL-4 but decreased production of interferon gamma by CD4+ T cells or increased production of TNF-a by CD8+ T cells. CD4+ and CD8+ T cells for *in vivo* proliferation and RNA isolation were purified from total T cells of the genital tract using magnet CD4+ T or CD+8 cell enrichment protocols. Quantitative PCR and ELISA methods were used to determine gene expression in T cells and production of cytokines in culture supernatants of T cells. Culture supernatants of stressed mice showed a decreased IFN-g and increased IL-4 production. Furthermore, elevated and reduced gene expression of GATA-3 and T-bet transcription factors respectively was obtained in CD4+ and CD8+ T cells of stressed mice. Increased production of TNF-a in CD8+ T cells of stressed and infected mice was observed. The production of low Th1-specific cytokine, IFN-g and high Th2-specific cytokine, IL-4 reflects the correlation between the expression of T-bet and GATA-3 in stressed mice. The results suggest that stress promotes a Th2 dominance over Th1 by the overexpression of GATA-3 and a less expression of T-bet. Furthermore, increased gene expression and production of proinflammatory of TNF-α by CD8 + T cells may promote pathology during chlamydia infection in mice under stressful conditions.

Introduction:

Chlamydia trachomatis is the most common bacterial sexually transmitted disease worldwide.[1] *Chlamydia trachomatis* is an intracellular pathogen that invades the epithelial cells of reproductive sex organs of men and women.[1,2] The spread of chlamydia genital infection by sexual activity.[1]

According to CDC reports, the prevalence of chlamydial infection is expressed in higher proportions among younger women. To increase the proper regiment of care during chlamydial infection its recommended than women who are sexually active should receive annual screenings testing for chlamydia trachomatis and other sexual transmitted disease.[1] Chlamydial cases of women ages 15-19 has increased drastically by 10.7% from the years of 2014-2017. [1]The reported cases in men ages 15-19 increased by 28% during 2014—2017. Reported cases of women aged 20-24 years old increased similarly to 10.9% from the year 2013-2017.[1] Chlamydial cases in men ages 20-24 years old increased by 30.1% from 2013-2017.[1] Awareness and screenings of chlamydia infections has increased greatly, but many women are still not diagnosed.[1] Complications of not receiving adequate healthcare in

chlamydial infections being unrecognizable in asymptomatic in women.[1] Chlamydial infections are 80% asymptomatic in women. The high rates of asymptomatic infections in women is a determine factor for the increase in prevalence of the disease over the years.[1] Long lasting of infections in women can lead to infertility, pelvic inflammatory disease, and susceptible to many other diseases. [1,2]

The disease essentially can affect any sociodemographic group, but low economic classes experiences higher prevalence of the disease.[1,3] African Americans are infected at higher percentages than any other race. Living in impoverished and stressful neighborhoods,[1,3] inadequate healthcare and educations, and insufficient resources are factors that can trigger stress in the population.[1,2,3] Stress can be a contributing factor to high incidence of Chlamydia trachomatis in African American populations.[1,3] Chlamydia genital tract infections when diagnosed can be treated with antibiotics, but a vaccine against the

obligate intracellular parasite is not available.[3,5] A vaccine for chlamydia would prevent the reinfections or infections of the disease worldwide.[5]

Chlamydia muridarum

In this study, *Chlamydia muridarum*, the mouse strain is being used because of the similarity of the disease to the human genital tract infection.[6] The usefulness of *C. muridarum* as model is its pathogenicity and immune response in mouse resembles pathogenesis and adaptive immune response of chlamydia genital tract infection in humans.[6] In contrast the *Chlamydia muridarum* infection in mice are much aggressive than then *Chlamydia trachomatits* infection mice. C*. muridarum* Infection is at its highest during days 3-7 producing between 10^6 to 10^7 IFU (inclusion forming unit) from the vagina.[11] Compared to C.*trachomatis* which produces 10^3 to 10^4 IFU from the vagina.[11] C. *muridarum* Infection travels to upper genital tract causing complications of hysosaplinx and infertility.[12] In experiment conducted by Chunfu Yang and colleagues vaginal tissue examined during the C.*muridarm* shows inflammation while the C.t*rachomatis* vaginal tissue shows none.[11]The C.*muridarm* and C.*trachomatis* infection both produced cell mediated immunity generated by T-cells. Important significance of the study is that a C.*muridarum* infection in mice are highly immune to reinfection but C.*trachmatis* expressed insufficient immunity.[11]

Cytokines and Transcription factors:

Cytokines are cell signaling proteins involved in many immune responses in the body.[13,14] CD4 and CD8 are two subsets of T lymphocytes that are involved in during protection or immunopathogenesis of chlamydia. [13,14,15]T helpers cells are known as CD4 T cells, which subdivided into to two groups TH1 and Th2 cytokines.[14,15] Cytokines are specific to types of immune response that are triggered by the response of intracellular pathogens.[15,16,17] Th1 cytokines produce IFN)-gamma, interleukin (IL)-2 and tumor necrosis factor (TNF)-beta.[16] Th2 responds to cell responses of extracellular parasite.[16,17] Th2 cytokines are IL-4, IL-

5, IL-6, IL-9, IL-10, and IL-13 are involved in promoting immune Reponses for atopic and allergic reactions. [16,17]

Specifically, in the experiment Th1 cytokines interferon gamma, and Th2 cytokines 1L-4 and IL-12 are trying to be understood because cellular cytotoxicity and cytokine-mediated functions because their involvement in CD4+ T-cells response in a host chlamydial infection.

Many signals can contribute to how activated T-cells differentiate.[13] The environment of the cytokines has been proposed to be a regulator in cells. Naïve T-cells in presence of IL-12 activate Th1 cells, whereas IL-4 will stimulate Th2 differentiation.[16,17] Transcription factors GATA-3 and T-Bet are regulators of t-helper differentiation.[16,17] T-Bet blocks Th2 cytokine activation, while Th1 is activated.[16,17] GATA-3 transcription factors block Th1 cytokines from differentiating while Th2 is activated. [16,17]

Significance of the study

The prevalence of chlamydia genital infection is at a high rate in minorities of low socioeconomic population, but the reasons are not known.[1] Stress and other underlying factors could be a reasoning on why chlamydia is prevalence is high in the populations of minorities.[1,2] The effect of stress on chlamydia genital infection and immune response is not well known. [2]

Objective and hypothesis

Stress is suspected to weaken the resistance to genital infections and in this study chronic stress generated by cold water is used to determine the production of cytokines. The objective is to determine the dynamics of cytokines during chlamydia infection in the mouse model. We hypothesized that stress leads to increased production interleukin-4 (IL-4) and gene expression of transcription factor, GATA-3 and decreased production of interferon gamma (INF- γ) and gene expression of T-bet gene in with or without *C. muridarum* infection.

Mice:

Mice are inexpensive and easy to handle compared to other models. Six to seven-week female BALB/c mice from Hilltop Lab Animals, Inc. (Scottsdale, PA.) will be used for the study. Mice will be housed in the Bluefield State College (BSC) vivarium. Bluefield State College Institutional Animal Care and Use Committee permits the procedures and protocols for the mice. Mice were checked daily as well.

Stressing Protocol:

Upon arrival, mice are allowed seven days to acclimate the new conditions. Mice will be stressed for five minutes daily in (4-5 ^{O}C) cold water for 21 days. Each water container is marked to ensure that water will cover the backs of the mice. Mice are closely watched to ensure their safety.[3] To prevent hypothermia mice are dried off with towels immediately after removal from the cold-water bath.[3] Mice are then placed back into the proper cages and back into normal temperatures. They are kept in normal temperatures and do not receive the stress protocol. The non-stressed mice serve as the control during the experiment.

C*hlamydia muridarum* inoculation:

On the 17th day of the stressing period non-stressed and stressed mice were injected subcutaneously with 2.5mg/mouse of progesterone in 100ul of phosphate buffered saline. Progesterone is used regulate the menstrual of the mice. A week later, preceding the stressing period, mice were infected with chlamydia muridarum intravaginally. Using a dull pipette mouse were infected intravaginally with 10^5 IFU of *C. muridarum* in 30 uL of PBS. The anesthetics Ketamine-Xylazine were used to induce anesthesia. Mice were put under anesthesia to make sure the mice did not feel any pain or uncomforted.

T cell isolation from spleen and genital tract:

After 48 h of infection, spleens and genital tracts from mice were removed aseptically and kept in complete medium of RPMI 1640. Spleen cells were minced, teased, and pressed through a 70 mm size. Mouse CD4+ T and CD8+ cells were isolated using EasySep negative selection kit from Stem cell Technologies following the manufacturer's instructions. Cell were counted and seeded in wells at 1 x 10^6 cells/ml for proliferation at 37^OC supplemented with CO2.

Enzyme Linked Immunosorbent Assay (ELISA): Elisa kits purchased from Invitrogen (Grand Island, NY) were used to determine the levels of specific cytokines in the culture supernatants in reference to standard with known concentrations. Procedure follows the manufacturer's instruction.

RNA isolation: Total RNA was isolated using a FastRNA Pro Green Kit and a fastprep instrument purchased from MP Biomedicals (Solon, OH). These kits and instruments were utilized following the manufacture's instructions.

Synthesis of cDNA and PCR Analysis: Theme Scientific Nanodrop (Thermofisher) instrument was used to measure the isolated RNA. A 5x iScript cDNA Synthesis Kit will be purchased from Bio-Rad (Hercules, CA) using manufacturer's instructions. Using the cDNA RT-PCR test will be performed using a 2x iTaq SYBR Purchasing of Green Supermix from Bio-Rad following the manufacturer's instructions. iScript is a revising Moloney murine leukemia virus reverse transcriptase that optimized for reliable cDNA synthesis over a wide dynamic range of input RNA. To obtain complementary DNA, and cDNA one microgram of RNA is added. iScript cDNA Synthesis Kit guides an two-step process to reverse transcription.

Results:

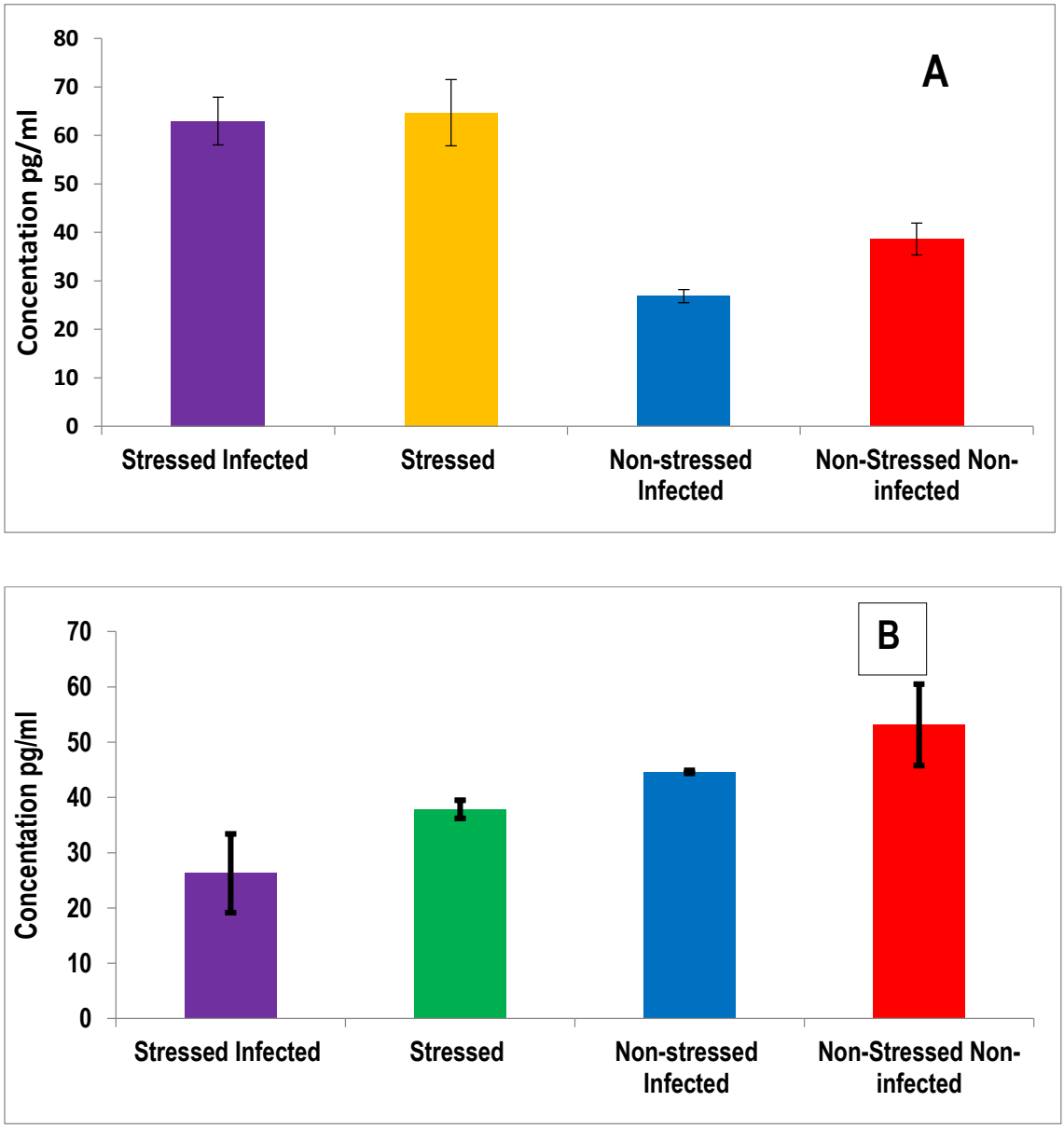


Figure1: Productions of IL-4 (A) and (INF-g (B) in T-cells isolated from the genital tract of stressed or non-stressed mice infected with *Chlamydia muridarum*

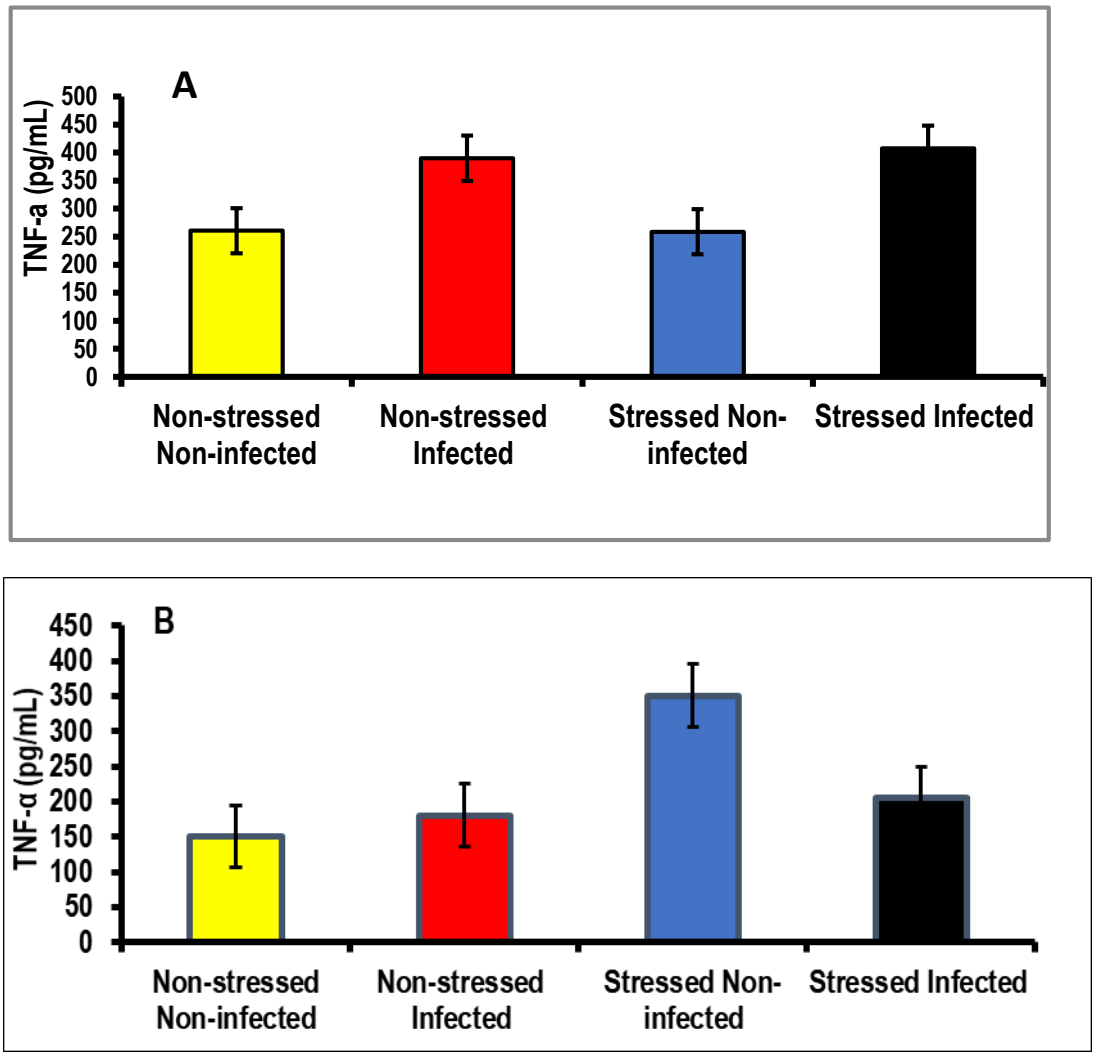
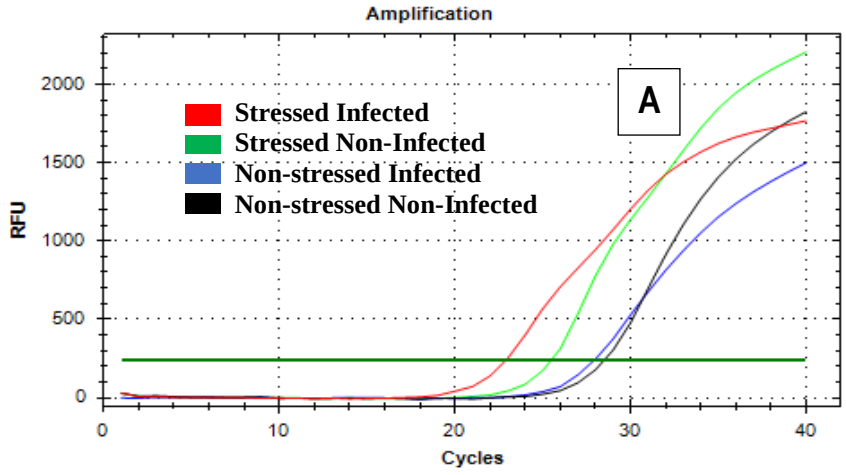
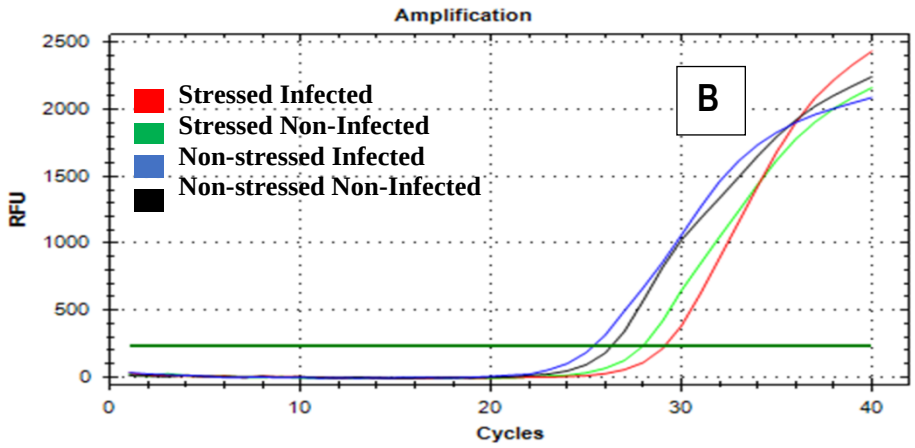
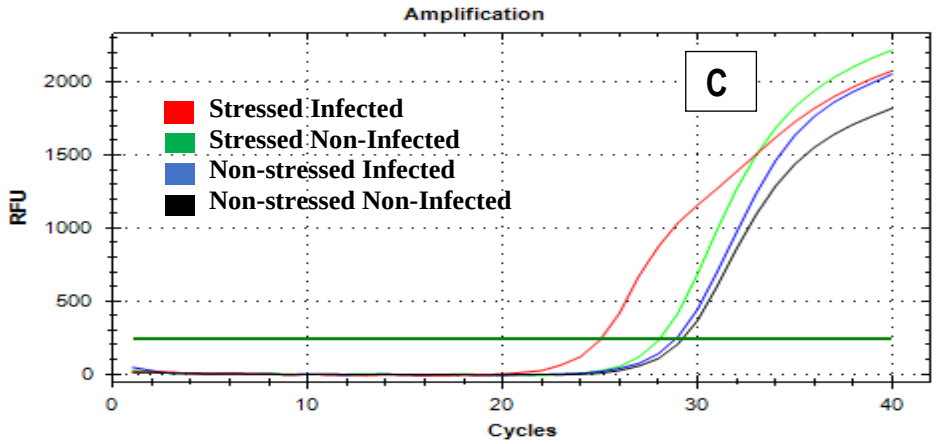


Figure 2: Production of Tumor Necrosis Factor-a by CD8+ T cells of the genital tract during 24 (**A**) and 48 (**B**) hours of proliferation




Amplification
B
Stressed Infected
Stressed Non-Infected
Non-stressed Infected
Non-stressed Non-Infected
RFU
Cycles
0
500
1000
1500
2000
2500
0
10
20
30
40


Amplification
C
Stressed Infected
Stressed Non-Infected
Non-stressed Infected
Non-stressed Non-Infected
RFU
Cycles
0
500
1000
1500
2000
0
10
20
30
40

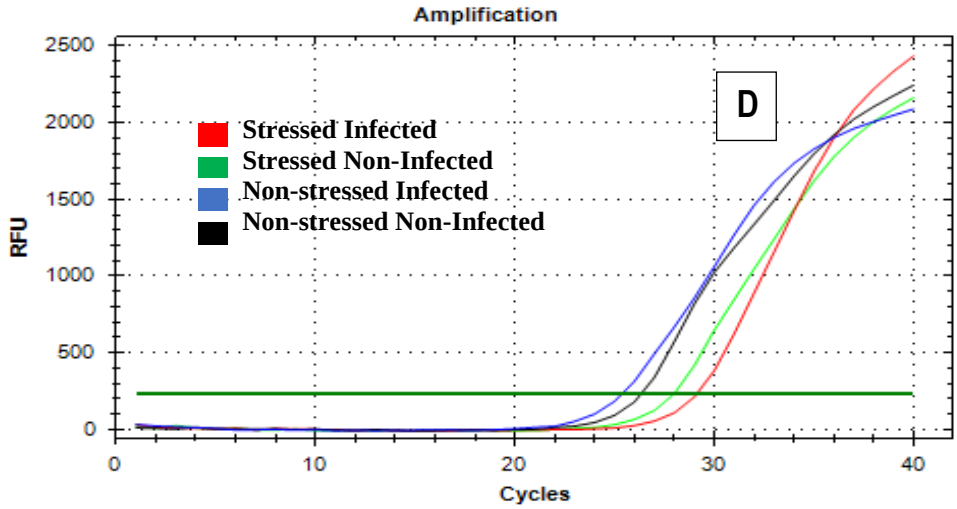

Amplification
D
Stressed Infected
Stressed Non-Infected
Non-stressed Infected
Non-stressed Non-Infected
RFU
Cycles
0
500
1000
1500
2000
2500
0
10
20
30
40

Figure 3. Gene expression profiles of GATA -3 (**A**), T-bet (**B**), IL-4 (**C**), Interferon-gamma (**D**) in T cells of stressed and non-stressed mice infected with *Chlamydia muridarum.*

Discussion and Conclusion:

Cytokine production profiles were analyzed using the ELISA. Figure 1 shows the protein secretion IL-4 and IFN-y in genital tract. According to the results stressed infected mice compared to the non-stressed non-infected (control group) had a higher production of IL-4. Also they analysis of non-stressed infected mice showed the production of IFN-y ,which is need for the clearance of chlamydial infections. Figure 2 shows the production of the Tumor Necrosis Factor-alpha (TNF-α) by genital tract derived CD8+ T cells during 24 and 48 hours of proliferation. Elisa was used to measure protein secretion. **Figure 2** Graph **(A) of** (TNF-α) in 24 hours we obtained that the stressed infected mice had the highest levels of of production, while the other groups of mice had the same production. **Figure 2 (B)** Production of (TFN-α) was expressed higher in the stressed noninfected mice, while stressed infected production decreased and non-stressed infected and non-stressed-infected decreased.

Gene expression profiles were analyzed using real-time polymerase chain reaction (PCR). **Figure 3** shows the gene expression of IL-4 IFN-y, T-Bet, and Gata-3. Graph (**A**) of gene expression of GATA-3 had high amplification in the stressed mice compared to the non-stressed non-infected mice. Graph (**B**) T-bet had decreased gene expression in the non-stressed infected mice. Graph (**C**) Gene expression of had high amplification in the stressed mice compared to the non-stressed mice. (**D**) Amplification of IFN-y had higher amplification in non-stressed non-infected mice.

Summary and Conclusions:

The ELISA and PCR results of IL-4, IFN- y ,GATA-3 and T-bet supports the hypothesis that cold-stress modulates immune response during a chlamydial genital infection. This is evidence supported by the

fact that increased production IL-4 is associated with increased gene expression of transcription factor, GATA-3. The results further suggest that possibly switching of Th1 to Th2 immune response in the cold-induced stress mouse model. It also suggested the up-regulation of Th2 cytokines and decreased production of interferon gamma (INF- γ) and gene expression of T-bet gene in with or without *C. muridarum* infection. The gene expression CD4+T cells of the genital tract showed high production of IL-4 and Gata-3 while a decrease in IFN-y and T-bet. We concluded from the experiment that stress is modulating the production of Th1 cytokines which is needed for chlamydial clearance to Th2 cytokine production. The analysis of the CD8+ T cell production of (TFN-α) showed increased gene expression of the production of proinflammatory cytokine (TFN-α). During the first 24 hours stressed infected produce higher production, but after 48 hours non-stressed infected had higher production. These results were not expected, and further research is needed to understand the decreased production (TFN-α) in stressed infected mice.

In conclusion the ELISA and PCR test showed strong correlation between the two. The results obtained from this study are comparable with previous stress-related experiments that have been conducted in the lab. Our results indicate that cold-induced stress may influence balance between Th1 and Th2 cytokines of stressed and infected mice, but further study is warranted.

Future Directions:

Use of a beta2-adrenergic receptor knock out mouse stress model to dissect to the regulation to CD4+ T cell subset differentiation and CD8+ cells. Our data shows in other experiments in the lab that Beta2-adrenergic is involved in the suppression of the immune system. By studying the mouse model that lacks the receptor, may give us insight about the regulation of the immune system during cold induced stress and chlamydial genital infection.

Acknowledgements:

This research work was supported by Concord University McNair Scholarship Program NIH Grant P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence (HSTA) NIH Grant P20GM103434 awarded to Bluefield State College and Dept of Applied Science and Mathematics at Bluefield State College

References

1. Chlamydia. CDC Center for Disease Control and Prevention. [Internet]. (MD): Center for Disease Control and Prevention. 24,July 2018; [cited November 21] www.cdc.goc/std/stats.
2. Anttila T, Saikku P, Koskela P, Bloigu A, Dillner J, Ikäheimo I, Jellum E, Lehtinen M, Lenner P, Hakulinen T, Närvänen A, Pukkala E, Thoresen S, Youngman L, Paavonen Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. JAMA. 3 Jan 2001; 285(1):47-51.
3. Tesfaye Belay, Anthony Woart, and Vincent Graffeo. Effect of cold water-induced stress on immune response, pathology and fertility in mice during Chlamydia muridarum genital infection Journals investing in science., 18 April 2017; 75, (5) 1-13.
4. Hackstadt, T. Cell biology, p. In R. S. Stephens (ed.), Chlamydia: intracellular biology, pathogenesis, and immunity. ASM Press, Washington, D.C Apr 2005; 73(4): 1939–1946
5. A new computer model for estimating the impact of vaccination protocols and its application to the study of Chlamydia trachomatis genital infections. de la Maza LM Vaccine. 1995 Jan; 13(1):119-
6. Thylefors, B., A. D. Negrel, R. Pararajasegaram, and K. Y. Dadzie. 1995. Global data on blindness. Bull. W. H. O. 73:115-121.
7. Anttila, T., P. Saikku, P. Koskela, A. Bloigu, J. Dillner, I. Ikaheimo, E. Jellum, M. Lehtinnen, P. Lenner, T. Hakulinen, A. Narvanen, E. Pukkala, S. Thoresen, L. Youngman, and J. Paavonen. 2001. Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. JAMA 285:47-51.

8. ↵Ohshige, K., S. Morio, S. Mizushima, K. Kitamura, K. Tajima, A. Suyama, S. Usuku, P. Tia, L. B. Hor, S. Heng, V. Saphonn, O. Tochikubo, and K. Soda. 2000. Behavioral and serological human immunodeficiency virus risk factors among female commercial sex workers in Cambodia. Int. J. Epidemiol. 29: 344-354.
9. Schachter, J. 1999. Infection and disease epidemiology, p. 139-169. In R. S. Stephens (ed.), Chlamydia: intracellular biology, pathogenesis, and immunity. ASM Press, Washington, D.C
10. Morrison RP, Feilzer K, and Tumas DB. Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in Chlamydia trachomatis genital tract infection. Infected Immunity. Dec 1995; 63(12):4661-8.
11. Chunfu Yang, William Whitmire, Gail Sturdevant, Kevin Bock, Ian Moore, and Harlan Caldwell. Infection of hysterectomized mice with *Chlamydia muridarm* and *Chlamydia trachomatis*. Infection and immunity. 2017, July; 85(7) e00197-17.
12. Shah AA, Schripsema JH, Imtiaz MT, Sigar IM, Kasimos J, Matos PG, Inouye S, Ramsey KH. 2005. Histopathologic changes related to fibrotic oviduct occlusion after genital tract infection of mice with Chlamydia muridarum. Sex Transmission Dis 32:49–56. doi:10.1097/01.olq.0000148299.14513.11
13. Habiba Chakir, Haiping Wang, David Lefebvre, John Webb, and Fraser W Scott. T-beta/Gata-3 ratio as measure of th1 and th2 cytokine profiles in mixed cell populations. Journal of Immunological Methods 278 (156-169)

14. Marilyn James. Th1 and Th2 response what are they. Science commentary 12, August 2000; 321() 1627-31
15. Jun-Ming Zhang, Jianxiong. Cytokines, Inflammation and Pain. Int Anesthesiol Clinic 2007; 45(2): 27–37.
16. Jeffery Perkel Distinguishing Th1 and Th2 Cells. The scientist 14 May 2001 https://www.the-scientist.com/technology-profile/distinguishing-th1-and-th2-cells-54709
17. Richard P. Morrison, Harlan D. Caldwell Immunity to murine chlamydial genital. Infection interferon and immunity. 11 Oct;70(6)2741-2751.
18. Habiba Chakir, Haiping Wang, David Lefebvre, John Webb, and Fraser W Scott. T-beta/Gata-3 ratio as measure of th1 and th2 cytokine profiles in mixed cell populations. Journal of Immunological Methods 278 (156-169)
19. Rank, R. G., K. H. Ramsey, E. A. Pack, and D. M. Williams. 1992. Effect of gamma interferon on resolution of murine chlamydial genital infection. Infected Immunity. 60:4427-4429
20. Byung Jun Kam, Harblan P. Jones. Epinephrine-primed murine bone marrow derived dendrite cells facillate production of IL-17A and IL-4 but not INN-y by CD4 t-cells. Brown behave immunity 24 Oct;24(7):1126-1136
21. Marcia G. Guerescni et al. Beta 2-adrenergic receptor signaling in CD4+ Foxp 3+ regulatory t-cells enhances their suppressive function in a PKA-dependent manner. European Journal of Immunology 2013;43 1001-1012

Climate-growth analysis of *Acer rubrum* and *Quercus palustris* in a Central Appalachian bottomland oak forest

John M. Cline

December 12, 2018

Geography, Department of Social Sciences, McNair Scholars Program
Concord University

Abstract

Bottomland oak forests provide ecosystem services such as nutrient exchange and pollution entrapment and generally improve aquatic and riparian habitats. These forests are declining due to human impacts (i.e., draining and development) and little is known about the development and resiliency of these plant communities. I used dendroecological methods to examine growth dynamics in canopy-dominant *Quercus palustris* and co-dominant *Acer rubrum* in a remnant forest patch on the floodplain of the Meadow River in West Virginia. I developed a 141-year (1876-2016) *Q. palustris* growth chronology using core samples extracted from 15 live trees and partial cross sections cut from 16 logs. I also developed an 85-year (1931-2016) *A. rubrum* growth chronology using core samples collected from 22 live trees. Temporal trends in summer moisture-growth relationships in both species indicate a shift in optimal growth conditions beginning in the late 1970s to early 1980s. Our results suggest that either increased summer moisture availability or changes in local hydrology have favored *A. rubrum* growth at the expense of *Q. palustris* at the study site.

Introduction

Bottomland hardwood forests

A bottomland hardwood forest, or riparian forest, is a plant community found in low lying areas of a watershed. In North America, these forests are primarily found in the Mississippi and Ohio River valleys. Huffman (1981) defines the size of bottomland hardwood forests ranging from broad alluvial plains to narrower areas that border headwater tributaries. These forests are the result of the hydrologic and geologic conditions of a surrounding drainage basin – processes of building up and losing soils through sedimentation. They are typically flat with slight differences in elevation and are inundated for most of the growing season (Naiman and Decamps 1971; Taylor 1990; Hosner 1963). It is a highly productive forest type found to contain more biomass than upland forests (Johnson and Bell 1976). The distribution of soil and tree species relies on the gradient of hydrologic conditions, which is subdivided starting near the stream [wettest] and transitions to the upland [driest] where moisture varies the most (Taylor 1990; Naiman and Decamps 1971). Biotic production and the quality of water downstream rely heavily on the hydrologic regime of bottomland oak forests as they have the unique ability to trap nonpoint-source pollution and exchange valuable nutrients during flooding (Naiman and Decamps 1971; Fischer et al. 2000). Within a landscape, bottomland oak forests act as the boundary between aquatic ecosystems and upland forests, connecting sections of landscape that are increasingly fragmented by human development (Fischer et al. 2000). Bottomland oak forests also function as wildlife corridors for terrestrial organisms, as well as plant seed dispersal, because they are linear and parallel to nearby streams (Fischer et al. 2000; Naiman and Decamps 1971).

Plant community composition (e.g., species diversity) and structure (e.g., tree density) in bottomland oak swamps are sensitive to changes in hydrology. These forests oscillate within a dynamic equilibrium annually, reacting to climatic variables and regime shifts (Sousa 1984). This balance is easily disrupted when the hydrology of the ecosystem has been altered by landscape disturbances such as flooding land use change (Stockton and Fritts 1973). The potential stability of riparian plant species relies on resilience to extreme climatic conditions (Naiman and Decamps 1971). Severe, infrequent events can greatly affect the resiliency of a system, influencing species composition and growth and causing the system to shift into an altered dynamic equilibrium (Beisner 2003; Taylor 1990; Naiman and Decamps 1971; Damasco-

Junior et al. 2004). Water stress is a push factor which can potentially force a system to shift toward a new set of parameters (Mistch et al. 1984). These parameter changes may either shift back to the original values based on the system's ability to return to normal functions (i.e., resiliency) or the system will continue to transition into various forms of stability (Holling 1973; Beisner 2003; Folke et al. 2004; Folke 2006). Nutrient rich water left behind from intense flooding acts as the pull factor, positively influencing growth for the following year (Mitsch 1984). The highly sensitive shifts and exchanges that occur in these ecosystems mark them as primary indicators of climatic change.

Climate-tree growth analyses

Dendrochronology [*dendro* - tree rings. *chronology* study of time] is a science and research method that studies the timing of events and rate of change on the landscape by observing tree growth morphology (Speer 2013). Tree growth is one of the most frequently used data proxy studied to assess past and present climate. Tree-rings record high-resolution climate data from the tree's highly sensitive feedback response. Climate regulates a tree's ability to photosynthesize and, therefore, the variability in ring-width typically correlates with variability in climatic drivers (Fritts 1965; Fritts et al. 1971). Using this method, I can assign an accurate calendar year to each ring and then assess relationships with instrumental climate data (Fritts 1971).

It is important for forest managers and climate scientists to understand the dynamic relationships between plant species and climate. Specific signals in tree rings allow for various types of reconstructions such as fire, flooding, and extreme weather events (Speer 2013). Tree rings also contain signifiers of ocean influences like the North Atlantic Oscillation, providing useful data for reconstructing past climatic change and allows scientists to model future climate (Saladyga et al. 2015). Networking these reconstructions across the landscape give us capabilities to determine current and future climate responses of forests (Speer 2013). However, in temperate forests, the climate-growth relationship can be difficult to assess due to widespread human induced landscape changes (Prentice 1992).

Climate-growth relationships rely on the basic geological concept of uniformitarianism, which assumes present-day relationships have been stable through time. However, trees may change their responses to climate as environmental factors change over time (Speer 2013). Recent research indicates that there is a shift in the forest to climate responses. D' Arrigo (2008)

provides a thorough review of literature regarding the reduction in tree growth response to temperature in high latitudes. The climate signal of northern forests has largely deteriorated with tree growth decreasing as temperature increases during the growing season (D' Arrigo 2008). It is theorized that the change in growth is attributed to direct temperature stress or drought stress from high temperatures (D' Arrigo et al. 2004; Barber et al. 2000). Growth responses appear to be deviating from precipitation as well (Driscoll et al. 2005: Lloyd 2017).

More recent research is shifting toward the deteriorating relationships between climate and tree-growth in the mid-latitudes. Globally, forest stands in the mid-latitudes offer a wide range of information regarding climatic changes, such as variations in growth responses to soil moisture, precipitation, and temperature. Climate is known to be a primary driver of forest composition and density. Though in temperate forests, competition is the most prevalent driver of radial growth, affecting the assessment of the relationship (Rubio-Cuadrado et al. 2018). Forests that are prone to drought are more sensitive to slight changes in climate (McCarthy 2001) and trees show high-sensitivity to local climate when compared to ecological changes within the stand in relation to the size of the dominant species (Mérian & Lebourgeois 2011).

In the Eastern deciduous forests of North America, a 'divergence' has been documented in various drought stressed forest stands. Trees have been responding differently to drought since the middle of the 20th Century, similar to temperature limited trees in the high latitudes (D' Arrigo 2008). In recent decades, it has been found that more trees are less sensitive to soil moisture, which may attribute to increased moisture availability. Tree growth is negatively impacted during extremely dry conditions; however, the linear relationship between soil moisture and growth appears to be deteriorating (Maxwell et al. 2016). Temperate forests are losing their ability to store carbon due to decreased water availability (Brzostek et al. 2014). This recent research indicates that more data needs to be collected in the mid-latitude Eastern Deciduous forests to elucidate these changes in climate-tree growth relationships.

Research Objectives

In this study, I assessed the growth dynamics of *A. rubrum* and *Q. palustris* within a remnant patch of bottomland oak forest in southern West Virginia. Specifically, my objectives were the following:

1) Develop annual ring-width chronologies for *A. rubrum* and *Q. palustris.*

2) Assess the strength of the climate-growth relationship during the common period for both species (1946-2016).

3) Assess the temporal stability of climate-growth relationships.

Methods

Study Site

The study site is located in the Meadow River Watershed near Crawley, West Virginia (**Figure 1**). The landscape is a low-lying basin in the ridge and valley ecoregion of the Appalachian Mountains, at 2,405 feet of elevation. The microtopography of the collection site ranges within four feet of the lowest point, from flat areas at equal elevation of the stream – which are inundated from early spring through mid-summer – to slightly elevated knolls. A drainage divides the landscape which slowly meanders into the Meadow River. Canopy dominant tree species include *Q. palustris* (pin oak), *A. rubrum* (red maple), *Acer saccharinum* (silver maple), and *Nyssa sylvatica* (black gum) –*Rhus radicans* (poison ivy) dominantly grows around *Q. palustris* trunks. *A. rubrum* is found mostly on the higher elevated areas and *Q. palustris* is found in the low-lying area of the study site. Other species in the forest include *Fraxinus americana* (white ash), *Fraxinus nigra* (black ash), and *Carya ovata* (shagbark hickory).

Crawley, West Virginia is situated in WV Climate Division 4, which is characterized as a humid continental climate with an average annual temperature of 9.8. The average January temperature is -2.0°C and the average July temperature is 20.4°C (NOAA 2018). Total annual precipitation is 124.4 cm. Precipitation peaks in July with an average total rainfall of 13.3 cm, while October is the driest month with an average total rainfall of 8.2 cm (NOAA 2018). Palmer Drought Severity Index (PDSI) calculates both positive and negative net surface soil moisture – integrating prior precipitation, moisture supply, runoff, and evapotranspiration (Palmer 1965). PDSI is autocorrelated so that previous precipitation affects current values which allows for a tightly fit correlation when compared at various timescales (Guttman 1998; Palmer 1965). Trends of divisional mean summer (JJA) soil moisture Palmer Drought Severity Index (PDSI) for the period of 1895-2016 indicate a sharp decrease in the region following the 1950s and has been increasing gradually after the 1970s (**Figure 2a**). Standardized Precipitation Evapotranspiration Index (SPEI) calculates precipitation and evapotranspiration, combined with the effects of temperature variability at different timescales. Involving temperature variability

allows SPEI to standardize local water deficits to be compared efficiently with PDSI (Vincent-Serrano et al. 2010). Gridded (SPEI) from 1905 to 2015 indicate generally the same trend as with a sharp decrease after the 1950s and has been increasing gradually since the 1970s (**Figure 2b**). Both of these drought indices are well known drought detectors measuring both positive and negative soil moisture across various spatiotemporal scales (Hayes et al. 1999; Vicent-Serrano 2010).

Field Methods

We established six 450 m^2 plots in a remnant patch of bottomland oak forest in March of 2016 for the purposes of assessing of forest dynamics. I extracted at least two cores from canopy dominant *Q. palustris* and co-dominant *A. rubrum* using an increment bore. I inserted the increment bore into the center of the stem at breast height, perpendicular to the slope of the terrain (Speer 2010). Twenty-two *A. rubrum* cores were collected from each tree above 10 cm in diameter at each plot. I targeted live and downed *Q. palustris* within and outside of each plot. A partial cross section was cut from 15 *Q. palustris* logs, while core samples were collected from sixteen live-standing *Q. palustris.*

Lab Methods

We dried, mounted and sanded each sample using progressively finer grit (300-600 grit) sandpaper so that the cells were clearly seen under a binocular microscope. I also sanded each cross section using the same grit sand-paper to see each cell clearly under the microscope (Speer 2013; Orvis and Grissino-Mayer 2002). I visually dated each sample using the skeleton plot technique, assuring accuracy with a reference chronology from a neighboring forest stand in Babcock, West Virginia (Saladyga 2017; Schweingruber et al. 1990; Yamaguchi 1991).

Tree-ring Chronologies

Digital images were captured using a high resolution 3200 (dpi) flatbed scanner. Annual rings were measured to the nearest 0.01 mm using image analysis software CoolRecorder 7.8 (Larsson, 2003). Each digital measurement was cross-dated again using CDendro 7.8 (Larsson 2003) for precision calendar years of each growth ring. A third cross-dating method is applied for accuracy and replication. COFECHA creates a master chronology using the raw ring-width and tests the relationship between each sample series providing an interseries correlation value. The interseries correlation value is the average correlation of each dated series when cross-dated with the other series (Speer 2013; Holmes 1983; Grissino-Mayer 2001). The raw-ring width

values were then processed using the software CRUST (Melvin and Briffa 2014). The raw ring-width measurements were standardized to 1.0 using a residual ring-width to reduce background noise from ecological events, so that growth was accurately analyzed with the climate variables (Melvin and Briffa 2014). I fitted a 30-year smoothing spline using the residual ring-width data to further reduce non-climatic background noise and to display the growth trends (Cook and Peters 1981).

Climate-growth analyses

We analyzed two indices to assure the result is not a function of the index's calculation. Each index was correlated to the annual growth of *Q. palustris* and *A. rubrum*. For our purposes of assessing the drought signal in *Q. palustris* and *A. rubrum,* I calculated the arithmetic mean of summer moisture months June, July, and August of both indices – PDSI and SPEI (Maxwell 2016). We calculated correlation and response function of annual growth of *Q. palustris* and *A. rubrum* monthly annual values of both drought indices individually from 1946-2016 using DendroClim2002 (Biondi and Waikul 2004). Previous growing season climatic values through current year growing season (pAug - Oct) were used in calculating static linear relationships between climate and drought. I used these months because (1) growth in the previous year can affect growth in the current year and (2) growth in this region continues until late October (Fritts et al. 1971). The correlations are based on univariate bootstrapped Pearson product moment coefficients (r). These values are then tested within the 95% confidence interval, to reduce the amount of erroneously significant values that may be a function how each index is calculated (Biondi and Waikul 2004). Previous and current year August-October was used to assess autocorrelation and to determine the months of the growing season that are significantly correlated with growth. In the program, calculated bootstrapped correlation coefficients from 1946-2016 for PDSI and from 1946-2015 for SPEI, the common period between each species' growth record. The months in the climate record that show the most significant relationship using the correlation values are analyzed in the moving interval analysis to display the relationship over time.

We assessed temporal trends in growth using DendroClim2002 (Biondi and Waikul, 2004). Dendroclim2002 processes 30-year moving interval analyses, correlating annual growth to each individual drought index, using 1000 bootstrapped samples. The temporal relationships were assessed within previous year growing season through current year growing season (pAug

– Oct) – correlations are based on Pearson (r) product moment coefficients (Maxwell 2016; Biondi and Waikul 2004). The moving interval shifts forward one year for each calculated value beginning in 1931-1961 as the beginning common period in each growth record. Each data point is an average of the previous 29 annual correlation values (Biondi & Waikul, 2004; Fritts, 1971). A shift is present in the relationship of summer moisture when individual months are tested. In this region, peak growing season is known as summer months June, July, and August (JJA), and, thus, drought index values corresponding to this annual period would provide an adequate assessment of the climate-growth relationship at this site (Maxwell 2016). I averaged PDSI and SPEI for JJA annually and ran the bootstrapped 30-year moving interval analysis against growth for each species. Then, I subtracted *Q. palustris* index correlation coefficients from *A. rubrum* correlation coefficients. Finally, I averaged the correlation coefficients of growth for each drought index to visualize the temporal transition in optimal growth conditions for each species.

Results

Ring-width chronologies

The *A. rubrum* chronology was developed from 22 live-standing trees. *A. rubrum* sample depth diminishes to 6 trees in 1950. Notable periods of below normal growth for *A. rubrum* are: 1950's 1970's and the early 2000's. *A. rubrum* raw values provided an interseries correlation of 0.489 [p = 0.001] (**Figure 3a**). Visually comparing timeseries of the calculated arithmetic mean of annual JJA PDSI and SPEI to annual growth of *A. rubrum* provided general similarities of trends in growth responses to moisture. Notable periods of abnormal dryness, as observed using both index calculations are the early 1930's, 1960's, and 1990's. Periods of below normal growth of *A. rubrum* are the 1960s, 1970s, and the early 2000s. Significant marker rings in a large percent of samples were found in the 1930s, late 1970s, and early 2000s. The 1960s is a period of significantly low soil moisture according to both indices. This period corresponds to significantly narrow marker rings ubiquitous among a large percent of sample in both species, however, *A. rubrum* responds positively to this period of negative soil moisture.

The *Q. palustris* chronology was developed from 16 cross-sections and 15 core samples. Sample size for *Q. palustris* extends to 1876 with sample size diminishing to 10 by 1900. *Q. palustris* raw values provided an inter series correlation of 0.571 (**Figure 3b**). Periods of below normal growth of *Q. palustris* are: 1910's, 1960's and early 2000's. Significant marker rings in a large percent of samples were found in the 1930s, 1960s, 1970s, and 1990s. Periods of below

normal moisture shown in the mid-1970s, indicated in both indices, corresponds to narrow annual growth-rings in the 1970s, ubiquitous among samples in both *Q. palustris* and *A. rubrum*. In contrast to *A. rubrum, Q. palustris* responds negatively to a period of dryness in the 1960s.

Climate-growth relationships

Bootstrap correlations of annual *A. rubrum* growth and monthly PDSI show that previous year November has a positive significant [p > .05] relationship (**Figure 4a**). This indicates that positive previous year soil moisture influences current year growth, as moisture is calculated by PDSI. When *A. rubrum* growth is correlated with monthly SPEI values, growth has a negative response to negative previous year December soil moisture (**Figure 4b**). When annual *Q. palustris* growth is bootstrapped correlated to monthly PDSI, there is not a significant relationship (**Figure 5a**). Annual growth responds positively to positive current-year August soil moisture, as calculated by SPEI. Negative current-year October SPEI impacts annual growth negatively (**Figure 5b**).

Temporal trends in the summer drought signal

When *A. rubrum* annual growth is correlated to annual June, July, and August PDSI, there is a significantly positive relationship that peaks in the 1970s with a sharp decline into the 1980's – followed by a gradual increase in the 1990s. PDSI summer months (JJA) follow a tightly fit orthogonal pattern (**Figure 6a**). SPEI *A. rubrum* growth responses follow generally the same trend as PDSI. The growth response to June SPEI declines in the early 1980s and does not recover until the early 2000's. July falls in the late 1970s and hovers a zero-relationship value to the early 2000s. While, August SPEI-growth response, is a zero-relationship in the late 1970s and increases dramatically in the mid-1980s (**Figure 6b**). The growth response generally recovers mid-1970s and is either positive or significantly positive from 1970 to present. Over time, the relationship between annual *A. rubrum* growth and June, July, and August SPEI is unstable.

When *Q. palustris* annual growth is correlated to annual June, July, August PDSI the relationship is unstable over time. There is a significant positive relationship from 1925 then declines to 1955. The growth response recovers gradually after the 1955 decline and fluctuates to a peak in the 1990s and an abrupt decline in the late 1990's preceding a subsequent disruption of the response to present (**Figure 6c**). The *Q. palustris* response to SPEI shows generally the same trend but is less tightly fitted and responds in various patters within each month (**Figure 6d**).

From 1930 to 1946, positively. Then the relationship to each month SPEI decouples within the 1960s. June begins to decline in 1975, while July and August decline much earlier, the mid-1960s. The responses to each SPEI in each month decline to either negative or significantly negative from the 1970s to present. Though, over time this relationship is unstable.

Difference in correlation coefficients

We averaged JJA drought indices and correlated those means with annual growth of both species using the 30-year moving interval analysis (**Figure 7a**). As shown in Figure 7a, *A. rubrum* has a negative relationship with each index from 1960 to 1980, and gradually responds positively as time progress. The opposite is seen with *Q. palustris'* response to each drought index. From 1960 to 1980, *Q. palustris* is responding posivitely to each index and gradually reduces to a zero-relationship, as *A. rubrum* response increases over time. Because this is a total shift in positive and negative coefficients, I subtracted *Q. palustris* correlation coefficients from *A. rubrum* correlation coefficients (**Figure 7b**). This resulted in a total interchange of response coefficients beginning in the late 1970's to the early 1980's. *A. rubrum* below zero coefficients shift gradually to above zero coefficients and become positive as time progresses into the present.

Discussion

Bootstrap correlations revealed insignificant relationships with most monthly mean annual drought index values. The 30-year moving interval analysis revealed the climate-growth relationship between *A. rubrum* and *Q. palustris* has weakened over time when calculated with both drought indices. PDSI shows a significant positive temporal relationship with both species but weakens as time progresses. The PDSI analysis also appears to be more tightly fit timeseries within each month, this is a function of the autoregressive nature of the PDSI formula – making it the most appropriate to assess a drought signal (**Figure 6a-6c**) (Guttman 1998). The annual growth response of both species to PDSI appear to be inverse of each other. In the common period, *A. rubrum* growth responses are negative at the beginning of the record, from 1940 through the 1970s, while *Q. palustris* is responding positively to positive measures of soil moisture. Annual growth of both species show the same general trends when correlated with SPEI, with orthogonal differences are shown as the individual months are acting independently (**Figure 6b-6d**). Likewise, growth responses to SPEI measures are inversed between each species. *A. rubrum* growth is negative from 1940 to the 1970s, as growth responses of *Q.*

palustris begin to decline. August SPEI appears to be the only individual month that shows a different trend. August SPEI, as indicated in the static bootstrapped correlation, positively impacts growth in both species, in the same trend over the time period – I see this on the landscape as trees in this region are moisture limited later in the summer when precipitation is lower (**Figure 6b-6d**). The trend in the growth response, present among both species in each index, up to present indicates that all trees could potentially desiccate within the decade (**Figure 6**).

Overall, *Q. palustris* growth trends began declining in the 1970s, while *A. rubrum* growth ascend in the same time frame. This may be a result of increased precipitation and surface soil moisture, which has been increasing since the 1970s – allowing for *A. rubrum* to out compete. Generally, optimal growth conditions each species have traversed at the Crawley study site. As this trend has been occurring since the 1970s, there appears to be an abrupt deterioration in growth responses of *Q. palustris* to PDSI in the late 1990s (**Figure 6c**). Regionally, streamflow was at an high on average as a result of a snow storm in January of 1996 – leading to more moisture on the surface following increased temperatures later in that month – and a flood in May of the same year. Event sequencing is detrimental to ecosystem hysteresis (Beisner 2004; Holling 1973; Folke et al. 2004). In this case, dramatically increased moisture on the surface late in the dormant season created an environment in which the dominant species could not uptake the excess water when the growing season began. Then, sequentially, a flood occurred in the region altering the topography and surface hydrology of the area. Pockets of water developed with change in surface soil from flooding, as well as fallen *Q. palustris* from an earlier event. *Q. palustris* is a species that thrives in higher levels of inundation (Minckler and McDermott 1960). However, *Q. palustris* growth and regeneration tends to decline as duration of flooding and level of moisture increases (Minckler and McDermott 1960). When the canopy opened, shade intolerant species such as *A. rubrum* established with little competition from *Q. palustris* which continued to decline with more moisture. *Q. palustris* recruitment decreased as stand density grew in favor of *A. rubrum* (Kabrick et al. 2005).

An of increase regional moisture leading to a decrease of xeric species and establishment of shade intolerant mesic species is known as "mesophication" (Nowacki and Abrams 2008). A shift from oak- dominated forest stands to mesophytic species dominance has been occurring throughout the Eastern deciduous forest since the 1950s (Nowacki and Abrams 2008).

Mesophication has been observed primarily on upland sites which lead us to attempt to evaluate dynamics and assess the climate-growth relationship of this bottomland site. Mesophication can potentially redistribute nitrogen deposition from altering precipitation regimes across Eastern US (Alexander and Arthur 2010). Our findings suggest that mesophytic species could out-compete even mesic oak species in bottomland riparian sites as a potential positive feed-back of on-going mesophication in upland forest stands.

Conclusions

Bottomland oak forests are a dwindling feature on the landscape throughout West Virginia due to human development. These forests are sensitive to climate and land-use changes due to their immediacy to neighboring stream. In this study, I have demonstrated that divisional drought indices have a significant relationship with annual growth of *A. rubrum* and *Q. palustris* in a Central Appalachian bottomland oak forest. Our results indicate a shift in optima growth conditions at the site in favor of shade intolerant mesophytic species at the expense of the canopy dominant *Q. palustris*. The deterioration of the response in each species within the most recent 30-year period indicates that both species are dying. The increase of soil moisture at the Crawley site can be attributed to regional land-use changes as well as the upward shift in regional precipitation since the 1970s. The site should be observed into the future to forecast forest structure and specie dynamics within these climatic parameters. Finally, more data should be collected from other bottomland forests located in West Virginia to further elucidate temporal dynamics of these highly sensitive ecosystems.

References

Alexander, H. D., & Arthur, M. A. (2010). Implications of a predicted shift from upland oaks to A. rubrumon forest hydrology and nutrient availability. *Canadian Journal of Forest Research*, 40(4), 716-726.

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., & Gonzalez, P. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest ecology and management*, 259(4), 660-684.

Barber, V. A., Juday, G. P., & Finney, B. P. (2000). Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. *Nature*, 405(6787), 668.

Beguería, S., Vicente, S., Latorre, B., & Reig, F. (n.d.). Global SPEI database. Retrieved from *http://spei.csic.es/database.html*

Beisner, B. E., Haydon, D. T., & Cuddington, K. (2003). Alternative stable states in ecology. *Frontiers in Ecology and the Environment*, 1(7), 376-382.

Bell, D. T., & Johnson, F. L. (1974). Flood-caused tree mortality around Illinois reservoirs. Transactions of the Illinois State Academy of Science.

Biondi, F., & Waikul, K. (2004). DENDROCLIM2002: a C++ program for statistical calibration of climate signals in tree-ring chronologies. *Computers & geosciences*, 30(3), 303-311.

Brzostek, E. R., Dragoni, D., Schmid, H. P., Rahman, A. F., Sims, D., Wayson, C. A., ... & Phillips, R. P. (2014). Chronic water stress reduces tree growth and the carbon sink of deciduous hardwood forests. *Global Change Biology*, 20(8), 2531-2539.

Burke, M. K., & Chambers, J. L. (2003). Root dynamics in bottomland hardwood forests of the Southeastern United States Coastal Plain. *Plant and Soil*, 250(1), 141-153.

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., ... & Jacobsen, A. L. (2012). Global convergence in the vulnerability of forests to drought. *Nature*, 491(7426), 752.

Cook, E. R., & Peters, K. (1981). The smoothing spline: a new approach to standardizing forest interior tree-ring width series for dendroclimatic studies.

D'Arrigo, R. D., Kaufmann, R. K., Davi, N., Jacoby, G. C., Laskowski, C., Myneni, R. B., & Cherubini, P. (2004). Thresholds for warming-induced growth decline at elevational tree line in the Yukon Territory, Canada. *Global Biogeochemical Cycles*, 18(3).

D'Arrigo, R., Wilson, R., Liepert, B., & Cherubini, P. (2008). On the 'divergence problem'in northern forests: a review of the tree-ring evidence and possible causes. *Global and Planetary Change*, 60(3-4), 289-305.

Damasceno-Junior, G. A., Semir, J., Santos, F. A. M. D., & Leitão-Filho, H. D. F. (2004). Tree mortality in a riparian forest at Rio Paraguai, Pantanal, Brazil, after an extreme flooding. *Acta Botanica Brasilica*, 18(4), 839-846.

Driscoll, W. W., Wiles, G. C., D'Arrigo, R. D., & Wilmking, M. (2005). Divergent tree growth response to recent climatic warming, Lake Clark National Park and Preserve, Alaska. *Geophysical Research Letters*, 32(20).

Fekedulegn, D., Hicks Jr, R. R., & Colbert, J. J. (2003). Influence of topographic aspect, precipitation and drought on radial growth of four major tree species in an Appalachian watershed. *Forest Ecology and Management*, 177(1-3), 409-425.

Fischer, R. A., & Fischenich, J. C. (2000). Design recommendations for riparian corridors and vegetated buffer strips (No. ERDC-TN-EMRRP-SR-24). Army engineer waterways experiment station Vicksburg, MS. *Engineer Research and Development Center*.

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., & Holling, C. S. (2004). Regime shifts, resilience, and biodiversity in ecosystem management. Annual Review of *Ecology, Evolution, and Systematics*, 35.

Folke, C. (2006). Resilience: The emergence of a perspective for social–ecological systems analyses. *Global environmental change*, 16(3), 253-267.

Fralish, J. S. (2003). The central hardwood forest: its boundaries and physiographic provinces.

Fritts, H. C. (1965). Tree-ring evidence for climatic changes in western North America.

Fritts, H. C., Blasing, T. J., Hayden, B. P., & Kutzbach, J. E. (1971). Multivariate techniques for specifying tree-growth and climate relationships and for reconstructing anomalies in paleoclimate. *Journal of applied meteorology*, 10(5), 845-864.

Grissino-Mayer, H. D. (2001). Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-ring Research*.

Guttman, N. B. (1998). Comparing the palmer drought index and the standardized precipitation index 1. *JAWRA Journal of the American Water Resources Association*, 34(1), 113-121.

Hayes, M. J., Svoboda, M. D., Wiihite, D. A., & Vanyarkho, O. V. (1999). Monitoring the 1996 drought using the standardized precipitation index. *Bulletin of the American meteorological society*, 80(3), 429-438.

Holling, C. S. (1973). Resilience and stability of ecological systems. A*nnual review of ecology and systematics*, 4(1), 1-23.

Holmes, R. L. (1983). Computer-assisted quality control in tree-ring dating and measurement. *Tree-ring Bulletin*.

Hosner, J. F., & Minckler, L. S. (1963). Bottomland Hardwood Forests of Southern Illinois--Regeneration and Succession. *Ecology*, 44(1), 29-41.

Huffman, R. T., & Forsythe, S. W. (1981). Bottomland hardwood forest communities and their relation to anaerobic soil conditions (pp. 187-196). J. R. Clark, & J. Benforado (Eds.). *Elsevier Science Publishing Co.*, Amsterdam.

Kabrick, J. M., Dey, D. C., Van Sambeek, J. W., Wallendorf, M., & Gold, M. A. (2005). Soil properties and growth of swamp white oak and Q. palustris on bedded soils in the lower Missouri River floodplain. *Forest Ecology and Management*, 204(2-3), 315-327.

Larsson, L. A. (2003). CooRecorder: image co-ordinate recording program. *Cybis Elektronik & Data AB*, Saltsjöbaden, Sweden.

Lorimer, C. G. (1980). Age structure and disturbance history of a southern Appalachian virgin forest. *Ecology*, 61(5), 1169-1184.

Lloyd, A. H., & Bunn, A. G. (2007). Responses of the circumpolar boreal forest to 20th century climate variability. *Environmental research letters*, 2(4), 045013.

Martin-Benito, D., & Pederson, N. (2015). Convergence in drought stress, but a divergence of climatic drivers across a latitudinal gradient in a temperate broadleaf forest. *Journal of Biogeography*, 42(5), 925-937.

Maxwell, J. T., Harley, G. L., & Robeson, S. M. (2016). On the declining relationship between tree growth and climate in the Midwest United States: the fading drought signal. *Climatic Change*, 138(1-2), 127-142.

McCarthy, J. (2001). Gap dynamics of forest trees: a review with particular attention to boreal forests. *Environmental reviews*, 9(1), 1-59.

McEwan, R. W., Dyer, J. M., & Pederson, N. (2011). Multiple interacting ecosystem drivers: toward an encompassing hypothesis of oak forest dynamics across eastern North America. *Ecography*, 34(2), 244-256.

McDonald, R. I., Peet, R. K., & Urban, D. L. (2002). Environmental correlates of oak decline and A. rubrum increase in the North Carolina Piedmont. *Castanea*, 84-95.

Melvin, T. M., & Briffa, K. R. (2014). CRUST: software for the implementation of regional chronology standardisation: part 1. Signal-free RCS. *Dendrochronologia*, 32(1), 7-20.

Mérian, P., & Lebourgeois, F. (2011). Size-mediated climate–growth relationships in temperate forests: a multi-species analysis. *Forest Ecology and Management*, 261(8), 1382-1391.

Mérian,, C. J., Orwig, D. A., & Abrams, M. D. (1994). Age structure and successional dynamics of a presettlement-origin chestnut oak forest in the Pennsylvania Piedmont. *Bulletin of the Torrey Botanical Club*, 13-23.

Minckler, L. S., & McDermott, R. E. (1960). Q. palustris acorn production and regeneration as affected by stand density, structure and flooding.

Miao, S., Zou, C. B., & Breshears, D. D. (2008). Vegetation responses to extreme hydrological events: sequence matters. *The American Naturalist*, 173(1), 113-118.

Mitsch, W. J., & Rust, W. G. (1984). Tree growth responses to flooding in a bottomland forest in northeastern Illinois. *Forest Science*, 30(2), 499-510.

Naiman, R. J., & Decamps, H. (1997). The ecology of interfaces: riparian zones. *Annual review of Ecology and Systematics*, 28(1), 621-658.

NOAA National Centers for Environmental information, Climate at a Glance: Global Time Series, published November 2018, retrieved on December 7, 2018 from *https://www.ncdc.noaa.gov/cag/*

Nowacki, G. J., & Abrams, M. D. (2008). The demise of fire and "mesophication" of forests in the eastern United States. *AIBS Bulletin*, 58(2), 123-138.

Orvis, K. H., & Grissino-Mayer, H. D. (2002). Standardizing the reporting of abrasive papers used to surface tree-ring samples.

Palmer, W. C. (1965). Moisture variability and drought severity. In Proc., Proceedings: *Thirteenth Annual Meeting of the Agricultural Research Institute*, October 12-13, 1964, Washington, DC.

Porter, T. J., & Pisaric, M. F. (2011). Temperature-growth divergence in white spruce forests of Old Crow Flats, Yukon Territory, and adjacent regions of northwestern North America. *Global Change Biology*, 17(11), 3418-3430.

Prentice, I. C. (1992). Climate change and long-term vegetation dynamics. *Plant succession: theory and prediction*, 293-339.

Rouvinen, S., Kuuluvainen, T., & Siitonen, J. (2002). Tree mortality in a Pinus sylvestris dominated boreal forest landscape in Vienansalo wilderness, eastern Fennoscandia. *Disturbance dynamics in boreal forests: Defining the ecological basis of restoration and management of biodiversity*.

Rubio-Cuadrado, Á., Camarero, J. J., del Río, M., Sánchez-González, M., Ruiz-Peinado, R., Bravo-Oviedo, A., ... & Montes, F. (2018). Long-term impacts of drought on growth and forest dynamics in a temperate beech-oak-birch forest. *Agricultural and Forest Meteorology*, 259, 48-59.

Ruzicka, K. J., Groninger, J. W., & Zaczek, J. J. (2010). Deer browsing, forest edge effects, and vegetation dynamics following bottomland forest restoration. *Restoration Ecology*, 18(5), 702-710.

Saladyga, T., & Maxwell, R. S. (2015). Temporal variability in climate response of eastern hemlock in the Central Appalachian Region. *Southeastern Geographer*, 55(2), 143-163.

Saladyga, T. (2017). Forest Disturbance History from 'Legacy'Pitch Pine (Pinus rigida) at the New River Gorge, West Virginia. *Natural Areas Journal*, 37(1), 49-57.

Schweingruber, F. H., Eckstein, D., Serre-Bachet, F., & Bräker, O. U. (1990). Identification, presentation and interpretation of event years and pointer years in dendrochronology. *Dendrochronologia*, 8, 9-38.

Speer, J. H. (2013). Fundamentals of tree-ring research. Tucson, AZ: *The University of Arizona Press.*

Sousa, W. P. (1984). The role of disturbance in natural communities. A*nnual review of ecology and systematics*, 15(1), 353-391.

Stockton, C. W., & Fritts, H. C. (1973). Long-term reconstruction of water level changes for Lake Athabasca by analysis of tree rings. *JAWRA Journal of the American Water Resources Association*, 9(5), 1006-1027.

Taylor, J. R., Cardamone, M. A., & Mitsch, W. J. (1990). Bottomland hardwood forests: their functions and values. *Ecological processes and cumulative impacts illustrated by bottomland hardwood wetland ecosystems*. Lewis Publ., Chelsea, MI, 13-86.

Vicente-Serrano, S. M., Beguería, S., & López-Moreno, J. I. (2010). A multiscalar drought index sensitive to global warming: the standardized precipitation evapotranspiration index. *Journal of climate*, 23(7), 1696-1718.

Warren, R. J., Rossell, I. M., & Moorhead, K. K. (2004). Colonization and establishment of A. rubrum (Acer rubrum) in a southern Appalachian wetland. *Wetlands*, 24(2), 364-374.

Yamaguchi, D. K. (1991). A simple method for cross-dating increment cores from living trees. *Canadian Journal of Forest Research*, 21(3), 414-416.

Young, P. J., Keeland, B. D., & Sharitz, R. R. (1995). Growth response of baldcypress [Taxodium distichum (L.) Rich.] to an altered hydrologic regime. *American Midland Naturalist*, 206

Tables and Figures

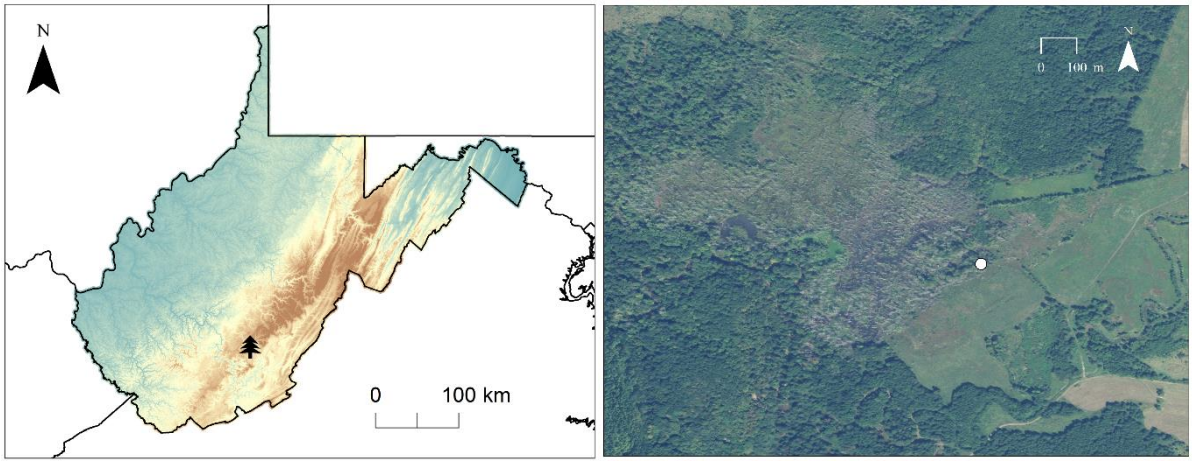


Figure 1. Study site in Southeastern West Virginia, located in Central Appalachia. Aerial view of the Crawley, West Virginia area study site.

Table 1. Cofecha output reporting accuracy of cross-dated raw ring-width measurements for *Q. palustris* and *A. rubrum*.

Species	No. series	Master-series	Total rings in all series	Total rings checked	Interseries. correlation	Avg. mean sensitivity	Flagged segments	Mean series length
Acer rubrum	40	85-years	2360	2358	0.489	0.321	2	59.0
Quercus palustris	61	141-years	5941	5940	0.571	0.164	14	97.0

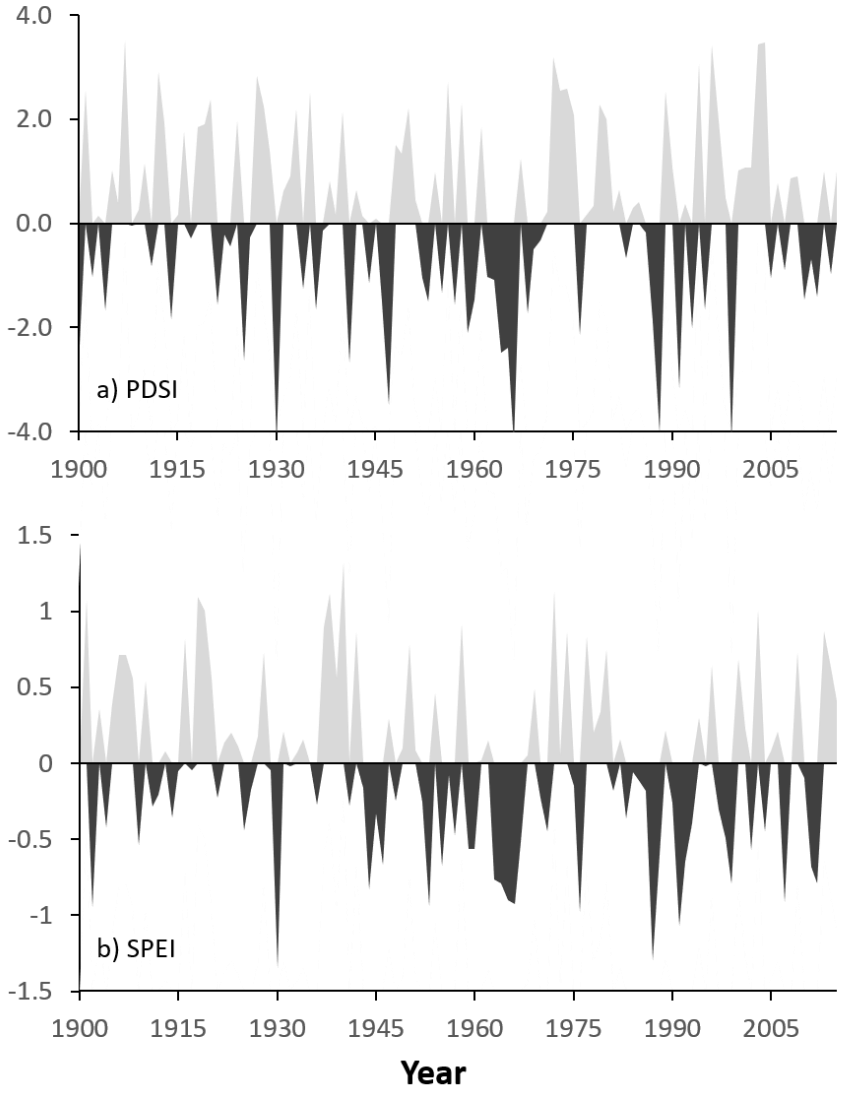


Figure 2. a) Palmer Drought Severity Index [PDSI] obtained from NCDC WV Climate Division 4 (NOAA 2017; Palmer 1965). **b**) Standardized Precipitation Evapotranspiration Index [SPEI] for coordinates 37.75 N, 80.75 W (Vincent-Serrano 2010).

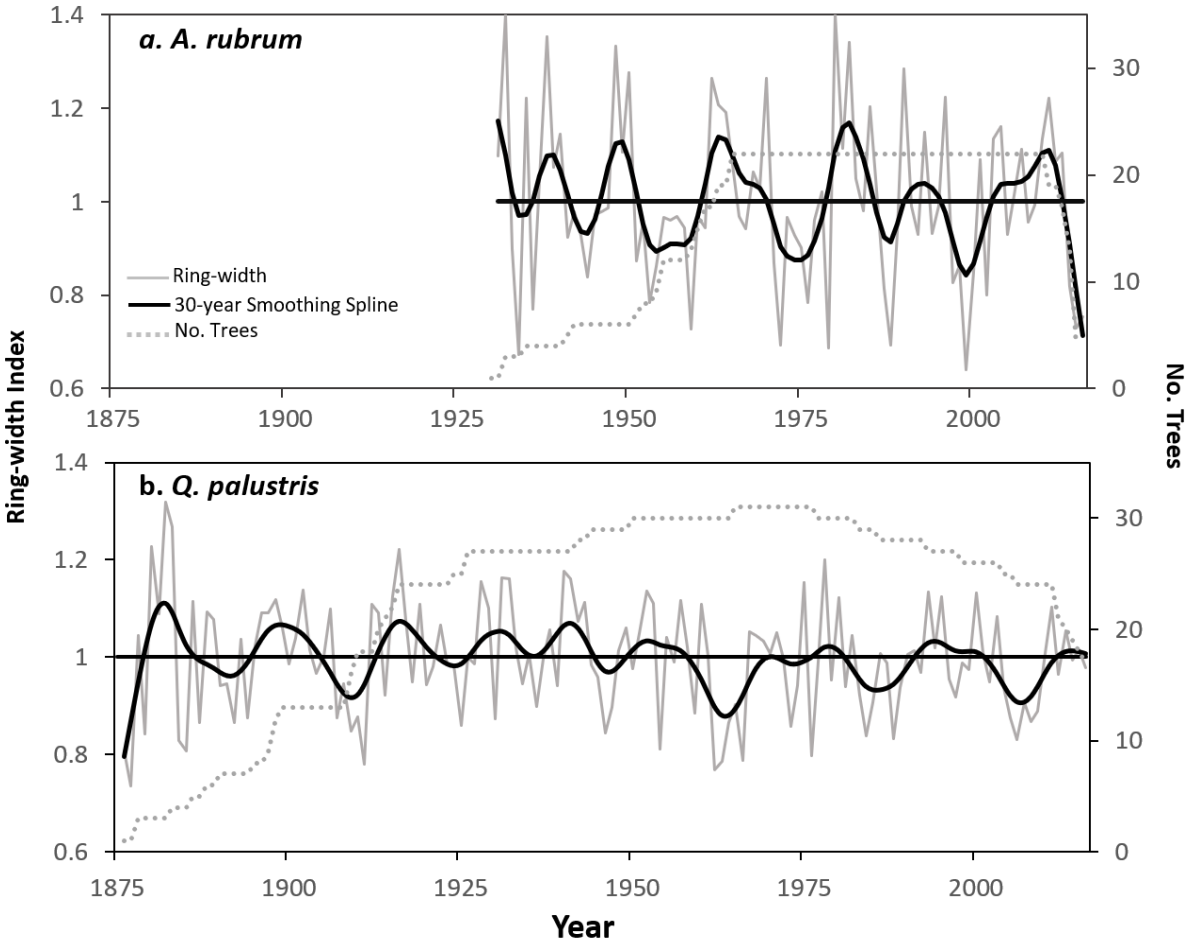


Figure 3. Residual (RES) ring-width indices for **a)** *A. rubrum* (1931-2016) with an interseries correlation of 0.489 (n = 22 trees/40 series) and **b)** *Q. palustris* (1876-2016) with an interseries correlation of 0.571 (n = 31 trees/60 series). Annual growth for both chronologies is standardized to 1.0.

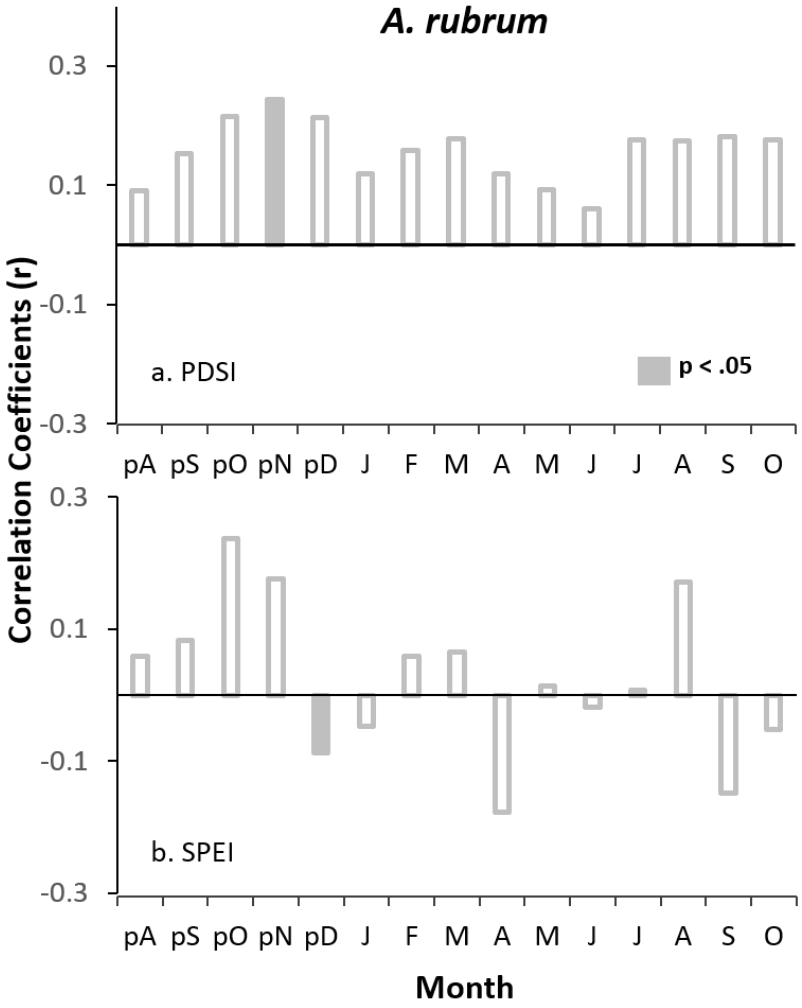


Figure 4. Bootstrapped correlation of previous growing season August through current growing season October Palmer Drought Severity Index (PDSI) and Standardized Precipitation Evapotranspiration Index (SPEI) calculated with annual growth of *A. rubrum* from **a)** 1946-2016 **b)** 1946-2015.

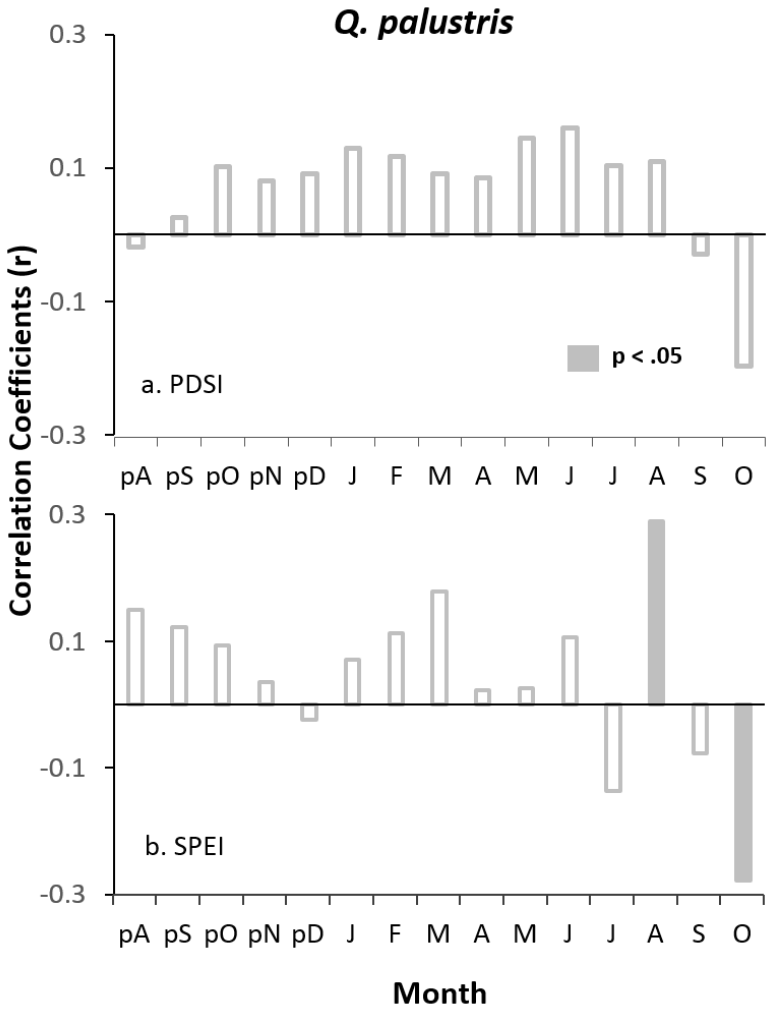


Figure 5. Bootstrapped correlation of previous growing season August through current growing season October Palmer Drought Severity Index (PDSI) and Standardized Precipitation Evapotranspiration Index (SPEI) calculated with annual growth of *Q. palustris* from **a)** 1946-2016 **b)** 1946-2015.

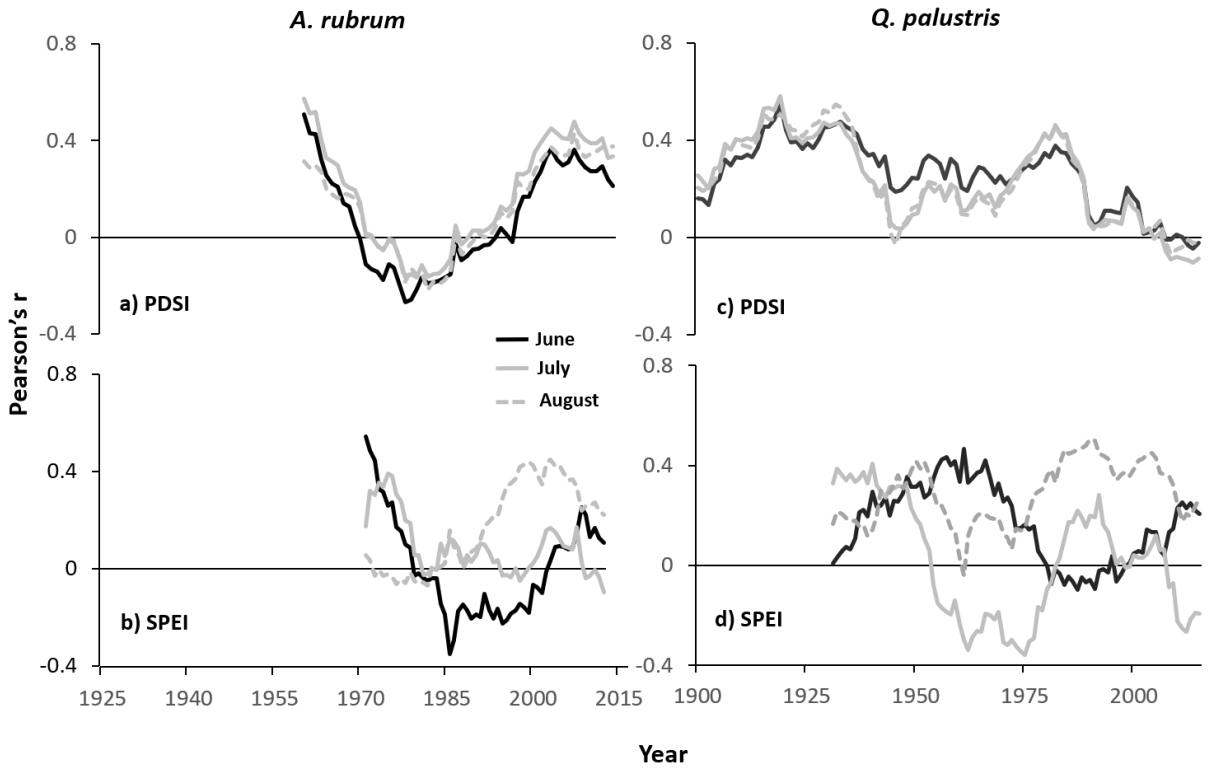


Figure 6. Bootstrapped 30-year moving interval analyses of *A. rubrum* and *Q. palustris* annual growth correlated to June, July, August Palmer Drought Severity Index (PDSI) and Standardized Precipitation Evapotranspiration Index (SPEI).

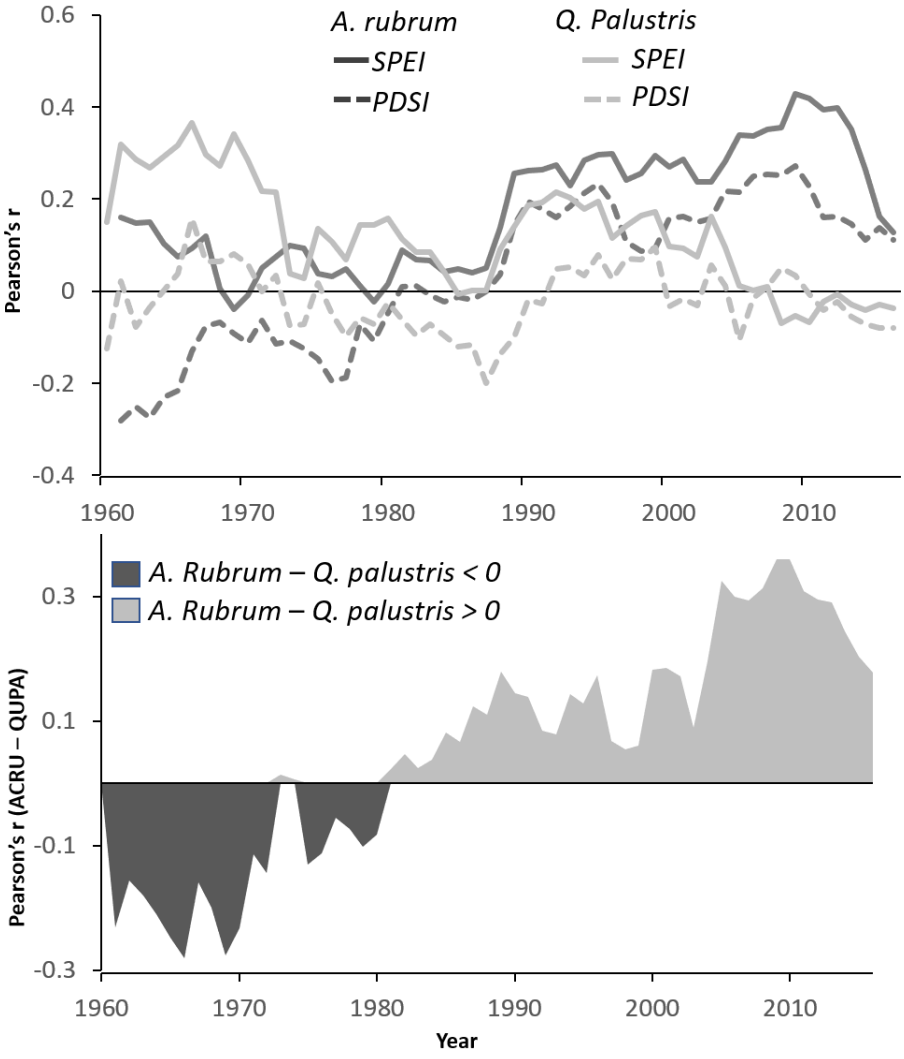


Figure 7 (a) Bootstrapped 30-year moving interval correlation analysis of *A. rubrum* (ACRU) and *Q. palustris* (QUPA) growth and JJA PDSI and SPEI. (b) Difference in average *A. rubrum* (ACRU) and *Q. palustris* (QUPA) 30-year moving interval correlation coefficients. In both figures, each data point represents the end of a 30-year interval.

How to Conduct Marketing Research For Free

Tyler Coy

William Deck, Ph.D.

Abstract

Web-based marketing research is quickly becoming an alternative to traditional methods, with survey sites such as SurveyMonkey gaining 25 million users since its founding and garnering 90 million survey responses a month. There are hundreds of free online survey tools with features that support interests ranging from companies collecting quantitative data on their customers to research-based surveys interested in identifying trends. The impact of these web-based surveys on research methodology is becoming more prevalent as we shift further into The Information Age and continue to rely on the internet for answers. Companies, researchers, and public institutions are all realizing the opportunity that free surveying sites offer can serve as a remunerative alternative to more cost-intensive traditional marketing research methods. The purpose of this study is to present a literature review to give context to our aim to provide a guide on how to conduct marketing research at minimal to no cost.

Introduction

To many, traditional marketing research methods are still superior to more modern options. Traditional methods are defined in this context as postal surveys, in-person interviews, and focus groups. However, research conducted by Schillewaert and Meulemeester (2005) supports that traditional marketing methods such as mail surveys, in-person interviews, and focus groups produce similar results in regards to response rates when compared to online data collection methods. However, cost efficiency, design difficulty, and time implications are significant variables to be considered when deciding on a data collection method. Modern research methods, in this context, refers to collecting data utilizing the internet. Research by Baron, Healey, & Ilieva (2002) suggests that web-based surveys have proved to be the most superior form of data collection in regards to marketing research. Patino, Pitta, Quinones (2010) argued the process of creating surveys and analyzing data can be advantageous compared to traditional methods, explaining that selecting question types, wording, and layouts, is fast and easy. The aim of this research is to evaluate the suggestions made by past researchers and to assess the feasibility of conducting marketing research utilizing free web-based survey programs, in particular to questioning theories that advocate for web-based survey cost effectiveness, accessibility, and response rates.

History of Online Web-Survey Tools

Technology has changed the way we collect and store data, especially considering companies are trying to leverage technology as a tool to expand their customer base or to gather feedback. Businesses are beginning to understand that data-backed decision making can stimulate growth substantially. Data has always been collected and utilized by organizations, but the tools we have available are changing the way we execute data collection and analysis. Online data collection and storage was initially encouraged by the creation of Microsoft Excel, a tool that makes it easy to input and analyze data. Cloud storage of data only further encouraged the growth of online data collection by protecting companies from breaches through using Virtual Private Networks (VPN). Major Online Surveying platforms SurveyMonkey and Qualtrics both utilize cloud based tools to protect customer data. Online Surveying Platforms have grown from a tool that allows you to simply input raw data and personally analyze it into an elaborate tool that can collect data, analyze data, and identify potential respondents all at the click of a button.

Pricing of Web Surveys

Cost of web-survey service varies depending on features and services, but basic features can be purchased for $20 a month on the website that is being analyzed and used for data collection, Survey Monkey (a web-survey service that offers authoring tools, server space, and automated survey analysis). Survey Monkey, much like most web-survey providers, has limitations. These include a limit of 1000 responses per month, and an additional 5 cent charge per survey response that is over the limit. In contrast, other businesses, such as KeySurvey, charge $670 a year for services comparable to what SurveyMonkey offers for $240 a year (Wright 2017). Other businesses also charge per survey and have additional features that provide consultation for the

web-survey design process. For those who have a research design and process, the basic version or less expensive options will be sufficient.

ADVANTAGES

COST

Online surveys were found to have minimal financial resource implications, with the scale of the survey not affecting financial commitment (Baron et al., 2002). Significant costs associated with traditional marketing research methods such as postal surveys include postage, photocopying, data entry, and clerical work. It was concluded that none of these costs were associated with an online survey. Prior to recent technological innovations, surveys could be costly because of technological and programming needs. The availability of web-based survey software and its ability to develop questionnaires has significantly lowered the cost of preparation (Evans & Mathur 2005). Survey development, distribution, and analysis are all significant cost to consider when performing marketing research. Web-based surveys have proved advantageous compared to conventional methods because of their ability to automate the creation of questionnaires, cost efficiently distribute invitations via e-mail, and automatically analyze data through data analysis software.

Flexibility

Online surveys have the ability to be distributed from anywhere in the world via e-mail, with low costs and large samples to gather data from, and they also offer the advantage to participants the flexibility to participate whenever they would like (Evans & Mathur, 2005). The author of this publication suggests that surveys can be created, distributed, and accessed from anywhere in the world. The internet affords a kind of interconnectedness that cannot be replicated by traditional marketing research methods

TIME

Wright (2017) suggests that survey research is still young and evolving with the growth of web services. This evolution of web-services is making the previously difficult task of creating surveys much easier with software that can perform a number of costly tasks. In recent years utilizing web-surveys has become more practical for researchers because of self-authoring services, eliminating the need for coding and web-design skills. Utilizing web-based survey services can be particularly fruitful for those looking for time-efficient research and access to large segments of society. Wright (2017) argues that as the cost of computer hardware and the popularity of the internet increases, online researchers will have access to an abundant segment of society. Utilizing traditional marketing research methods to gain access to comparable segments that the internet provides would not be realistic because of time restraints related to research.

Accessing more geographically diverse segments with real-time access to interactions provide many advantages to online surveys. Online survey participants can respond to surveys by clicking URL's that are sent by e-mail and can be quickly transported to web-survey tools that are "directive and powerful" (Evans & Mathur 2005). The convenience provided by these

surveys through "one-click access" are superior to conventional distribution methods that require more time and cost to acquire responses.

An additional time-saving advantage of web surveys is data-analysis software that is available for online surveys (SPSS, SAS, Excel), which was noted to "alleviate time restraints for data interpretation and analysis" (Baron, Healey, & Ilieva 2002). Data analysis can be automated, alleviating time restraints and also providing a reliable place for data storage within your software.

Online surveys may also save time by allowing researchers to collect data while they work on other tasks (Llieva, Baron, & Healey, 2002). To increase efficiency, invitations should be administered online via chat boards, newsgroups, and message board communities. Once invitations are distributed, data collection becomes autonomous, permitting the researcher to work on other aspects of their project.

DISADVANTAGES

Requires a Pull Strategy

It is important to contrast web-based surveys to alternatives such as paper-based surveys, which Landoy and Repanovici (2009) suggest are translated into electronic formats such as e-mail surveys. This suggestion raises the question if e-mail surveys are considered the modern equivalent to paper-based surveys, how do web-based surveys compare? A key advantage stated by Landoy was that e-mail surveys provide the "push" affordance, while web-based surveys require a "pull" effect to attract people to the survey. This disadvantage is important to consider when constructing web-based surveys to effectively serve as an alternative to paper-based and e-mail surveys. The conclusion that e-mail surveys are superior to web-based surveys could be challenged had the research provided survey invitations, which could have been administered via e-mail, thus creating a similar "pull" effect that was suggested.

Data Quality

With the advent of web-based surveys, consumers have gained the opportunity to obtain incentives for completing web-based surveys. While incentives can be an effective method to increase response rates, they can be detrimental to data quality, potentially compromising data integrity. The risk of respondents inputting inaccurate data for incentives has become increasingly challenging for companies who are utilizing incentivized web-surveys (Locket 2004). This indicates that while incentives can be useful to create a higher quantity of data, data quality is risked.

Traditional marketing methods, such as paper-based surveys data quality cannot be completely replicated by electronic formats (Andrews et al., 2003). This is largely due to privacy and confidentiality issues related to the internet, causing online survey results to become unreliable. Automated mailing lists and nonpublic online communities are particularly problematic when attempting to gather data online (Cho & LaRose, 1999). These unwanted emails or "spam" are seen as invasive and not conducive to building trust between the participants. Transparency,

credibility, and appropriate distribution methods are all essential to building the trust that is required to produce quality results from participants.

Junk Mail Image

Online surveys have the tendency to be perceived as junk mail (Evans & Mathur 2005). Mailing lists are commonly used as a distribution method of online-surveys, or to extend invitations to web-based surveys. "In May 2004, MessageLabs, an internet security firm, found that 692 million out of 909 million scanned e-mail messages (76 percent) sent to its US customers were screened as spam "(Evans & Mathur 2005). This research suggests that well over half the invitations that marketing researchers are distributing are marked immediately as spam. Marketing messages are becoming more difficult to distribute because of internet protection agencies As a result, potential respondents become increasingly difficult to contact due to lack of invitation methods, in turn dramatically affecting response rates.

Demographic Issues

According to an article headed by Dorine Andrews, intense internet users with stronger internet skills (comparative to the general population) typically participate in online surveys, affecting the ability to generalize the total population with the online population. This observation suggests that those with fewer internet capabilities are less likely to participate in online surveys. The online population is generally associated with fairly higher-income households, younger people, and often from White and less African American and Hispanic compared to the general population (Andrews et al., 2003). This exclusion of particular demographics directly affects data quality when conducting online surveys and creates gaps in research due to economics, age, and ethnicity. With only a third of the population being internet "literate" (Wyatt, 2000), there is a clear limitation to gathering data online. In summary, traditional marketing methods have the ability to effectively gather data from a more diverse segment of social classes because of internet capability and accessibility restrictions.

Optimizing Web-Surveys

Ideal Investigators

Optimizing your web-survey is largely dependent on your needs as a researcher, with factors such as your budget and internet literacy levels being prominent factors. Ideal web-survey investigators are those with a limited budget and sufficient web skills.

Investigators should be aware of the potential for their target-group to be underrepresented on the internet to ensure they are gathering quality data. There is significant evidence supporting the idea that the internet population is demographically skewed (Evans & Mathur 2005). With this in mind, minimizing data quality risk could be done by screening prospects via email to ensure a diverse group is identified.

Identifying prospective respondents

Ideal respondents should be avid internet users (Wyatt, 2000), with their e-mail addresses recorded for future reminder messages (Evans & Mathur 2005). Respondents should have the opt-in option when e-mailing surveys, to adhere to privacy standards, whilst respecting their privacy. Doing these things will increase response-rates and ideally increase the likelihood of responses without incentives.

How to Create Online Marketing Research Surveys

Marketing Research Process

The table below identifies the steps in the Marketing Research Process:

Define the problem	• Identify purpose of study • Understand how info gathered will impact decision making • Can be done through interviews with industry experts
Develop an approach to the problem	• Formulate an objective for the research • Generate research questions • Discussions with management can be done to assist in this process
Formulate the research design	• Create a blueprint for conducting the project • Detail necessary procedures for obtaining desired information • Purpose is to design a study that tests hypotheses
Data Collection	• Collect data utilizing selected methodology (Online Survey Tools)
Data Preparation and Analysis	• Edit, code, transcription, and verification of data • Questionnaires are inspected and edited
Report Dara	• Document results in a written report • Identify major findings • Prepare report in comprehensible format

Research Design

The three most prominent research designs are exploratory, descriptive, and causal.

First, you must determine whether you are doing primary market research or secondary marketing research. Primary research is gathering new data for your project, using secondary data is using data that's already been produced. If primary research is your selected method, the next step is determining your target audience.

Determining your target audience is key to producing quality data from online surveys. If you are a retail store, maybe you have a customer list or an already developed social media following

to pull from. You must ensure your survey analyzes your target market so you can improve the quality of your future decisions.

Once target audience is identified, you must determine research design, data collection format (which is going to be online, but you have to determine which online survey tool to use), determine the sampling plan, pretest your final survey draft, then distribute survey and analyze the results from the data you collected.

For the purpose of the research conducted, SurveyMonkey was used to formulate an online survey. Collecting data utilizing Survey Monkey is made easy with pre-made templates, a globalized panel to reach from, and extreme accessibility and free survey-analysis software. Before attempting to use SurveyMonkey to conduct marketing research, you should consider limitations that apply to the free version such as the number of items you can have in your questionnaire (10), and the number of responses you can have (100). Although there are other limitations, these will be the most significant when attempting to utilize SurveyMonkey to gain insights on your customer base. After creating an account with Survey Monkey, you select whether you will be using their surveys for Business, Academic, Government, or Personal purposes so they can optimize their services to fit your needs. For the purpose of this research, business was selected. Additional pre-screening questions include job titles, your company's objective, and ultimately what kind of survey you would like to send.

Pre-Testing your survey

It is crucial to pre-test your survey. This can easily be done with a small portion of your target audience, but if you cannot contact them directly then use co-workers or friends to help find glitches and unexpected errors in the survey design. Keep in mind that pre-testing your potential respondents prior to the actual test can affect the way they respond to your survey questions.

SurveyMonkey allows you to examine your survey prior to distribution by clicking the "Preview & Score" section of your survey. You can view your survey design and ensure that all aspects of the survey are functioning properly. Reviewing your survey before distribution is especially important when distributing online-surveys due to the complex environment your survey will be distributed to. You can view your survey in desktop, tablet, and phone modes to ensure your survey is compatible across all platforms.

In addition to these features, SurveyMonkey encourages you to comment and gain feedback by inviting people to your SurveyMonkey web link to collaborate. You can share your survey preview in most popular collaboration apps. SurveyMonkey recommends sharing a preview of your survey to Slack, a workflow website, to gain feedback from a like-minded community.

Survey Invitations and Follow-ups

According to Edwards (2016), contacting respondents prior to survey data collection via invitations and after attempts to collect data via follow-ups, you can increase response rates by utilizing these tools. Research concluded there were substantial benefits by inviting participants then following up with them to remind them to take their surveys.

After sending email invitations on SurveyMonkey, you can send reminders to ensure respondents take your survey. You can also follow up with a thank you email to thank them for their responses. Sending reminder emails can be crucial to optimizing your response rate, as they help you to encourage potential respondents who haven't completed the survey.

You have two options for reminder emails, the most effective being automated email reminders. Automated reminder emails contact potential respondents after a certain number of days after the initial invitation is sent. If you invite more respondents later, reminders will be automatically sent.

One-off reminder emails can also be programmed. You can send reminder emails anytime, or you can schedule it for a specific date and time. If you invite more respondents to take your survey later, you have to manually send other reminder emails.

To manage follow up emails on SurveyMonkey you can utilize the Follow-up Emails section of the collector overview tab to track your automated emails once they are set up. Follow-up emails can be toggled on or off to disable emails.

Designing the Survey

After personalizing templates based off of your information Survey Monkey allows you to name the survey, upload pre-designed questions if you have already created the survey, and even select the survey category (ranging from demographics to customer satisfaction). Next, you will design the survey. Survey Monkey offers recommended questions, but also allows for a fully customizable survey, equip with a copy and paste feature that allows you to input questions from past surveys for further collection. You can alter how your target audience responds to your questions, with checkboxes, multiple choice, ranking, and text boxes for open-ended questions. Additional features include the ability to customize the appearance of your survey. You can add a custom watermark or logo, footers, and alternatively choose from themes designed and provided by SurveyMonkey.

If you choose to design your own survey, you must understand how to create an effective survey. When designing an effective online survey, you must make your purpose clear. You have to know the purpose so once customer input is received you can take action on your goals. For example, it is important to understand if you are trying to gain customer insights, or understand the value of making certain changes.

It is important to ensure your questions are concise and avoid ambiguous terminology. It is important to use simple terms so that you do not confuse potential respondents. You should never assume that survey respondents understand the slang or terms you use. It is also important that your questions are as specific and as direct as possible. For example, if you are curious about how your customers feel about your bathrooms, you should ask "How satisfied are you with the bathroom conditions?" instead of "How clean are the bathrooms?" All of these aspects play into survey length, which is another important consideration when designing your survey. You want to ensure your survey does not get too long to promote higher response rates and more

quality responses from the questions you have included. So if you are debating on additional questions, it is usually intelligent to forgo any information that is not crucial to your objective.

Qualitative vs. Quantitative Research

It is important to consider what kind of data you want to collect when selecting a survey design. Types of data can generally be split into two categories, quantitative and qualitative data. Quantitative data is designed to be structured and statistical evidence from which conclusions can be drawn typically illustrated and supported through numbers. Qualitative data seek opinions from people. It tends to dive deeper into topics and gain insight into people's thinking. Qualitative data is typically better when you need to gain an understanding of your consumers and their motives, but its broad questioning makes it much more difficult to analyze.

Exploratory Research

You would use exploratory research to gain further insight and determine data collection methods, so it is crucial to determine if it is necessary before creating your survey on SurveyMonkey. Exploratory research can be conducted on SurveyMonkey by using open-ended questions to further your understanding of a topic. While this method is typically not statistically measurable due to its qualitative nature, it can assist you in formulating a hypothesis to be tested by the other research methods.

Descriptive Research

Descriptive research is used to make conclusions and is typically used to gather quantitative data that can be analyzed to make these conclusions. Descriptive research is used to assist in defining the attitude held by a group on a specific subject. For example, you may want to determine the level of customer satisfaction in your business. Descriptive research would be used to define their opinions and make a conclusion based off of the results. You could perform descriptive research on SurveyMonkey by surveying respondents on their attitudes and opinions regarding your product and using the results for further analysis to make a conclusion.

Causal Research

Causal research, much like descriptive research is much like descriptive research in its structured design and conclusive nature. However, the purpose of causal research is to establish a cause and effect relationship between variables. For example, if you are a business owner and you want to figure out the correlation between your branding and your product sales you would use causal research. Causal research could be performed in this instance using an online survey by attempting to measure the percentage increase in sales using different branding as the independent variable. A current customer list could be used to distribute surveys determining whether specific branding is worth the resources being utilized or if it is even significant in relation to sales.

Visual Design of Survey

When designing a survey, you must always consider the context of the questions you are asking. First, you must determine what kind of information you desire. If asking for private or sensitive

information, exuberant colors can throw respondents off and may cause their trust to falter. Consider using neutral colors that match the context of your questions in this instance. No matter what questions you use, ensure your color patterns match the context of the questions.

Usage of Logos

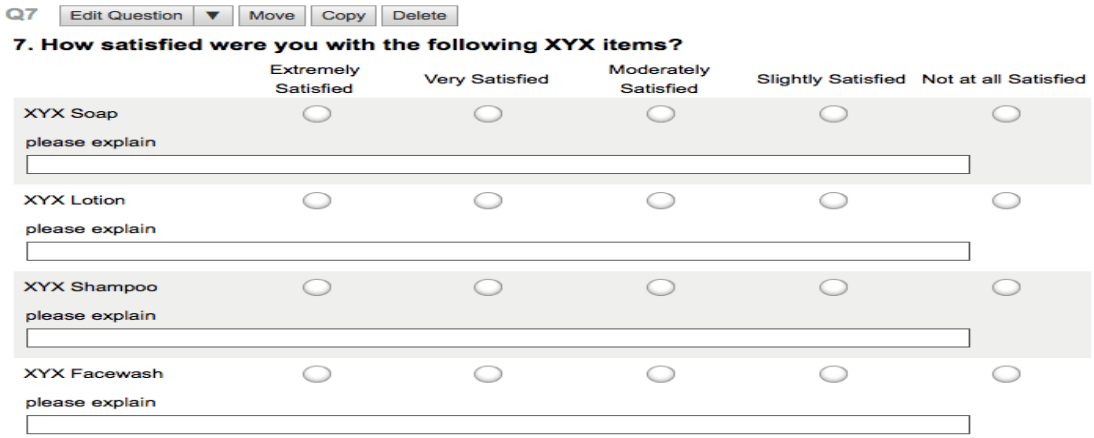
If your brand image is important to you, use your logo in your Survey header, but also use your company colors in the header and throughout the survey to keep the presentation clean and consistent.

Prioritizing Content

Ensuring that your content is easy to read is essential, whether it be using a legible font or increasing text size. According to SurveyMonkey, Arial, Helvetica, and Verdana fonts work best for web-based surveys. To optimize the visibility of your content you should minimize your usage of colorful backgrounds so your color contrasting is effective.

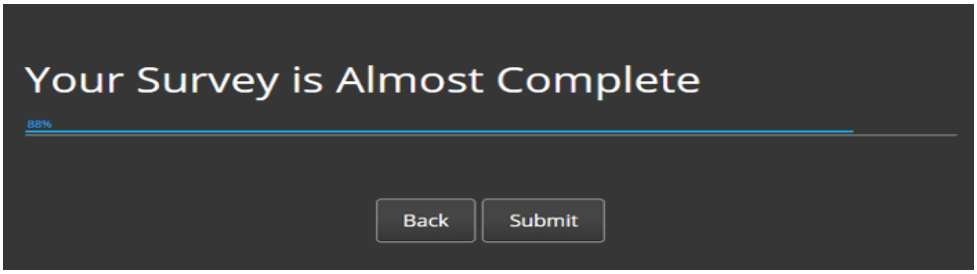
Utilize comment boxes

Comment boxes allow you to collect long open-ended responses. Some questions can be restrictive, particularly ranking and rating scales. Utilizing comment boxes enables respondents to give a more complete response, telling you about their motivation for their decision. Using comment boxes also shows respondents that your survey design is legitimate and you are interested in their opinions.



Utilize Progress Bars and Page Indicators

You can optimize your response rate by keeping your survey as short as possible, but in case your survey spans more than one page, it is important that you break up your survey into pages and use progress bars. Progress bars not only manage their progress but reward them with a visual of their work. Another way to manage survey progression is to indicate survey length by page when the survey begins, then enable the page indicator tool on SurveyMonkey to ensure respondents understand their time commitment from the start.



Avoid double-barreled questions

Ensure that you are asking one question at a time. In most cases, avoid using the word "and" in questions, as it usually identifies questions that have two parts. For example, if you are interested in the customer service and reliability of a cell phone provider, you would want to ask that in two different questions instead of asking "Do you feel the cell service provider has top notch customer service and reliability?" An effective way to split these questions would be to ask two separate questions, "Do you feel the cell service provider has effective customer service?" and "Do you feel the cell service provider has excellent customer service?"

Double-barreled questions:
Was the customer service representative helpful and kind?

Correct format:
Was the customer service representative helpful?

Was the customer service representative kind?

Avoid Leading and Biased Questions

When creating your survey, you will want to avoid using questions that lead the respondent to a specific answer. You should state all potential alternatives in the question answers, and identify respondents with a significant distance from the topic to avoid biases. It is especially important to identify respondents with distance from the topic when reviewing the pretest so that errors can be exposed.

Leading questions:
The customer service representative was helpful, right?

Correct format:
Was the customer service representative helpful?

Random Assignment

Random assignment is a crucial factor to consider when conducting marketing research, particularly if you are trying to establish a cause and effect relationship. In online surveys, this

refers to randomly assigning each respondent to one randomly selected question. This ensures that each sample has an equal chance of being exposed to the variables.

It should be noted that Random Assignment, or "A/B Tests" as SurveyMonkey defines them, is a paid feature that must be purchased. At the time of this writing, an annual fee of $384 will give you access to randomization capabilities, along with a plethora of features including unlimited surveys, questions, responses and much more. In contrast to the average cost of marketing research studies, utilizing the "Advantage" paid plan through SurveyMonkey could offer you features that could give you the insight you need to grow your business.

Avoid Using Grids or Matrices for Online Respondents

Respondents typically do not fill in grids correctly or accurately, and most sites grids, including SurveyMonkey, are not very mobile-friendly.

Below is an example of a matrix question taken from a SurveyMonkey survey:

Utilizing Advanced Branching with SurveyMonkey

Rules can be inserted into your survey during the design process to ensure that errors are minimized. For example, if respondents select an answer, but also select "none of the above", the rule that survey creators should enable for that question will trigger an error message notifying the respondent that the "none of the above" answer cannot be selected with other answers.

Advanced branching can be accessed by clicking the page logic tab, selecting advanced branching logic, then selecting the "add a new rule" option. Once this is selected you can customize your rules and even create your own error messages. It is important to avoid creating rules that will elicit favorable results for your research, as this will delegitimize your survey results.

Advanced branching also enables the survey creator to skip respondents ahead and hide questions based off of their responses. For example, branching questions will only be shown to the respondent if conditions are met, otherwise, they will be hidden. Conditions for these branching are typically answering questions in a certain way. For instance, if respondents are asked if they consume eggs and select yes, an effective question to branch to would be "how

many eggs do you eat per day?" The condition that needed to be met was answering yes to consuming eggs, and the branching question was asking the respondent about their daily consumption habits. The utility of this feature is to ensure you are not asking questions to individuals that are not applicable to them, as indicated by former questions.

Ensure you are not adding too many conditions to each question. This can make managing your questions and analyzing the data extremely tedious. Minimize your conditions but have a strategy for employing them when needed.

Collecting Responses

Collecting responses to surveys can be automated or done manually with SurveyMonkey. If the survey creator wants to upload an e-mail list that has been acquired from the target audience, SurveyMonkey will distribute the survey to potential respondents. Additionally, SurveyMonkey can embed the survey on your website and elicit responses from website visitors via pop up surveys or pop up invitations. SurveyMonkey also suggests acquiring a web-link for free to distribute via social media to increase respondents. Other ways to collect data utilizing SurveyMonkey include Kiosk Surveying, which can turn your mobile device into a survey station- enabling you to collect responses nearly anywhere you go. Utilizing chat rooms such as Reddit to collect survey responses could also be effective, especially if you are a member of an online community and already have a well-developed influence on your platform. You can also upload offline responses manually by entering the data into response sheets on the website. All of these response collection methods are included as free features for registered members on SurveyMonkey but are limited to 100 responses per survey.

Analyzing Data from Online Questionnaires

Survey data analysis is required to make any conclusions after collecting responses. Before you can present or report data, data must be coded, tabulated, and edited to ensure there were not errors and to ensure the data is easy to interpret.

Prior to data analysis software, researchers had to edit, code, and tabulate data manually. This would require them to edit each survey to minimize errors in the data analysis step of the marketing research process. Editing survey responses requires the investigator to identify mistakes by respondents throughout the survey.

Coding surveys requires the investigator to assign numeric values or codes to represent a response. This is easier with closed-ended questions, but can be difficult with open-ended questions. Coding open-ended questions requires the investigator to read through responses and identify potential response categories. This may require the investigator to combine or narrow down response categories for the purposes of data analysis. Finally, you can create codes to assign to these response categories for seamless data analysis.

Once physical surveys are coded, you can tabulate the information into excel to create a spreadsheet for data presentation and analysis. This step can be skipped if a survey has been created online. If a survey is formulated online, results are automatically tabulated.

In addition to providing free-survey software, SurveyMonkey has free survey analysis software that determines the effectiveness of your questions before distributing it. Based off of the sample survey created, the estimated completion rate of a survey with 10 questions was 89%. The analysis software provided recommendations to add a custom logo to the survey and estimated a time to complete the survey, which averaged 8 minutes. In addition to an analysis of the effectiveness, SurveyMonkey provided feedback on each question, indicating whether it was closed or open-ended and providing advice on how to improve the questions.

Summary

Online Survey tools are growing and becoming more capable as the digital world expands. As these survey tools become more capable, they are exceeding their predecessors (traditional data collection methods) in cost, capability, and even their potential to reach respondents. SurveyMonkey enables businesses with no marketing research experience to gain quantitative and qualitative data with minimal effort and little to no cost. SurveyMonkey has tools that will help to optimize your survey, ensure you are targeting the appropriate audience, and even assist you in distributing your survey across multiple platforms. SurveyMonkey assists with word choice, survey design, and goes far enough to suggest when alternative question formatting is necessary, such as inserting branching questions to identify respondents who can appropriately answer specific questions. Overall, Online survey data collection companies are an effective tool for users looking to gather data from an already identified list of respondents (i.e. customer list for customer satisfaction surveys). Traditional data collection methods are slower, costlier, and can be less effective, while the tools provided by SurveyMonkey take the hassle out of the survey building and distribution process.

Anthony Patino, Dennis A. Pitta, Ralph Quinones, (2012) "Social media's emerging importance in market research", Journal of Consumer Marketing, Vol. 29 Issue: 3,pp.233-237, https://doi.org/10.1108/07363761211221800

Evans, J. R., & Mathur, A. (2005). The Value of Online Surveys. Internet Research,15(2), 195-219. doi:10.1108/10662240510590360 ·

Kevin B. Wright; Researching Internet-Based Populations: Advantages and Disadvantages of Online Survey Research, Online Questionnaire Authoring Software Packages, and Web Survey Services, Journal of Computer-Mediated Communication, Volume 10, Issue 3, 1 April 2005, JCMC1034, https://doi.org/10.1111/j.1083-6101.2005.tb00259.x

Llieva, J., Baron, S., & Healey, N. M. (2002). International Journal of Market Research. Online Surveys in Marketing Research,1-3. doi:https://doi.org/10.1177/147078530204400303

Schillewaert, N., & Meulemeester, P. (2005). Comparing Response Distributions of Offline and Online. International Journal of Market Research,47(2), 163-178. doi:10.1177/147078530504700203

Allison Garrett

The Microbiome of a Pierced Belly Button Compared to a Non-pierced Belly Button

Dr. Thomas Ford

ABSTRACT

We compared the bacterial microbiome from nonpierced innie belly buttons and pierced innie belly buttons by using sterile swabs with a buffer on them to gently swab the belly button. Then, comparing the two by performing DNA extraction using a PowerSoil kit by completing Gel Electrophoresis, restriction enzyme digestion and we used tRFLP (terminal Restriction Fragment Length Polymorphism) to determine the different types of bacteria within both pierced and nonpierced belly buttons. Results show that pierced belly buttons on average have a larger amount of bacteria than non-pierced belly buttons. There was a large quantity of the nonpierced bacteria all around the same area, 0.0 on both NMDS1 and NMDS2 while the community composition for pierced are around 0.0 on NMDS1, but anywhere from -0.5 to 0.5 on NMDS2. These differences were there because of the piercing and how it collects bacteria within the belly button. It allows for the bacteria to leave minimally and causes there to be a very diverse amount in the belly button of pierced belly buttons. There is also a lot of effects that these piercings can have on the belly button such as infections, scarring, pain, and edema in or around the naval.

INTRODUCTION

Bacteria is present everywhere upon the body. Normally, bacteria congregate in places that are moist and closed, like elbows, vaginas, and belly buttons (Brookshire, 2018). The best way to clean the body is to use soap and water because alcohol will disturb the pH balance of any of these given the area (Pennisi, 2008). Bacteria is generally placed into two different groups: Resident microbes and transient microbes. Resident microbes are normally fixed groups of microorganisms that are found in and on the skin. They are also not harmful and very beneficial to the host. Transient microbes are not on the surface, they are within the environment and may persist from hours to days. Although both transient bacteria and resident bacteria can

both be beneficial to the host they can colonize and cause disease. Most microbes thrive in a large community and only a minority of bacteria prosper in isolation (Kong, 2011). The microbes that are living in the belly button are a part of the host's microbiome, meaning that there are those microscopic organisms like bacteria, viruses and fungi that all live on and in all animals and plants within their microbiomes.

Without microbes the body will not function properly. Bacteria is mostly harmless and will protect the body from other infectious diseases. Bacteria is always present on the body, there are over one hundred trillion different bacteria upon the skin. These trillions of bacteria, viruses, fungi, archaea, and small arthropods colonize on the skin surface, creating the skin microbiome. These creatures are so tiny that you could place twenty-five thousand of them on top of each other and it would be about an inch tall. Some may help us make use of nutrients and of waste, some may even help to not let harmful pathogens eat on the nutrients we need for healthy cells. Then, there are some that are produced by scientists in a lab to make medicines and vaccines. Microbial studies have linked these different bacteria to human health and disease (Wolfe, 2012).

The belly button microbiome is one of the most diverse areas on the body. A biodiversity project was done where they tested sixty different belly buttons where they swabbed them and found two thousand three hundred and sixty-eight different species of bacteria within these belly buttons. Among these there were one thousand four hundred and fifty-eight that were thought to be new to science, one even being a bacterium that was only found in soil in Japan, and others that were only found in the ice caps and thermal vents (Rainforest of bacteria' live in our belly buttons, 2012).

Ninety percent of belly buttons are innies. Most innies have very diverse mixes of bacteria and fungi, while outies have two general kinds, *bacillus* and *staph*. Age, sex, and

ethnicity do not influence in which species live in the belly button microbiome. Adults have more types of bacteria than most children do (Wolfe-McClathy, 2012).

Bacteria around a piercing is normally increased in number. Piercings have been around for a very long time. Many ancient cultures had piercings on their ears, navels, nipples, tongues, and genitals, which is not so different from people today. Infections arise from when there is a piercing. The object that pierced it, or the type of metal the piercing is can all attract bacteria to this site. Some bacterial infections may result in nipple rings causing abscesses, surgical irritation, or causing the piercing to close completely (Tweeten, 1998). Most of these bacterial infections will become less irritated, which will decrease the infection if taken care of properly. Some of these infections can even become life threatening if not treated as needed.

Tongue piercings have many complications when someone first gets them, such as bacterial infections, pain, swelling, prolonged bleeding, and difficulty in swallowing, speech, and mastication. Later, after having the piercing for a while, complications include chipped and fractured teeth, gingival trauma, localized periodontitis, persistent difficulties in oral functions, and swallowing of the piercing. A study was done that took eighty-five people that had a tongue piercing. They had to wear the piercing for six months before they could participate, excluding anyone with issues within the mouth. Each participant wore a new, clean tongue piercing for two weeks to test and see the microbiological samples from the tongue. There was an increase in the amount of bacteria that was taken from around the piercing compared to the sample that was taken before the piercing was placed within the mouth (Kapferer,2010).

The belly button has a large amount of bacteria that is living within its microbiome, and piercings attract a higher number of bacteria. The object for this project is that a naval piercing will have different diversity and community composition than the belly button itself. I will be

testing this by collecting microbial DNA samples from belly buttons that have the piercing and that do not have the piercing. Once the skin microbiome is altered due to a piercing more bacteria will congregate towards the opening. The opening acts as a new home for these different bacteria. They will come in greater number and in a more diverse quantity. This could cause infections and diseases due to the bacteria that is now around the piercing and unless it is cleaned very thoroughly every day the bacteria will continue to increase and disrupt the microbiome.

The belly button has a large amount of bacteria that is living within its microbiome, and piercings attract more bacteria. The theory for this project is that a naval piercing will have more bacteria than the belly button itself because of how much bacteria is attracted to an opening in the skin and also because the piercing traps a large amount of bacteria within the belly button.

METHODS AND MATERIALS

Swabbing belly buttons that have the piercing and that do not have the piercing are both done by taking a swab and placing it in the belly button and swirling it to get as much bacteria as possible. Everyone had to fill out a questionnaire, the non-pierced people just had to state their gender, age, how many times they shower a week, when the last time hey showered was, and if they had taken any antibiotics over the last year and when. The pierced people had more questions, theirs were gender, age, how long they had their piercing, how many belly button piercing they had, how many times they shower a week, when the last time they showered was, what their cleaning routine for their piercing was, if they had any infections in their belly buttons, and if they had taken any antibiotics over the last year and when. In this experiment there will be 15 pierced belly buttons and 15 non-pierced belly buttons that will be swabbed. Each sample will be done using a Qiagen PowerSoil extraction kit on them to extract the DNA. Then Polymerase Chain Reaction (PCR) will be performed on the extracted DNA using the

primers 9F-FAM and 907R, where they were then put in the thermocycler under the stream setting and left to sit for 24 hours. From there the restriction enzyme protocol was done using Dde1, Msp1, Cutsmart Buffer, and the purified DNA. Gel Electrophoresis then happens using a Hyperladder 50bp where one can see where the DNA occurs under UV light. When preparing the digested PCR samples for t-RFLP submission each sample had Hi-Di Formamide and the dilution. Every sample had two dilutions one was a 1:1 dilution and one was a 1:50 dilution.

RESULTS

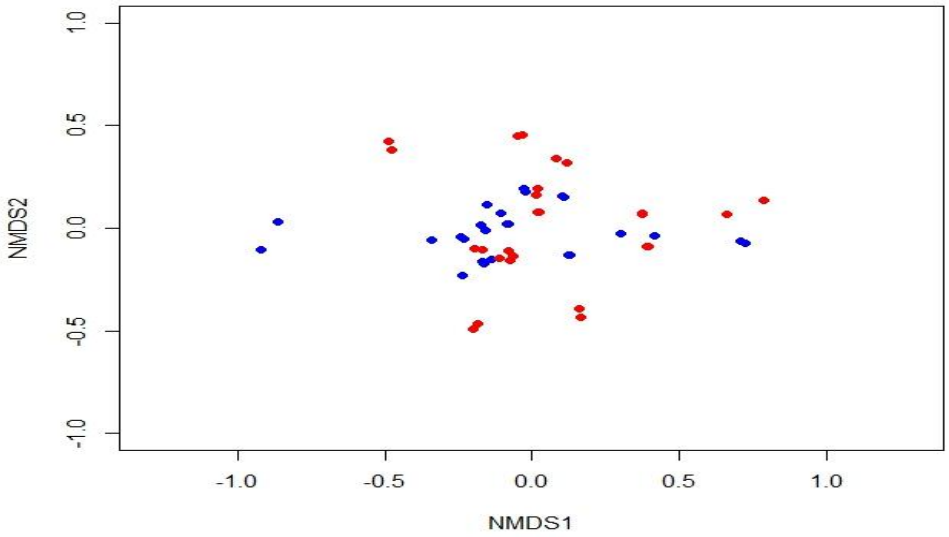


Figure 1. The Taxonomic Diversity (# operational taxonomic units – OTUs) of Pierced and Non-Pierced Belly Buttons. This graph shows the difference in community composition between pierced subjects (red) and non-pierced subjects (blue).

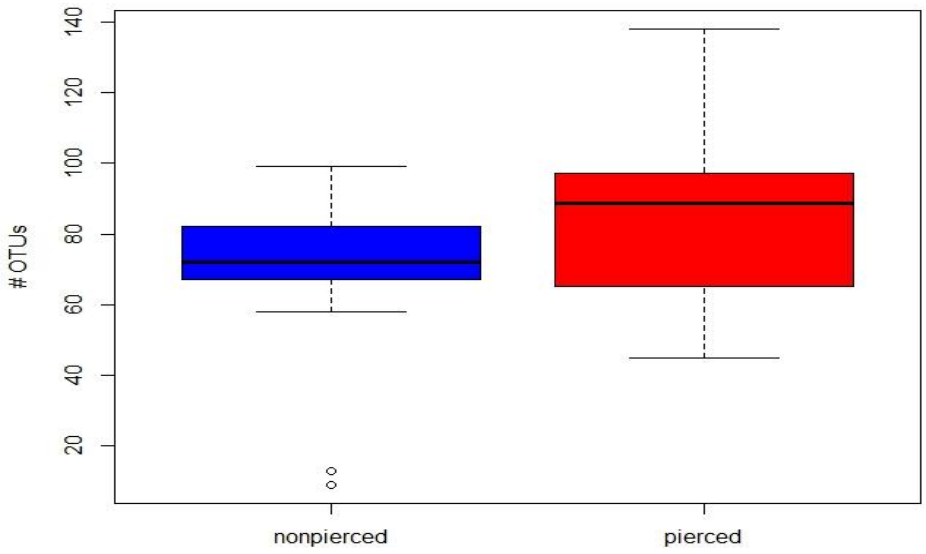


Figure 2: Multivariate Analysis of Community Composition (Non-Metric Dimensional Analysis – NMDS) of Pierced (red) and Non-Pierced (blue) Belly Buttons. This graph shows the number of bacterial taxonomic groups for pierced subjects compared to non-pierced subjects.

RESULTS

The number of bacterial taxonomic groups in figure 1 for nonpierced belly buttons is 71.20 while the mean of the pierced is 84.96, showing that there is higher taxonomic richness in the pierced belly buttons compared to the non-pierced belly buttons, a T-Test indicated t=-2.2994, df=49, and p=0.026. The minimum for the pierced was 45 and the maximum was 138 while the minimum for the nonpierced was 9 and the maximum was 99. Figure 2 shows that the number of bacterial taxonomic groups is much larger in pierced than nonpierced, showing a difference in bacterial composition among the two. The F. Model is 1.9464 and p-value associated with the F statistic is 0.043, PERMANOVA F=1.94, df=1,49, p=0.43.

DISCUSSION

The results were probably different due to hygiene, when the last time their belly buttons had been washed, whether they had infections in their belly buttons, or how long they had their piercing. More testing should be done to ensure these results. The bacterial taxonomic groups show that there is a larger diversity in pierced than non-pierced. This is because the piercing attracts bacteria and traps the bacteria within the belly button. The community composition was different, the non-pierced community was much more compact than the pierced community which was much broader. Most people that participated in this study said they shower daily, but some of the people with piercings had infections in their belly buttons, or they do not clean their belly buttons specifically because their piercing is constricting the bacteria and preventing it from leaving. The presence of metal was an aspect of interest because it was unsure whether the metal would reject the bacteria or if it would just trap the bacteria within the belly button. The study showed that the metal did not reject the bacteria as once thought, but it could be rejecting certain taxonomic groups. These results have many implications on the skin health in the belly button. Research shows that people who have piercings have inadequate sanitation. Many study's state that there is infection, scarring, pain, and edema related to naval piercings. Also, depending on the type of metal that the piercing was made of may change what bacteria is within the belly button.

BIBLIOGRAPHY

- Brookshire, Bethany. "Which Bacteria Hang out in Belly Buttons? Here's a Who's Who." MasterFILE Premier, 15 May 2018.
- Kapferer, Ines, et al. "Tongue Piercing: The Impact of Material on Microbiological Findings." 22 July 2010, Adolescent, Body piercing/adverse effects, Humans, Tongue piercing, Tongue/microbiology.
- Kong, Heidi H., and Julia A. Segre. "Skin Microbiome: Looking Back to Move Forward." 22 Dec. 2011, jidonline.org.
- Pennisi, Elizabeth. "Bacteria Are Picky About Their Homes on Human Skin." 23 May 2008.
- 'Rainforest of bacteria' live in our belly buttons." Asian News International, 16 Nov. 2012. Infotrac Newsstand, http://link.galegroup.com/apps/doc/A308671413/STND?u=viva_vpi&sid=STND&xid=294c62c9. Accessed 14 June 2018
- Tweeten, Samantha M., and Leland S. Rickman. "Infectious Complications of Body Piercing." 26 Mar. 1998, watermark.silverchair.com/26-3-735.pdf?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAZowggGWBgkqhkiG9w0BBwagggGHMIIBgwIBADCCA
- Wolfe-McClathy Tribune, Warren. "Belly Buttons Breed Bacteria." 16 Dec. 2012.
- Wolfe, Warren. "`Jungle' living in belly buttons." 18 Dec. 2012.

Perceptions of Music Education

Different Perceptions of the Value and Use of Music Programs in Schools

by Different Education Professionals

Kristen Knight

McNair Scholars Program

Research mentor: Dr. Timothy Mainland

Concord University

Literature Review

Music has played numerous roles in every society across the span of the centuries. It has been used to lead worshippers to salvation and taught children the letters of the alphabet. Music is used to share and spread stories, feelings, information, and ideas. It is more than just the noise that spurts out of our car radios; it is an artform that is thought to help us understand our way of life. "Throughout history many theories concerning music-whether musical, philosophical, theological, sociological, or political-share the conviction that music moves or tangibly influences its audience in an ethical or moral way" (Riethmuller).

Even so, people perceive music in very different ways. Some people think of music as an enjoyable activity, but nothing more. Others, such as our country's music teachers, see it as their livelihood. Some even see music as nothing but an annoyance. In education, we have this same difference in perceptions, and it can cause problems with giving students a quality education.

The music teacher is not the only one responsible for the quality of the music program; they must have the full support of the school's administrators and other faculty to thrive. "Within a school, the principal often facilitates the implementation of the curriculum and monitors its ability to meet broad educational goals. Teachers often depend on the support of the principal to meet their specific objectives and enhance their programs. This assistance is especially crucial in music education programs, where the building principal can help establish schoolwide support for the music curriculum" (Abril). The purpose and value of music and its funding in public education is viewed differently by principals, higher administrators, music teachers, and teachers of other subjects. This could explain possible impasses in the delivery of these programs and contribute to further research. To prevent these impasses, we must understand the perceptions of different educators and find a way to keep them all on the same page.

Principals and higher administrators oversee the curriculums taught in each class. They divide the funding where needed and try to maintain curriculum standards. "Today, principals, school boards,

and other community leaders are responsible for making curricular decisions based on a variety of beliefs and rationales" (Abril). Because of the current educational emphasis on high test scores, most schools put more effort into maintaining standards for STEM classes which leaves the Fine Arts as an afterthought. "Recent rhetoric emphasizing STEM (science, technology, engineering, and mathematics) seems to signal continued, challenging times for the arts" (Njoora).

These STEM classes are a huge asset to society because they contribute to advancements in technology, architecture, medicine, etc. Even so, the arts need to keep their own place in our educational system. "In a time of great scientific and technological advance, it is important that we maintain an appropriate balance in the curriculum…Music and the arts must be given an increasingly important place in the curriculum of the schools for they play an increasingly important role in the contemporary society" (Barbour). Music and the arts are a necessity for students to have at least a base knowledge that they can rely on to help them make judgements of artistic productions as well as the use of the arts in the media. "All secondary-school students… need experience in understanding music, the visual arts, the theatre arts, the industrial arts, and home economics. Otherwise they base their decisions [of artistic quality] on stereotypes and prejudices which can easily be manipulated by the mass media and by superficial shifts in fashion." (Barbour). There is still a gap in the interest of different educators regarding the importance of music in schools. "[Music] teachers indicated that music should be taught as an academic subject while administrators did not; and music teachers suggested that students were not given enough opportunities to create their own music, whereas administrators remained uncertain" (Abril). Music teachers recognize the importance of teaching music to the younger generations. They know that "[music] speaks through a kind of common language which transcends some of the difficulties of the spoken word in communicating deep human feelings which are common to all men" (Barbour). Many administrators feel like music is just a recreational course. According to Abril and Gault, "[principals] believed that winning athletic teams were more effective at fostering

improved school-community relations than outstanding musical performing groups… Music teachers thought that music had greater potential for building community relations, fostering creativity, and curricular equality" (Abril).

Numerous studies have been done to investigate the different perceptions of educators towards music education. According to Abril and Gault, most tests have only investigated areas with a maximum range of a single state, but they believe "[a] national survey could help the profession to better understand the state of secondary school music programs in the United States from the perspective of the school principal" (Abril). Their own test in the middle to late 2000s involved sending surveys to measure the different perceptions principals had of their music programs. Their results indicated that most schools, of the ones that answered, had some sort of music education classes. Most schools had some sort of ensemble, such as choir, band, and jazz. A small amount had in-depth classes that helped better students' musical knowledge, such as composition or theory. There were some noticeable gaps in the musical knowledge of principals. The study found that "principals consider performing to be a creative activity" (Abril). In a way, this is true, but musical creativity should be explored less with ensemble performances and more with composing and improvisation skills. "Although they may frequently see evidence of performing in their music programs, observing students develop skills as composers is usually less obvious to principals who do not observe the everyday activities of a music classroom (Abril).

Another study done was by Richard L. Barbour in the state of Oregon in 1967. He limited his research to principals and music teachers. His data indicated that one problem with different perceptions between the two groups deals with age and experience differences. The participants who agreed more with the survey statements, meaning they seem to support a vast, vibrant, and in-depth music program, seemed to be older and more experienced music teachers and younger, less experienced principals. They were labeled T1. The participants that least agreed with the statements

were labeled T2. This age/experience difference can be huge when the two parties try to communicate due to the generational gap. "It is this kind of difference that makes communication between groups difficult. This finding is one factor that can be added to the list of differences compiled by other studies on the lack of communication. It is apparent from this study and others, that if communication between the two groups is to take place, these kinds of differences must be compensated for or, if possible, eliminated…through re-education in formal class situations or during in-service training in professional organizations of the two sub-samples." (Barbour)

Another flaw Barbour noticed in the results in his data were that the music teachers in T1 taught in more "traditional schools" while the principals from the same group were located in "more flexibly-organized schools" (Barbour). Barbour states that "music teachers and principals have difficulty communicating because the like groups do not exist in similar situations" and believes that "[efforts] might be made to re-educate these two groups or to bring together in one school individuals with similar commitments to innovate or change" (Barbour). Having teachers and principals together who express different beliefs is good and bad; having both views in a school is a possible way to find the right mix of both styles to create a better system, but it is more likely to cause a struggle in progress because the two parties cannot agree.

In conclusion, music is an important subject that contributes to a well-rounded education. However, for a successful program, music teachers and administrators must be on the same page; there must be proper communication for the school to progress. Timothy K. Njoora states, "I would argue that for a commodity such as music, which is so meaningful to us whether old or young, a good deal of investment in time is required to ensure that it (music) remains part of our social structure" (Njoora).

References

- Abril, Carlos R. and Brent M. Gault. "The State of Music in the Elementary School: The Principal's Perspective." Journal of Research in Music Education, vol. 54, no. 1, Spring2006, pp. 6-20. EBSCOhost, login.ezproxy.lib.vt.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=mah&AN=21650541&scope=site.
- Barbour, Richard L. "A Study of Perceptions of Selected Innovative Statements in Music Education by Principals and Music Teachers in Oregon Secondary Schools." University of Oregon, 1968.
- Njoora, Timothy K. "More Than Just Good Feelings: Advocacy for Music Among Mainstream Subjects." Complementary Index, Ephost, 2015, login.ezproxy.lib.vt.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=edb&AN=108975542&site=eds-live&scope=site.
- Riethmuller, Albrecht. "Music beyond Ethics." JSTOR, www.jstor.org/stable/25162422.
- Walker, Loretta Niebur. "Do You Really Want to Know? Elementary Music Programs and Potential in Utah." MasterFILE Premier, EBSCOhost, 2015, Do You Really Want to Know? Elementary Music Programs and Potential in Utah.

Perceptions of Music Education

Different Perceptions of the Value and Use of Music Programs in Schools

by Different Education Professionals

Research Proposal and Conclusion

Description

My research project is meant to compare the perception of music programs among music teachers, teachers of other subjects, school principals, and other administrators. The purpose of this study is to expand the knowledge of possible reasons for failing music programs in public schools and reasons for underfunding.

Methods and Materials

To complete this study, my mentor and I created a 33-question survey meant to be taken by a music teacher, a teacher of another subject, a principal, and another administrator (with an option for others as well) for each school in the study. Schools were chosen by county's Per Capita Income (PCI). 80 West Virginia schools were contacted to participate. They were chosen to be 40 schools from the top PCI counties and 40 schools from the bottom PCI counties to ensure a wide range of backgrounds and income. The surveys were sent to educators through email and were sent back by the teacher by their own preferred method, whether it be through email, fax, or mail.

Hypothesis

My hypothesis was that the perception of music programs for different individuals would be significantly different. For the data I collected, my hypothesis was that music teachers would be concerned about details of funding and school support for an essential program while principals would say their program is properly funded and serves only a general recreational purpose. I expected the

latter to also seem more disconnected towards the program compared to their connection to core subjects. My expectation for other administrators was the same as principals. The data from other teachers what I expected to be the most diverse. Their perceptions of the music program would likely depend on their own interest in music and music education.

Conclusion

I was unable to get a conclusive answer to my hypothesis. I had too few participants to be able to prove or disprove my hypothesis. Out of the 80 schools that were contacted, only 20 responded to me. Overall, I only had 41 participants after over 320 educators were contacted. Out of those 41 participants, 30 were non-music teachers, 6 were music teachers, 4 replied with other, 1 was a principal, and no other administrators responded. The participants answered mostly how I expected them to. The principal somewhat agreed with the statement "Students do not get educational benefit from taking elective courses such as music". Even so, there were not enough participants to create a definitive answer.

Effects of Bisphenol A (BPA) and Two BPA Derivatives on Radish (*Raphanus raphanistrum* subsp. *sativus*) and Bean (*Phaseolus vulgaris*) Seed Germination and Growth

Nicole Matthews

McNair Program

Mentor: Dr. Darla Wise

Concord University

Abstract

Bisphenol A (BPA) is used in the production of plastic and resins. The chemical has been found to leach from the packaging into food, waterways, and the soil. This research will examine the effects of BPA and two derivatives of BPA on the germination of radish and bean plants as well as the overall growth of bean plants. The seeds were watered with various concentrations of each compound over the duration examined. The dry weight and length of the roots, stem, and leaves were measured to determine which compound had the most significant effects on plant growth. Both the radish and bean seeds had the lowest amount of growth for seed germination when watered with the 100mg/L solution of BPA. With regard to the overall growth of the bean plants, the 10mg/L solutions of BPA and BPS had the shortest juvenile leaves, and the 10mg/L solution of BPS had the shortest stems. The bean plants watered with the 100mg/L solution of BPA had the lowest amount of root growth.

Introduction

Through the production of everyday products, potentially harmful by-products can be produced as well. These products include the known human endocrine disrupting compound bisphenol A (BPA). Multiple studies done on this compound have led to the use of other similar compounds that are derivatives of BPA to produce BPA-free products (1). These include bisphenol S (BPS) and bisphenol F (BPF), which could potentially be just as harmful as BPA because of the similar structures the three compounds share. With a limited amount of research on these derivatives, it leads to questions about using them as replacements for BPA as safer options. The new discovery of another potential derivative for BPA, bisguaiacol F (BGF), could be a much safer option because it has been found to not interfere with the endocrine system (5). The effects of all of these compounds on animals have been thoroughly studied, but there is little research on the effects these compounds have on plants, especially those used as food sources. The radish seeds were chosen in this experiment due to the quick seed germination time. Pan et al. found that radish plants had decreased root length when exposed to 46mg/L solutions of BPA (3). The bean seeds were used due to the popularity of the plant as a food source in West Virginia. Studies done by Xia et al. and Sun et al. found that soybeans had an increase in root growth when exposed to low doses of BPA. Giuseppe et al. found that when watered with BPA solutions a decrease in dry weight and the length of the overall plant (6). This study will examine the effect of different concentrations of BPA and the different concentrations of BPF and BPS on the seed germination of radish and bean seeds, as well as the overall growth of bean plants.

Materials & Methods

Working Solutions

Stock solutions of the three compounds, Bisphenol A (BPA), Bisphenol S (BPS), and Bisphenol F (BPF) (Stigma Chemical, St. Louis, MO) were made by dissolving 100mg of each compound in a small amount of 70% ethanol and heating the solutions until the compound completely dissolved; this was then added to 100mL of tap water. Three working solutions of each compound were prepared in the concentrations of 100mg/L, 10mg/L, and 1mg/L. The solutions were stored in glassware at room temperature.

Seed Germination

Whatman filter paper was placed in each of the 100 glass petri dishes used. Two hundred fifty radish seeds and 250 bean seeds were randomly dispersed into groups of five and placed into a petri dish (Figure 3). Each control and experimental group received five petri dishes per seed type. The Whatman filter paper was saturated with 2mL of the corresponding solution on the first day and a cover was placed over each petri dish to prevent the seeds from drying out. For the remaining seven days 1mL of corresponding solution was pipetted onto the filter paper daily. The petri dishes were kept under fluorescent lights. When the seeds sprouted roots and/or stems, both were measured in millimeters. Data was collected over seven days at approximately the same time each day. The average lengths of both the stem and leaves and the roots were analyzed and compared using a t test from GraphPad Software (2).

Overall Growth

One hundred paper 12oz cups were used and filled partially with soil. Two hundred fifty seeds were dispersed randomly amongst ten cups per concentration (Figure 4). Five cups

contained three bean seeds and five cups contained two bean seeds. The seeds were all placed at the same depth and then watered with 20mL of corresponding solution on Monday and Friday. The cups were placed under fluorescent lights. Once the beans sprouted the length of the stems and juvenile leaves were measured daily. On the twenty-second day the plants were uprooted, and the length of the stems and leaves were measured. The plants were left to dry out over a two-day period and then the dry weight of the plants were obtained. The average lengths of the stems and leaves were analyzed and compared using a t test from GraphPad Software (2). The average dry weight for each concentration was also analyzed and compared using a t test from GraphPad Software (2).

Results

Seed Germination

The average lengths of roots and stems of 250 radish seeds (Figure 1) and the average root lengths of 250 bean seeds (Figure 2) were measured over a seven-day period. The radish and bean seeds watered with 100mg/L solution of BPA had the lowest amount of growth. The radish seeds watered in the different concentrations of BPF had much shorter roots and stems compared to the other experimental and control seeds. After collecting the data, a t test was performed using GraphPad Software (2). The *p* values that were below 0.05 were significant. These values can be seen in Table 1.

There was a significant difference between the tap water and the 100mg/L and 10mg/L solution of BPA for both roots and stems for radish seeds. There was also a significant difference between the tap water and the 10mg/L solution of BPS for both root and stem growth for radish seeds. All the BPF solutions had a significant difference compared to tap water in root growth

for radishes. There was also a significant difference between the 100mg/L and 10mg/L solutions of BPA and 10mg/L and 1mg/L solutions of BPA in both root and stem growth for radishes. For the bean seeds there was a significant difference between tap water and the 100mg/L solution of BPA in root growth.

When measuring the seeds, some bean seeds in certain compounds were observed to have thicker roots. The 1mg/L solution of BPS had thicker roots than the other two BPS concentrations (Figure 5). The 100mg/L solution of BPA had thicker roots than the 10mg/L solution of BPA. However, water had the thickest roots overall.

The radish seeds in certain compounds had more root hairs than others on average. The BPA concentrations of 1mg/L and 100mg/L had less root hairs that the 10mg/L of BPA solution and water (Figure 6). The BPF concentrations had less root hairs than the other solutions apart. The 100mg/L solution of BPS also had less root hairs compared to water and the 1mg/L solution of BPS.

Overall Growth

The average lengths of stems and juvenile leaves of 250 bean seeds were measured over a twenty-two day period. The seeds were watered with the 10mg/L solutions of BPA and BPS had the lowest amount of juvenile leaf growth (Figure 7). The seeds watered with 10mg/L solution of BPS had the lowest amount of growth for stem. The seeds watered with 100mg/L solution of BPA had the lowest amount of root growth. After collecting the data, a t test was performed using GraphPad Software (2). The *p* values that were below 0.05 were significant. These values can be seen in Table 2. After the plants dried out for two days the average dry mass was

measured (Figure 8). After collecting the data, a t test was performed using GraphPad Software (2). The *p* values that were below 0.05 were significant. These values can be seen in Table 3.

Significant differences were found when analyzing the different lengths and mass of the plants in each compound. When compared to tap water there was a significant difference in stem length to the 10mg/L BPA and all three concentrations of BPS. There were also significant differences in stem length between the different concentrations of each of the three compounds. There was no significant difference in the differences in juvenile leaf length between tap water and the three compounds. However, there were significant differences in juvenile leaf length between the different concentrations of each of the three compounds, except between the 100mg/L and 1mg/L solutions of BPS and the 10mg/L and 1mg/L solutions of BPF. There were significant differences in root length between tap water and the two lower concentrations of BPA and the two higher concentrations of BPS. There were also significant differences in root length for the different concentrations of BPA between the two higher concentrations, as well as the highest and lowest concentrations. Significant differences in root length were also found between the 10mg/L and 1mg/L solutions of BPS, and the 100mg/L and 1mg/L solutions of BPS.

When analyzing the mass for the plants in the different compounds significant differences were found. When compared to tap water, the 10mg/L solution of BPA, the 100mg/L and 10mg/L solution of BPS, and the 1mg/L solution of BPF had significant differences. When comparing the different concentrations to each other, the 10mg/L and 1mg/L solutions of BPA, the 100mg/L and 1mg/L solutions of BPA, the 100mg/L and 10mg/L solutions of BPS, the 100mg/L and 1mg/L solutions of BPS, and the 100mg/L and 1mg/L solutions of BPF.

Once the beans were sprouted and the measuring began, three sprouts in the 1mg/L BPF solution were observed to be growing upside-down with the cotyledons in the soil and the roots

in the air (Figure 9). On Day 18, one of these sprouts began to grow correctly. On Day 22, when the plants were uprooted one of the plants that were growing upside down had developed a second set of roots that were growing beneath the soil (Figure 10). The 1mg/L BPA solution had three sprouts with thicker stems than the other compounds (Figure 11). At least one sprout in the 100mg/L BPF solution and 100mg/L BPA solution had deformed or shriveled juvenile leaves (Figure 12). One sprout in the 100mg/L BPF solution had only one juvenile leaf growing (Figure 13). Some sprouts in the grew mature leaves but did not grow juvenile leaves on Day 18.

Conclusions

The production of plastics and resins also produce harmful by-products that are human endocrine disrupting compounds, such as bisphenol A (BPA). Research has found that BPA is harmful and has led to the introduction of BPA derivatives to replace BPA. These derivatives include bisphenol S (BPS) and bisphenol F (BPF), which are structurally similar to BPA. There is a limited amount of research to test the effects of these derivatives.

This experiment examined the effect BPA, BPS, and BPF have on seed germination in radishes and beans and the overall growth of bean plants. The seed germination portion of this experiment found that the root and stem lengths of radishes watered with tap water were significantly different to the higher concentrations of BPA, the highest concentration of BPS, and all concentrations of BPF (Figure 1) (Table 1). There was a significant difference in root growth of bean seeds between tap water and 100mg/L solution of BPA (Figure 2) (Table 1). The overall growth portion of this experiment found that the stem lengths of the bean plants watered with tap water were significantly higher than all concentrations of BPS, and the 10mg/L solution of BPA (Figure 7) (Table 2). The root length of the bean plants watered with tap water were significantly lower than the two lower concentrations of BPA and the two highest concentrations of BPS. The

dry mass of the plants watered with tap water was significantly higher than the 10mg/L solution of BPA, the two higher concentrations of BPS, and the lowest concentration of BPF (Figure 8) (Table 3). This data suggests that the three bisphenol compounds have significant effects on seed germination and overall growth of plants. Further research is being conducted to determine the effect of another derivative, bisguaiacol F (BGF) that is considered a much safer option on seed germination and the overall growth of plants.

Citations

1. Chen D, et al. (2016). Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity—A Review. *Environmental Science & Technology*, 50, 5438-5453.

2. GraphPad Software. 2018. QuickCalcs. https://www.graphpad.com/quickcalcs/ttest2/. Accessed 9-27-18.

3. Li X, Wang L, Wang S, Yang Q, Zhou Q, and Huang X. (2018). A Preliminary Analysis of the Effects of Bisphenol A on the Plant Root Growth via Changes in Endogenous Plant Hormone. Ecotoxicology and Environmental Safety, 150, 152-158.

4. Sun H, Wang L, and Zhou Q. (2012). Effects of Bisphenol A on Growth and Nitrogen Nutrition of Roots of Soybean Seedlings. Environmental Toxicology Chemistry, 32(1), 174-180.

5. Szafran AT, Stossi F, Mancini MG, Walker CL, and Mancini MA. (2017). Characterizing Properties of Non-Estrogenic Substituted Bisphenol Analogs using High Throughput Microscopy and Image Analysis. *PLos ONE*, 12(7), e0180141.

6. Xia B, Wang L, Nie L, Zhou Q, and Huang X. (2017). A Pathway of Bisphenol A Affecting Mineral Element Contents in Plant Roots at Different Growth Stages. Ecotoxicology and Environmental Safety, 135, 115-122.

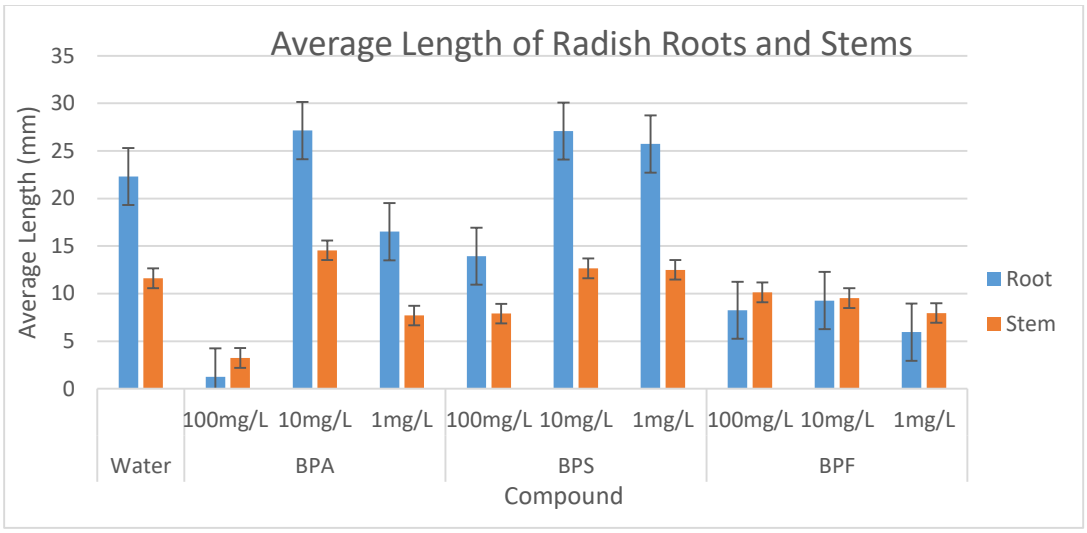


Figure 1: The average lengths (± SD) of 250 radish roots and stems were measured and recorded over a seven-day period. The seeds were watered daily with 1mL of corresponding solutions of tap water, or one of the three concentrations of bisphenol A (BPA), bisphenol S (BPS), or bisphenol F (BPF). The 100mg/L of BPA showed the lowest amount of growth in both roots and stems.

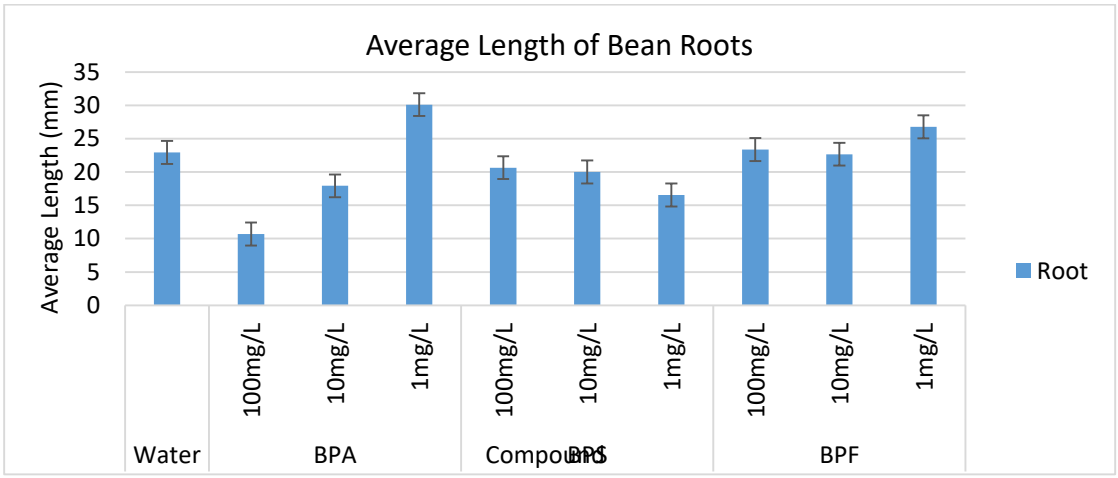


Figure 2: The average lengths (± SD) of 250 bean roots were measured and recorded over a seven-day period. The seeds were watered daily with 1mL of corresponding solutions of tap water, or one of the three concentrations of bisphenol A (BPA), bisphenol S (BPS), or bisphenol F (BPF). The 100mg/L of BPA showed the lowest amount of growth.

Setup for Seed Germination Experiment

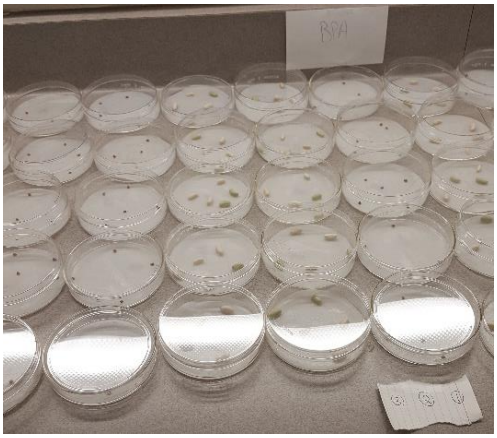


Figure 3: Two hundred fifty radish and two hundred fifty bean seeds were randomly dispersed into groups of five and placed into one of one hundred glass petri dishes. The petri dishes had a piece of Whatman filter paper that was saturated with 2mL of the corresponding solution. The petri dishes were covered and placed under fluorescent lights.

Setup for the Overall Growth Experiment

Figure 4: Two hundred fifty bean seeds were randomly dispersed between one hundred 12oz cups. Each concentration had ten cups and twenty-five seeds. The cups were filled partially with soil and then the seeds were placed on top of the soil and then covered soil so that all the seeds were at the same depth. The seeds were watered with 20mL of corresponding solution and placed under fluorescent lights.

T Test Values for Seed Germination

Group	Radish		Bean
	p Value		
	Root	Stem	Root
Water & 100mg/L BPA	0.0499	0.0526	0.0461
Water & 10mg/L BPA	0.0367	0.0316	0.0663
Water & 1mg/L BPA	0.1092	0.0545	0.1155
Water & 100mg/L BPS	0.0493	0.043	0.0773
Water & 10mg/L BPS	0.1317	0.0876	0.1014
Water & 1mg/L BPS	0.2539	0.3915	0.0619
Water & 100mg/L BPF	0.0494	0.0597	0.9352
Water & 10mg/L BPF	0.0498	0.054	0.709
Water & 1mg/L BPF	0.0483	0.0444	0.0733
100mg/L BPA & 10mg/L BPA	0.0471	0.0453	0.0739
10mg/L BPA & 1mg/L BPA	0.0444	0.0344	0.0855
100mg/L BPA & 1mg/L BPA	0.107	0.121	0.0793
100mg/L BPS & 10mg/L BPS	0.0624	0.0386	0.6655
10mg/L BPS & 1mg/L BPS	0.3095	0.887	0.0858
100mg/L BPS & 1mg/L BPS	0.0931	0.0871	0.1571
100mg/L BPF & 10mg/L BPF	0.0599	0.2078	0.7126
10mg/L BPF & 1mg/L BPF	0.0609	0.1141	0.0872
100mg/L BPF & 1mg/L BPF	0.066	0.0928	0.1714

Table 1: The t test *p* values of the three different compounds and water for radish and bean seeds. The significant *p* values are highlighted.

Comparison of Bean Root Thickness in Different BPS Concentrations

Figure 5: When observing the seeds, the 1mg/L solution of BPS had thicker roots than the other two BPS concentrations. A) bean in 100mg/L BPS solution; B) beans in 10mg/L BPS solution; C) bean in 1mg/L BPS solution.

Comparison of Radish Root Hairs in Different BPA Concentrations

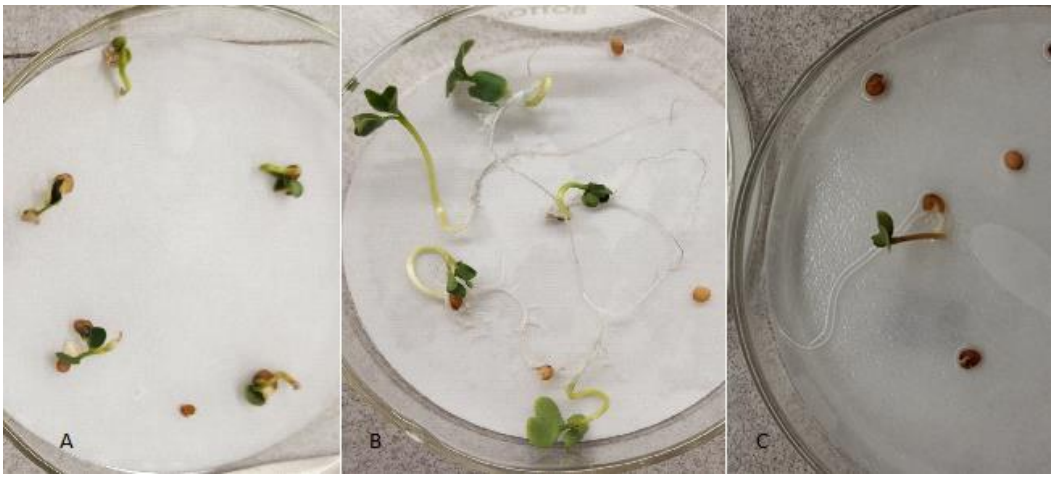


Figure 6: When observing the radish seeds, the number of root hairs on the seeds in the different BPA concentrations varied. The 10mg/L solution of BPA had more root hairs than the other two concentrations of BPA. A) bean in 100mg/L BPS solution; B) beans in 10mg/L BPS solution; C) bean in 1mg/L BPS solution.

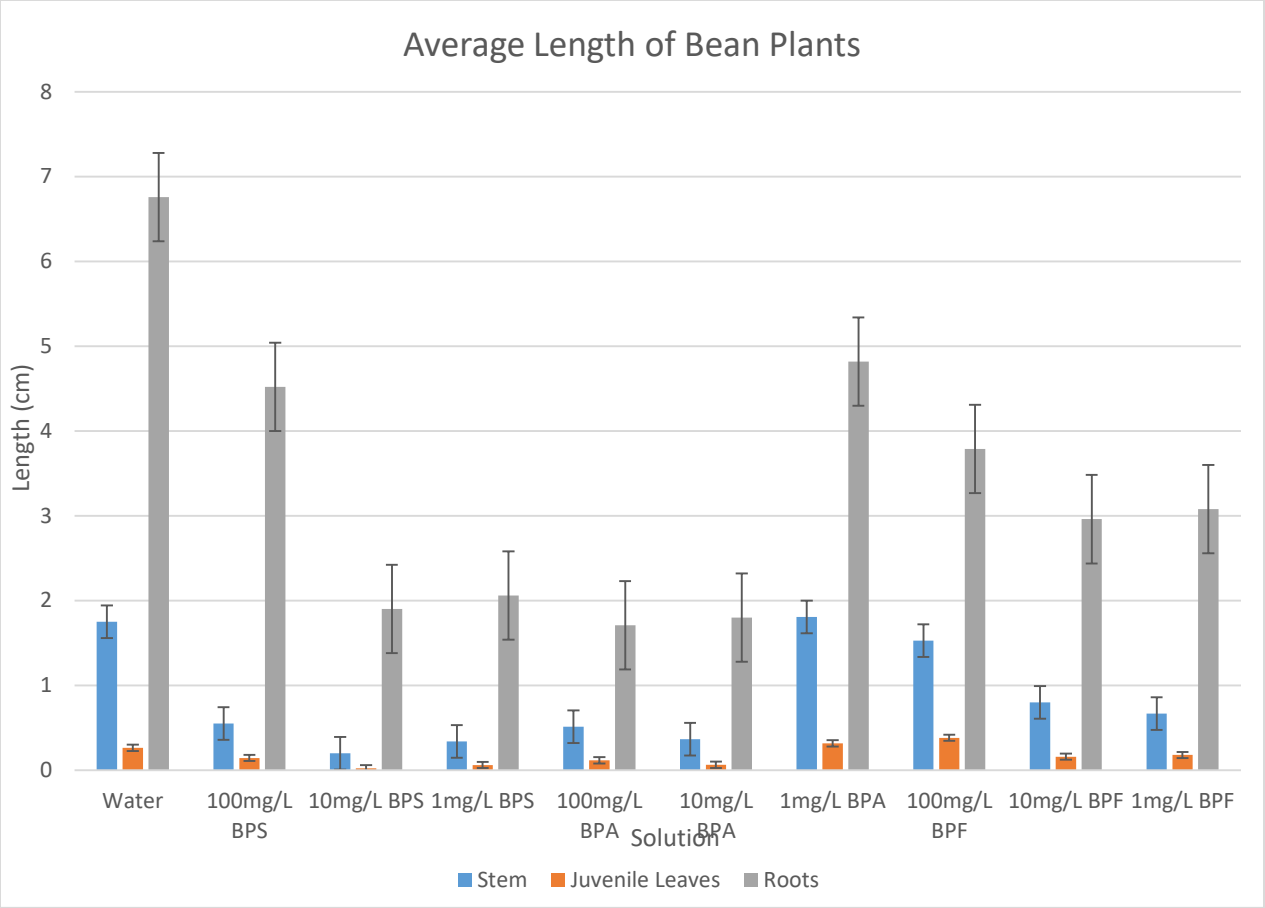


Figure 7: The average lengths (± SD) of 250 bean steams, roots, and juvenile leaves we measured over a twenty-two day period. The plants were watered on Monday and Friday with 20mL of corresponding solutions of tap water, and one of the three solutions of bisphenol A (BPA), bisphenol S (BPS), or bisphenol F (BPF). The 10mg/L solution of BPS had the lowest growth for juvenile leaves and stems with the 10mg/L solution of BPA having slightly more growth. The 100mg/l solution of BPA had the lowest amount of growth for roots.

T Test Values for Overall Growth

Group	*p* Value		
	Stem	Juvenile Leaves	Roots
Water & 100mg/L BPA	0.0660	0.0984	0.0997
Water & 10mg/L BPA	0.0455	0.0697	0.0008
Water & 1mg/L BPA	0.9224	0.0890	0.0011
Water & 100mg/L BPS	0.0313	0.2699	0.0036
Water & 10mg/L BPS	0.0318	0.0951	0.0045
Water & 1mg/L BPS	0.0328	0.7407	0.3494
Water & 100mg/L BPF	0.8004	0.4422	0.1403
Water & 10mg/L BPF	0.2294	0.8434	0.0696
Water & 1mg/L BPF	0.1670	0.5417	0.0894
100mg/L BPA & 10mg/L BPA	0.0011	0.0001	0.0064
10mg/L BPA & 1mg/L BPA	0.0004	0.0001	0.7988
100mg/L BPA & 1mg/L BPA	0.0012	0.0001	0.0032
100mg/L BPS & 10mg/L BPS	0.0165	0.0473	0.8710
10mg/L BPS & 1mg/L BPS	0.0079	0.0131	0.0097
100mg/L BPS & 1mg/L BPS	0.0272	0.0771	0.0147
100mg/L BPF & 10mg/L BPF	0.0011	0.0010	0.3781
10mg/L BPF & 1mg/L BPF	0.0001	0.3917	0.9328
100mg/L BPF & 1mg/L BPF	0.0001	0.0001	0.5490

Table 2: The t test *p* values of the three different compounds and water for the different lengths of the stem, juvenile leaves, and roots of the bean sprouts. The significant *p* values are highlighted.

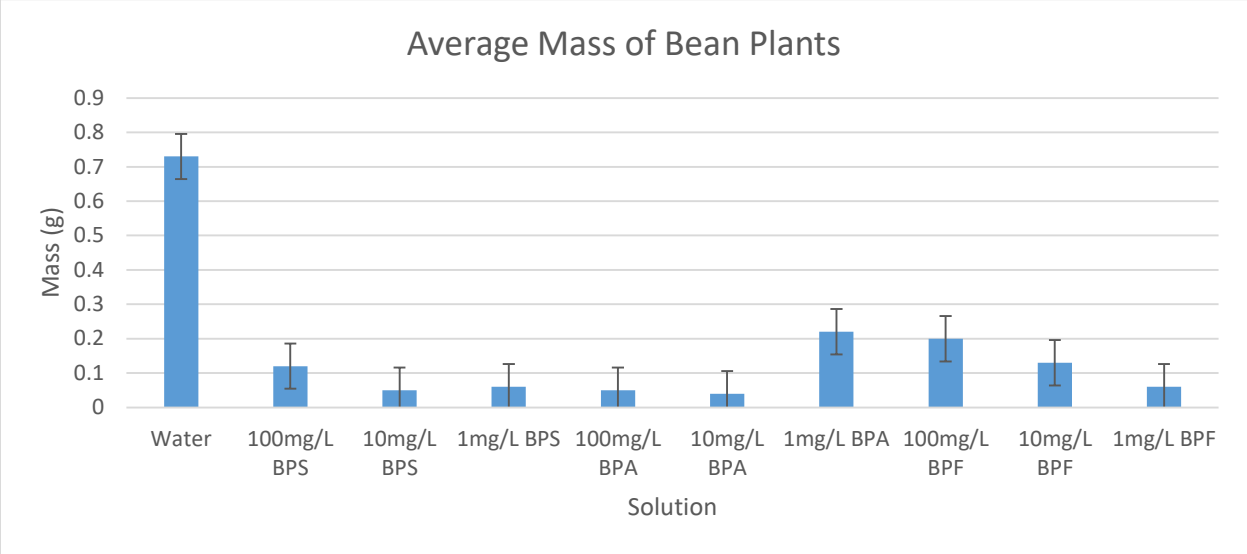


Figure 8: The average masses (± SD) of 250 bean plants were measured and recorded over a twenty-two day period. The plants were watered on Monday and Friday with 20mL of corresponding solutions of tap water, or one of the three concentrations of bisphenol A (BPA), bisphenol S (BPS), or bisphenol F (BPF). The 10mg/L of BPA showed the lowest mass.

Mass T Test *p* Values for the Different Compounds

T Test	
Group	P Value
Water & 100mg/L BPA	0.0752
Water & 10mg/L BPA	0.0436
Water & 1mg/L BPA	0.0501
Water & 100mg/L BPS	0.0441
Water & 10mg/L BPS	0.0403
Water & 1mg/L BPS	0.0971
Water & 100mg/L BPF	0.1125
Water & 10mg/L BPF	0.0822
Water & 1mg/L BPF	0.0460
100mg/L BPA & 10mg/L BPA	0.4650
10mg/L BPA & 1mg/L BPA	0.0047
100mg/L BPA & 1mg/L BPA	0.0109
100mg/L BPS & 10mg/L BPS	0.0016
10mg/L BPS & 1mg/L BPS	0.2863
100mg/L BPS & 1mg/L BPS	0.0030
100mg/L BPF & 10mg/L BPF	0.0974
10mg/L BPF & 1mg/L BPF	0.2808
100mg/L BPF & 1mg/L BPF	0.0140

Table 3: The mass t test *p* values of the three different compounds and water. The significant *p* values are highlighted.

Plants in the Lowest Concentration of BPF Growing with the Roots in the Air

Figure 9: Once the plants begun sprouting, some plants in the 1mg/L solution of BPF were growing upside-down with the roots in the air and the cotyledons in the soil.

Second Set of Roots Found on the Plants Growing Upside-Down in the 1mg/L Solution of BPF

Figure 10: After the plants were uprooted on Day 22, a second set of roots were found to be growing in the soil.

Differences in Stem Thickness Between the Different Compounds

Figure 11: A) plant watered with the 1mg/L solution of BPA; B) plant watered with the 100mg/L solution of BPF; C) plant watered with the 10mg/L solution of BPS; D) plant watered with the tap water. When measuring the plants, I noticed that plants 1mg/L solution of BPA had thicker stems than the other concentrations.

Shriveled or Deformed Juvenile Leaves on Plants in Certain Compounds

Figure 12: A) plant watered with the 100mg/L solution of BPA; B) plant watered with the 100mg/L solution of BPF. When measuring the plants, I noticed that some plants in the 100mg/L solutions of BPF and BPA had shriveled or deformed leaves.

Plant in the 100mg/L Solution of BPF Grew with Only One Juvenile Leaf

Figure 13: Once the plants were sprouted, one plant in the 100mg/L solution of BPF only had one juvenile leaf that grew.

Utilizing a GI transit Assay in Zebra Fish Larvae to screen for Autism Spectrum Disorder Related Genes

Brady Shrader, Dr. James Walters, Syed. M. Ali

Bluefield State College, Department of Applied Science and Mathematics, Bluefield, WV 24740

Abstract

Autism spectrum disorder (ASD) is a prevalent neurodevelopmental disorder. Patients with ASD often have comorbid digestive disorders including lower rates of intestinal motility and constipation. My project utilized an intestinal transit assay and forward genetic approach to develop a screen for genes linked to ASD. We fed 7 days post-fertilization larvae, larval food mixed with 1.0 μm FluoSpheres polystyrene beads for a duration of 3 hours. Larval intestines were scored every 3 hours for passage of the fluorescent microsphere. The average duration of intestinal transit was 18 hours. We then screened for a known gene that is linked to Autism Spectrum Disorder to validate our assay

Introduction

Autism Spectrum Disorder

Autism spectrum disorder is a comorbid disorder, this means both gastrointestinal and neurological dysfunctions are involved in Autism. [9] Autism is the most common neurological disorder worldwide, in fact, one in every one hundred and fifty children is diagnosed with ASD. Patients of Autism suffers from low intestinal motility, and constipation. [9] My project will be to utilize zebrafish in a transit assay of the gastrointestinal tract to screen for the gene that causes the Autism phenotype.

Why We use Zebra Fish

Zebrafish are used in studies on stress response, the cardiovascular system, metabolism, cancer, gastrointestinal disorders, neurological dysfunctions, and many more. [8] Seventy percent of zebrafish genes are humanlike. This makes zebrafish the perfect model animal for human disease. [8]

Studying zebrafish would allow for an essential step in future drug development. [9] One pair of zebrafish can produce upwards of one hundred to three hundred larvae in a year. [9] Embryonic development is rapid in zebrafish. Embryos execute evasive maneuvers upon touch within 24 h post fertilization. Organs including the gut are fully developed within five days post fertilization (dpf). Zebrafish larvae and early adults are optically transparent, this is useful for observing *in vivo* internal organs. [9] Zebrafish embryos are permeable to small molecules and drugs, providing easy access for drug administration. [9]

Materials and Methods

GI Transit Assay

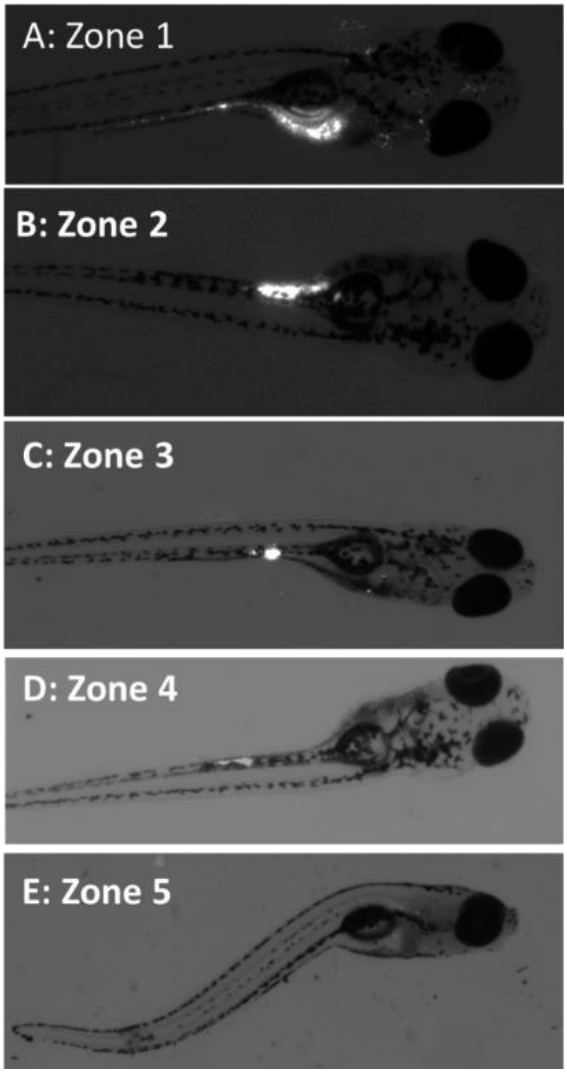
The GI Transit Assay was started by making a fluorescent tracer. The tracer was made by using a micropipette to mix 50uL of Deionized water with 2uL polystyrene microspheres (2% solid solution yellow green fluorescence), and100mg of Zebrafish Larvae food (Clarval AP 100 Micron Zeigler Bros inc.) making sure to measure the correct amount using a weigh boat and a scale (tearing the scale before using it) to form a paste. The paste was then spread on a piece of watch glass and left in a dark area overnight (to protect the florescence of the beads). The next day the tracer was scraped off of the watch glass using a razor blade and placed into an Eppendorf tube until further use.

The tracer was then fed to 58 zebrafish larvae aging at 7 days post fertilization. The tracer was placed in a Petri dish along with embryo media. The larvae were then allowed to swim in the petri dish with the fluorescent tracer/food for 1 hour. Only twelve larvae was placed in the petri dish at a time until all 58 larvae swam in the tracer The zebrafish where then placed in a Caron Refrigerated Incubator for three hours so that the tracer can fill the gut of each zebra fish larvae. Each larva was imaged to ensure that the zebrafish had a full gut before starting the transit assay of the gastrointestinal tract. Zebrafish without a full gut of tracer could not have been utilized in the assay.

The transit assay was continued by then imaging and scoring the larvae based upon where the tracer was in the Gastrointestinal Tract. The scoring was done based on the zones seen in (Figure 1). This was done at 3 different timepoints on a timescale of 20 hours. After the 20^{th} hour was imaged and scored, a chart was made using excel showing what larvae was in what zone, at what time point.

Figure 1: The zones of the Zebra fish larval Gastrointestinal tract

Figure 1: The zones of the zebra fish larval Gastrointestinal tract: (A)The first picture shows zone one in zebrafish larvae. The signal from the FluoSpheres in the intestine is right beside the swim bladder and gall bladder. Florescence in zone one must be distinguished from the natural florescence in the gall bladder. Therefore, we used beads that fluoresced in a channel with a longer wavelength than the background fluoresced. (B) The second picture displays zone two. Zone two begins right above the swim bladder and this is the zone where food will remain for most of the time and be digested properly. (C) Zone three starts where the swim bladder ends. (D) Zone four shows food on its way to exiting the digestive system. (E) Zone 5 shows the absence of fluorescent beads.



Zeiss discovery V8 Fluorescent Stereoscope

The microscope used to image the larvae was a Zeiss discovery V8 Fluorescent Stereoscope (see figure 2a) The stereoscope helped us take high quality images of the larvae by using the attached filters to enhance the fluorescence of the FluoSpheres seen in the images of the larvae. There are four attached filters on the Zeiss discovery V8 Fluorescent Stereoscope. (1) GFP 470 (2) Ds – Red (3) CY5 (4) M cherry. (see figure 2b) We chose which filter to use by first looking at the FluoSpheres and the larvae food under the microscope. We chose the filter that allowed the FluoSpheres to fluoresce the most while also allowing the food to darken the most. The filter that we chose to utilize is the M cherry filter because the filters excitation and emission allowed us to see the FluoSpheres the best.

Figure 2: Four filter set were tested to optimize fluorescent signal

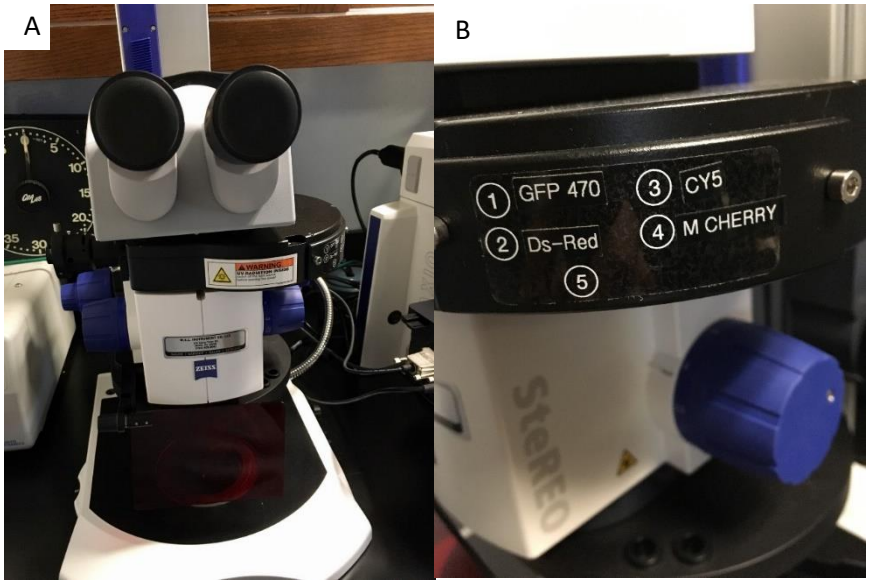


Figure 2: The microscope and filter set utilized to detect fluorescent microspheres: (A) The microscope used was a Zeiss Discovery V8 Fluorescent Stereoscope. This microscope helped us capture high quality images of the zebrafish during transit. (B) The microscope had 4 different filters that could have been utilized for this project. all of them are different excitations and emissions. This means that each lets in and out a certain amount of light.

Genomic Extraction

To do the genomic extraction, we must extract the DNA from the larvae tissue to use it as a DNA sample. In order to do this, we placed each piece of zebra fish larvae tissue in an individually labeled microcentrifuge tube containing 100 uL NaOH (the liquid should be submersive enough to allow for covering of the tissue) The tissue was then heated to 95°c or until the tissue was noticeably friable (15-20 minutes). The tubes was then cooled to 4°c by placing the tubes on ice, then adding $\frac{1}{10}^{\text{th}}$ volume of Tris to neutralize the basic solution. The tube was centrifuged and a pellet then formed at the bottom of the microcentrifuge tube. All of the liquid was then removed off of the pellet and placed in a different Eppendorf tube. The sample was then ready to be used for the PCR process.

Polymerase Chain Reaction

The primer used in the Polymerase Chain Reaction (PCR) was created with a mixture of 40 µL of Ultra-Pure H2O, 5 µL stock fw primer (795), and 5 µL stock fw primer (796) The mixture was a 1:10 dilution and was mixed using a 200 µL beaker. The beaker was then labeled and covered with foil until further use.

Dreamtaq master mix was then mixed with 1.0 µL of the primer and the 1 µL of prepped DNA samples the mixture was placed in separate Eppendorf tubes. The tubes where labeled 1-58 each number corresponding to a specific larva DNA. The tubes where placed into the thermocycler on Program 83.

Program 83 heats the DNA to 92°c causing the hydrogen bonds to break in the DNA otherwise knowns as the denaturation stage. Program 83 then cools down to 54°c this causes primers to attach to a specific location on the single-stranded template DNA by a way of hydrogen bonding,

this stage is called annealing. Program 83 then heats up to 72°c which enables the new DNA to be made by the dreamtaq polymerase enzyme which adds DNA bases. Program 83 cycles through these steps 35 times eventually stopping the cycles at 72°c. the thermocycler then cools down to 8°c to end the PCR. The samples where then placed in the freezer until further use.

Agarose Gel

1.5g of metaphor Agarose and 0.6 grams of regular Agarose for a total of 2.1g of Agarose was mixed with 40µL of TAE Buffer into a 200µL beaker. The beaker was then placed on a magnetic stirrer with a hot plate. A magnet was dropped in the beaker to maintain constant stirring. The beaker was heated until the Agarose was clear. Then was placed at a temperature of 65°c to cool before pouring the gel. Placing the beaker back to the magnetic stirrer every 15 minutes to get rid of the bubbles.

The Agarose gel was then poured into a gel mold. Combs was added to the mold, assuring that the combs where not touching the floor or the walls of the mold (this will cause a whole going through the gel). The combs will create wells in the Agarose gel to place the DNA samples in. It takes approximately 30 minutes for the gel to solidify.

Once the gel had solidified the comb was gently removed, making sure not to damage the wells or the gel while removing the comb. The gel was then placed into the gel electrophoresis machine (used for running gels). TAE buffer was added to each side of the gel electrophoresis machine allowing the TAE Buffer to cover the gel partially. A gel map was created showing where in the gel each sample will be located. A 100 base pair ladder was added to the first well in the gel using a micropipette. The DNA samples where then added to the gel from left to right, making sure the micropipette tips were changed between each sample. A wild type control was added into one of the wells to ensure that we get accurate results when genotyping the Wildtype

larvae. The top of the gel electrophoresis machine was then attached and the power source was plugged in and turned on. This caused an electrical current to shoot into the TAE buffer causing small bubbles to appear in the TAE. The negative end of the gel electrophoresis machine pushed the samples through the gel towards the positive end of the machine. This process takes approximately 2 hours to complete.

Once the samples finished running through the gel, they had to be taken out of the gel electrophoresis machine and stained using Ethidium Bromide ($C_{21}H_{20}BrN_{3)}$. The gels had to be stained to brighten the fluorescence seen in the samples, this makes it easier to image the gels and determine the genotype of the larvae.

Genotyping

Genotyping the Larvae was done by imaging the stained Agarose Gels and analyzing the bands of DNA in each sample. The Wild Type control told us that a Wild Type Genotype should have a lower band, where as a Mutant should have an upper band, and a heterozygous genotype should have both an upper and a lower band. However, we did not have any mutant genotypes, only Wildtypes and heterozygous larvae where utilized in this transit assay. The bands where labeled to show what band(s) belonged to which larvae (see figure 3). The results where charted down into an excel file and a bar graph to show the differences between genotype transit times.

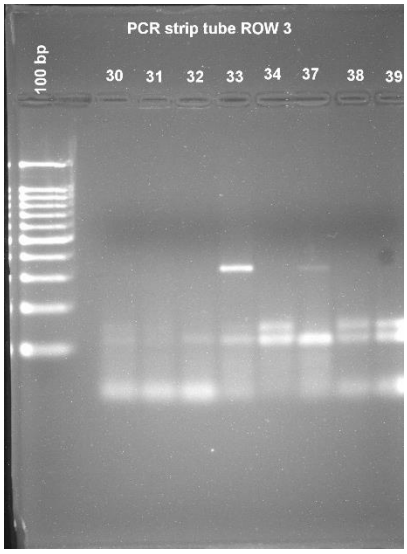


Figure 3: Agarose Gel

Results

The results of the genotyping were charted onto an Excel chart and analyzed carefully looking for fluctuations in transit time. One note that was made was that a few of the genotyped larvae was not passing the tracer properly. For example, a heterozygous larva would start at zone 1 during time point 1 then jump to a zone 4 at timepoint 2, finally at timepoint 3 the larva would go back to zone 2. This could be because of three reasons, the larvae defecated then consumed the tracer again causing the transit assay to restart, the larvae had an intestinal blockage and the larvae's GI tract pushed the tracer back up, or the larvae GI tract is working backwards.

Table 1: The chart in Table 1 shows how many of the Wildtype larvae (+/+) was in each zone of transit and at which time point.

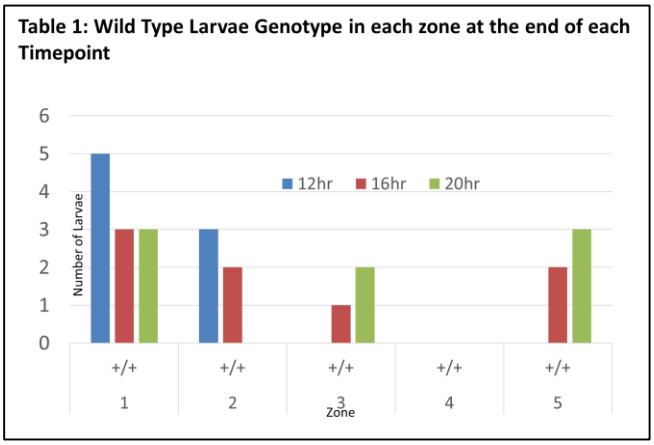
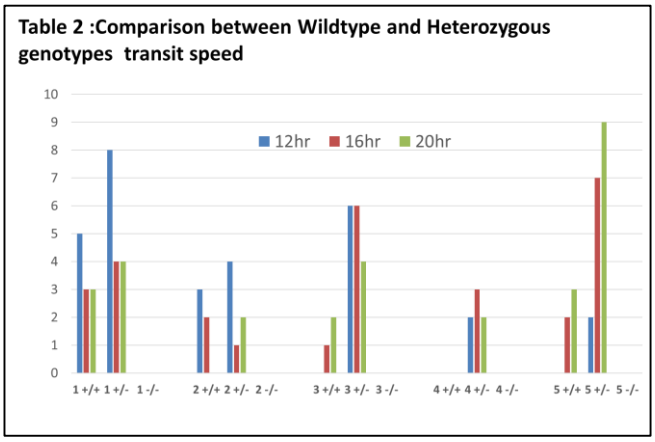


Table 2: The Table 2 chart compares the transit times of the heterozygous and Wild type larvae zebra fish. We are looking for slow transit times, which tend to occur more in the heterozygous genotype. By looking at the results you can see a fluctuation in between transit zones among the heterozygous larvae. (e.g. a zone 5 to a zone 2).



The ChD8 Gene

The known Autism Spectrum disorder gene that we think may be linked to the DNA is the ChD8 gene (Chromodomain Helicase DNA Binding Protein 8). [4] The Gene is a protein coding Gene, meaning that it is caused from a nucleotide deletion. People who have a mutation in the ChD8 gene are very likely to be diagnosed with Autism Spectrum Disorder, and these people will have autism marked with Gastrointestinal symptoms as well. [4]

Future Directions

Screening for a known ASD gene

Our future directions for this Transit assay experiment includes screening for the ChD8 gene (a gene linked to Autism Spectrum Disorder) using a reverse screening process. To do this we have to look at the nucleotides in a strand of DNA to find where the nucleotides form a mutation in the Gene. (see Figure 4)

Figure 4: Screening for mutated genes

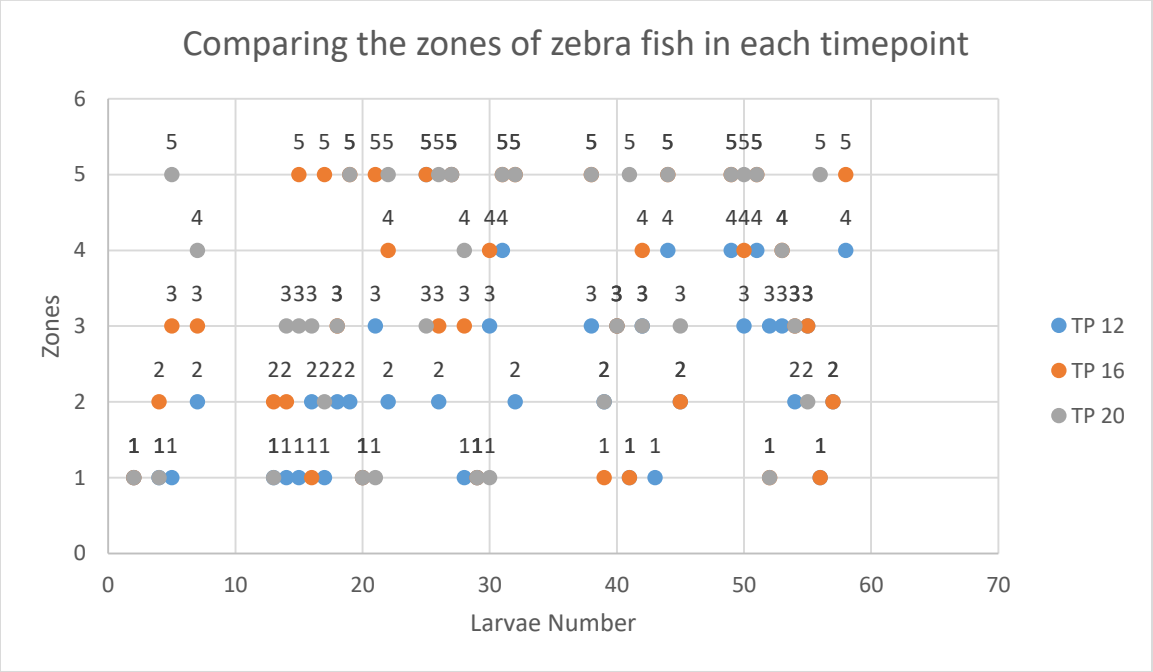
A	**Unmutated Strand of DNA** **ATGCAATGCAATGCAATGCAATGCAATGCAATGCAATGCAATGCAATGAA**
B	**Mutated Strand of DNA** **ATGCAATGCAATGCATTGCAATGCATTGCAATGCAATGCAATGCAATGCAA**

Figure 4A shows what the nucleotides in a normal strand of unmutated DNA looks like. However, Figure 4B shows what the nucleotides in a mutated strand of DNA does to form a mutation. There is a deletion in the DNA strand causing a mutation in the DNA strand.

Conclusion

In conclusion, Autism Spectrum Disorder is one of the most common neurological disorders worldwide. [9] In the United States 1 in every 150 children are diagnosed with autism This being said it is critical to research how to increase the diagnosis rate of Autism Spectrum Disorder.

Appendix A: Comparing the zones of zebra fish in each timepoint



Appendix A is a chart that shows the different zones that each individual zebrafish during each timepoint. The chart shows that there are multiple of the zebra fish larvae that may suffer from gastrointestinal issues such as larvae 15. Larvae 15 shows the larvae starting in Zone 1, then at TP 16 it moves to Zone 5 but the at zone 20 the tracer moves backwards to zone 3. This shows the gastrointestinal system may not be operating properly and is a sign of Autism.

Appendix B: Experiment Timeline

Experiment Pre

Step	Time Required	How Many Times	Total Time Required
Raising Larvae	**7 days**	**Once per trial**	**7 Days**
Making Florescent Tracer	**24 hours**	**Once per trial**	**24 hours**

Experiment

Step	Time Required	How Many Times	Total Times Required
Feeding the larvae, the tracer in a Petri Dish	**1 hour**	**5**	**5 hours**
Placing the Larvae in individual wells in a twelve well plate	**1 hour**	**Once per trial**	**1 hour**
Letting the tracer fill the larvae's gut	**3 hours**	**5 (but the times was staggered)**	**7 hours**
Image all of the larvae	**1 hours**	**Once per trial**	**1 hour**
Transit assay	**20 hours**	**Once per trial**	**20 hours**
scoring	**3 hours**	**Once per trial**	**3 hours**
Genomic extraction	**3 hours**	**Once per trial**	**3 hours**
Polymerase Chain Reaction	**5 hours**	**Once per trial**	**5 hours**
Making the Agarose Gel	**4 hours**	**Per gel**	**4 hours**
Loading samples into the Gel	**20 minutes**	**Per gel**	**20 minutes**
Running the gel	**2 hours**	**Per gel**	**2 hours**
Staining the gel	**1 hour**	**Per gel**	**1 hour**
Imaging the gel	**20 minutes**	**Per gel**	**20 minutes**
Genotyping	**5 minutes**	**Per gel**	**5 minutes**
Making a chart of results	**1 hours**	**Once per trial**	**1 hour**
Analyze results	**30 minutes**	**Once per trial**	**30 minutes**
Total time			**10 days** **10 hours** **15 minutes**

Appendix C: Material Reference

Material	Can Purchase From
• Agarose	**Lonza** **Rockland, Maine, USA** **207-594-3400** **www.Lonza.com**
• "Thermoscientific" Dreamtaq Green PCR Master Mix (2X)	**Thermos Fisher Scientific Baltics UAB** **V.A Graiciuno 8. L.T.02241 Vilnius, Lithuania**
• Ethidium Bromide stock solution 10 mg/ml in aqueous solution, Bio Technology Grade	**VWR International** **28600 Fountain Parkway, Salon Ohio, 44139**
• FluoSpheres Carboxylate 0.02um yellow/green (505/515)	**Life technology corporation, 29851, Willowcreek RD, Eugene, Oregon**
• Metaphor Agarose	**Lonza** **Rockland, Maine, USA** **207-594-3400** **www.Lonza.com**
• NaOH	**Lonza** **Rockland, Maine, USA** **207-594-3400** **www.Lonza.com**
• Primers	**Lonza** **Rockland, Maine, USA** **207-594-3400** **www.Lonza.com**
• 10X TAE Buffer	**VWR International** **28600 Fountain Parkway, Salon Ohio, 44139**
• tris	**VWR International** **28600 Fountain Parkway, Salon Ohio, 44139**
• 100 base pair ladder	**VWR International** **28600 Fountain Parkway, Salon Ohio, 44139**

References

1 A, Rich. (2009). A new high-content model system for studies of gastrointestinal transit: the zebrafish. a US Government work

2. Anna V. Golubeva, S. (2016). Microbiota-related Changes in Bile Acid & Tryptophan Metabolism are Associated with Gastrointestinal Dysfunction in a Mouse Model of Autism. Elsevier

3. Brady, C. (2017). Intestinal Transit Time and Cortisol-Mediated Stress in Zebrafish. ZEBRAFISH Volume 14, Number 5, 6

4. Database, G. (n.d.). CHD8 Gene (Protein Coding). Retrieved from https://www.genecards.org/cgi-bin/carddisp.pl?gene=CHD8

5. Field, H. A., Kelley, K. A., Martell, L., Goldstein, A. M., & Serluca, F. C. (2008). Analysis of gastrointestinal physiology using a novel intestinal transit assay in zebrafish,

6. Kitazawa, T., Yoshida, M., Teraoka, H., & kaiya, H. (2017). Does motilin peptide regulate gastrointestinal motility of zebrafish? An in vitro study using isolated intestinal strips. Elsevier Inc

7. Reddy Bhaskara Lakshmi, Saier Milton H, (2015) Autism and Our Intestinal Microbiota, Journal of Molecular Microbiology and Biotechnology

8. G. Karl, Rodeck Ulrich, Dicker P Adam An Emerging Model System for Human Disease and Drug Discovery

9. Burke Elizabeth, August 8th 2016 Why Use Zebrafish to Study Human Diseases? What are zebrafish?

10. Santacroce Luigi, Intestinal Motility Disorders 2017, Medscape Information

Acknowledgements

The Walters Lab is funded by WV-INBRE Grant# 2P20GM1.3434_14 P1500699. NIH Grant P20GM103434 to the West Virginia Idea Network for Biomedical Research Excellence. We would like to thank the McNair Scholars Program for support of Brady Shrader's research in the Walters Lab.

Gender, Age, and Sports Affiliation as Predictors of Body Dysmorphia

Keira Wickline

Mentor: Karen Griffee, Ph.D.

Concord University

McNair Scholars Program

Gender, Age, and Sports Affiliation as Predictors of Body Dysmorphia

Body Dysmorphic Disorder, also known as Body Dysmorphia, is a relatively little-known mental disorder that is becoming more prevalent in today's time. BDD (Body Dysmorphic Disorder) is now recognized in the DSM V as on the Obsessive-Compulsive Disorder (OCD) Spectrum (Dehbaneh, 2018). This disorder is defined by Katherine Phillips who quotes the American Psychiatric Association, "Body dysmorphic disorder (BDD), classified in DSM-5's new chapter of Obsessive-Compulsive and Related Disorder, is characterized by distressing or impairing preoccupation with one or more perceived defects or flaws in appearance that are not observable or appear only slight to others." One that is diagnosed with BDD will have an irrational obsession with one or more parts of his/her body that impairs their daily functioning. The obsession could be with any one part of the body and it varies from person to person. However, the body part that is the focal point is typically not one that is large, prominent, or unusual enough to be noticed by everyday people (Grace, 2018).

The prevalence of Body Dysmorphic Disorder seems to be rising (Taqui, 2008). Current research shows that the prevalence rate of BDD in the general population ranges from 0.7-2.4% (Nieuwoudt, 2015). Research also show that the rates are increasing for many reasons, one of which is that it is being recognized as a diagnosable disorder which has led to an increase in the number of cases being reported. Another possible reason for the increase in the number of cases being reported may be the effect of advertising, in which increasing numbers of ads being put out onto the Internet, especially those embedded into social media. These ads, along with the typical onslaught of television ads, may induce both young and older people to look at themselves more critically. For example, Taqui conducted a study on BDD that included males and females. One of the factors that was examined was whether the participants compared his/herself to people that

were on television. Eighty-five percent of the female participants reported that they had compared their bodies to those that they saw on television (Taqui, 2008). The same study also examind gender as a factor in the amount of BDD symptoms that were present in the students. The study consisted of 156 students, 67 male and 89 female. Of the 156 participants, approximately 78% reported that he/she was not satisfied with their body (Taqui, 2008).

Many different parts of the body can become the focus of BDD. Grace (2017) reported that some of the most common parts of the body of concern were the face, skin, hair, and nose. As for the symptoms of Body Dysmorphic Disorder, there are some common patterns. The following behaviors have been the most reported: mirror checking, excessive grooming, excessive makeup, skin cleansing, seeking reassurance, and comparing oneself to others (Dehbaneh 2018, Grace 2017).

The cause(s) of Body Dysmorphic Disorder are not known, however some research has investigated this question. For example, neurological research conducted by Grace (2008) has shown that in some patients there seems to be abnormal visual processing occurring in the brain. She quotes Deckersback et al. (2000) in saying that, "…neuropsychological data demonstrates abnormal patterns of visual information processing in BDD patients, including the selective recall of details rather than global features, of visual information from faces and figures." In other words, the visual processing pathways in BDD patients have a different way of viewing images all together. Instead of seeing the general idea, or bigger picture, of an image, the patient sees the details first. She states that this abnormal way of visual processing could be leading the patients to focus on one detail of their body/face instead of seeing their body/face as a whole.

Another researcher, Dehbaneh (2018), suggested an environmental cause. She suggested that early maladaptive schemas and dysfunctional attitudes could lead to Body Dysmorphic

Disorder. A schema is a thought process that allows a person to process information. So, a maladaptive schema is one that is created that is abnormal and that has negative effects on a person. Dehbaneh suggests that the maladaptive schemas could be formed from negative childhood experiences or from societal influences. She states that maladaptive schemas that are formed while young tend to be activated subconsciously when the person is older. A dysfunctional attitude is a way of thinking that does not fit in with the norms of society. Dehbaneh's research suggests that people with maladaptive schemas and dysfunctional attitudes correlated with one having BDD symptoms.

Similarly, Menees (2013) found that those who received critical comments and criticisms from those close to them were more likely to have/obtain symptoms of Body Dysmorphic Disorder. This was found to be true especially in the male population of the study, which found that males who received criticisms about their body from their parents were more dissatisfied with their body than those who did not.

Those diagnosed with BDD tend to have higher rates of depression associated with suicide and suicidal thoughts. Also, because of shame and a desire for privacy, most people diagnosed with BDD do not report their symptoms. This surely contributes to the low rate of diagnosis and mistreatment of the disorder (Grace 2017). There have been a couple of treatment options that have been investigated. For example, one study examined the effect of schema therapy on those diagnosed with Body Dysmorphic Disorder. It was found that schema therapy generally increases one's positive thoughts about his/her body (Debhaneh 2018). However, no such other research has been found. If one of the underlying causes of BDD is because of maladaptive schemas, then schema therapy would make sense as a treatment option. However, if the other explanation of BDD, abnormal visual pathway processes, is the underlying cause of

BDD then schema therapy may not be the best option for the patients. In contrast, if both of the explanations mentioned are underlying causes of Body Dysmorphia symptoms, then perhaps therapy and a type of medication that could be used to help counteract BDD symptoms. Further research needs to be conducted on the treatment of Body Dysmorphic Disorder.

Few studies have identified any variables associated with the diagnosis of BDD. Regarding the possible effects of gender, Taqui (2008) found that while more women reported being dissatisfied with their bodies, men reported more symptoms of BDD. That finding is particularly interesting, given that women are more likely to be diagnosed with anxiety disorders (McLean 2011). Age has not been examined in relation to BDD; however, age has been studied in anxiety disorders such as Panic Disorder and Generalized Anxiety Disorder. In research about those disorders, it was found that generally the disorders begin in the younger years, typically during adolescence (Ramsawh 2011). Another variable that would seemingly relate to BDD is sports affiliation. This variable has not been researched as a correlation to BDD, with the exception of weightlifting. Weightlifting in adult males was shown by Nieuwoudt (2015) to correlate with Muscle Dysphoria, which is considered a subcategory of Body Dysmorphia. Muscle Dysmorphia is defined as a fixation on how much muscle one has that interferes with his/her daily life. The study found that the number of participants that were at risk of BDD in his study was comparable to the amount of people diagnosed with BDD in the general population.

Methods

Participants

There were 61 participants in the current study; 22 males and 39 females. The participants were students at Concord University. The average age of the participants was 20 years old. The students were gathered via convenience sample.

Design

The experiment was a survey/questionnaire with a between groups design. There was one independent variable for the experiment and that was the score on the YBOCS-BDD questionnaire. Demographic information was acquired to test against the score on the questionnaire. The demographic information included age, gender, and sports affiliation.

Procedure

The experiment was approved by the campus HSRB. All the participants received and signed an informed consent form before taking the two-part questionnaire. The first section of the questionnaire was the demographics questionnaire asking for the participant's age, gender, and if the student participated in any sports. The second section of the questionnaire was the YBOCS-BDD (Yale Brown Obsessive-Compulsive Scale for Body Dysmorphic Disorder). The YBOCS-BDD was a 12-question survey to assess symptoms of the disorder in the participants. The questionnaire was in no way diagnostic in nature in the experiment, it was used to see how many symptoms were present, if at all, in the participants.

The questionnaire was given by the researcher to the students either during or after the class lecture that the students were in at the time. The informed consent form was distributed

first and once those forms were collected, the questionnaire was given out to the participants. The participants had approximately 10 to 15 minutes to complete both sections of the questionnaire, with time varying according to the professor where the questionnaire was distributed. The finished survey was then collected by the researcher and taken to be analyzed.

Results

There were three hypotheses for the current experiment. The first was that males would have a higher rate of Body Dysmorphic symptoms than females. The second was that the younger participants (ages 18-20) would have a higher rate of Body Dysmorphic symptoms than the older participants (ages 21 and up). The last was that those affiliated with sports would have a higher rate of Body Dysmorphic symptoms than those that were not affiliated with sports.

The data collected did not support any of the three hypotheses. The first hypothesis that males would have a higher rate of Body Dysmorphic symptoms was not supported. Males had a mean YBOCS-BDD score of 8.64, while females had a mean score of 14.05. An Independent Sample T-Test was used to analyze the data in two ways. The first was gender and the score of the YBOCS-BDD questionnaire, $t(59) = -2.26$, $p=0.43$. The second analysis was gender and the number of symptoms the participant logged on question 6 of the YBOCS-BDD questionnaire, $t(59)= -4.61$, $p=.615$. The second hypothesis, that younger participants would have a higher rate of Body Dysmorphic symptoms was not supported. A Pearson Correlation test was conducted on the data in two ways. The first was a correlation between age and YBOCS-BDD score, $r=0.083$, $p=.523$. The second was between age and the number of symptoms logged on question 6 of the YBOCS-BDD questionnaire, $r=-.041$, $p=.951$. The third hypothesis, those affiliated with sports would have a higher rate of Body Dysmorphic symptoms was not supported. An Independent Samples T-Test was conducted on the data in two ways. The first was the score of the YBOCS-

BDD questionnaire and whether the participants were affiliated with a sport, t (59) = 1.118, p=.224; there was no significant difference. The second was the number of symptoms logged on question 6 of the YBOCS-BDD questionnaire, t (59) = 1.825, p=0.073. A Multivariate Analysis of Variance was conducted to assess if there was an interaction between whether a participant played a sport and his/her gender. There was no significant interactions between sport and gender were found, both Fs (1.57) ≤ 2.55, p ≥ 0.11.

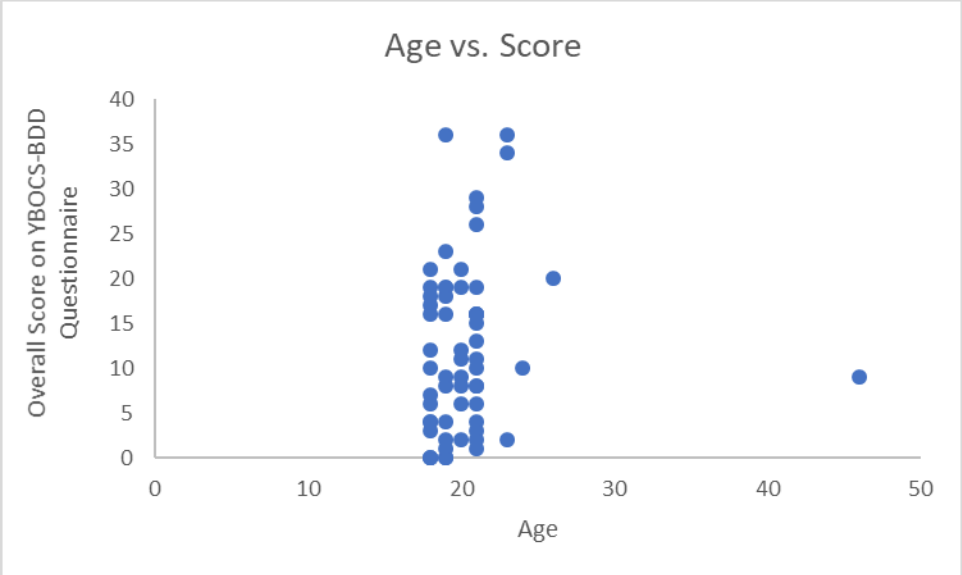


Figure 1. The graph shows the relationship between the age of the participants and the overall score on the YBOS-BDD questionnaire. There was no significant difference found between the two: r=0.083, p=.523.

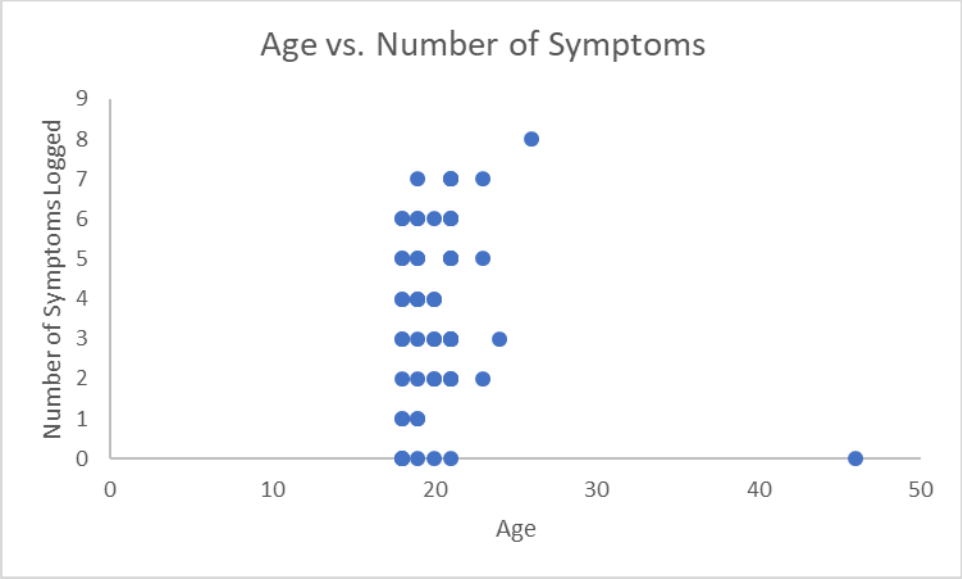


Figure 2. The graph shows the relationship between age and the number of symptoms logged by the participants on question number 6 on the YBOCS-BDD questionnaire. There was no significant difference found: r=-.041, p=.951.

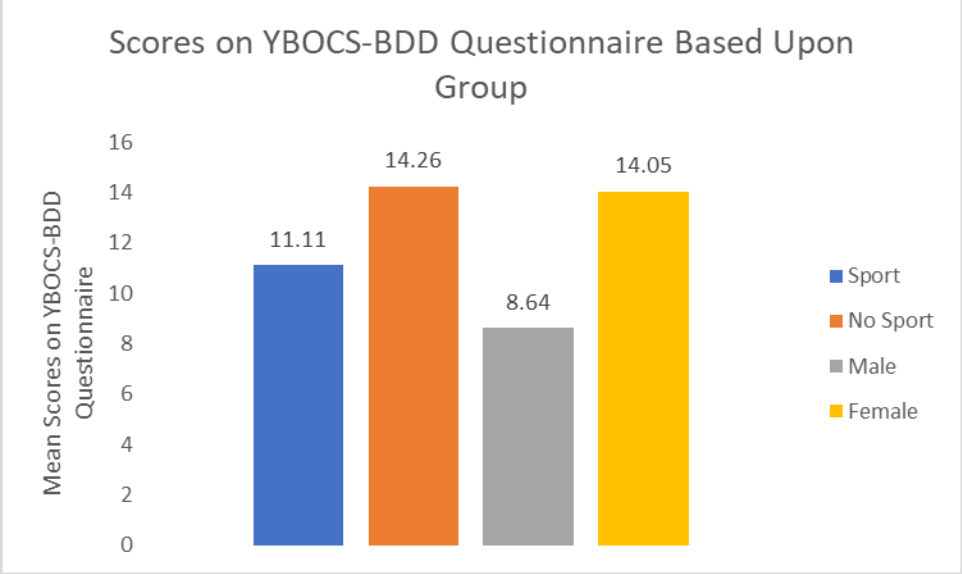


Figure 3. The graph portrays the relationship between groups sport, no sport, male, and female. The bars show the group mean scores on the YBOCS-BDD questionnaire for each group. Group Sport had a mean score of 11.11. Group No Sport had a mean of 14.26. Group Male had a mean

score of 8.64. Group Female had a mean score of 14.05. The graph visually shows that no significance was found between the average scores in males and females and in participants who logged that he/she played a sport and those that did not log that he/she played a sport.

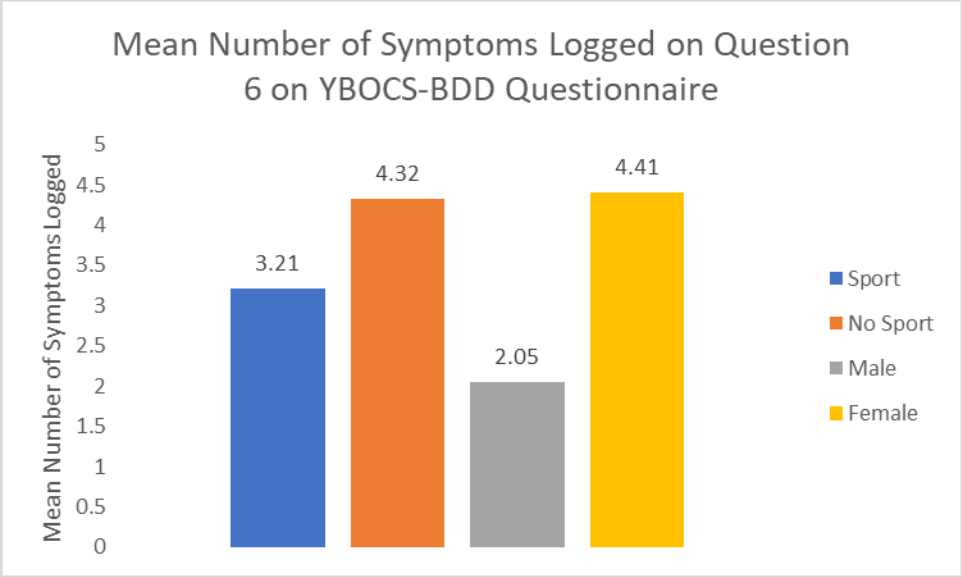


Figure 4. The graph shows the mean number of symptoms logged on question number 6 of the YBOCS-BDD questionnaire. The graph further portrays that no significance was found within Group Gender and Group Sport. Group Sport had a mean number of symptoms of 3.21. Group No Sport had a mean number of 4.32 symptoms. Group Male had a mean of 2.05 symptoms. Group Female had a mean score of 4.41 symptoms logged.

Discussion

The data did not support the three hypotheses in the current experiment. The data showed that there were no significant effects found between age, gender, or sports affiliation. The results from the current experiment did not coincide with research conducted by Taqui and collegues (2008), which stated that while women were overall more dissatisfied with their bodies, the men noted more Body Dysmorphia symptoms. The data also did not coincide with Ramsawh (2011)

which stated that younger people, generally those in adolescence, were more likely to be diagnosed with Anxiety Disorders. The data did not agree with Nieuwoudt (2015) which found that male weightlifters logged having more Body Dysmorphia related symptoms than those that did not participate in weightlifting.

There were a couple of reasons why these results could have occurred. The first is that the subjects did not seem to take the survey seriously. Multiple students were noticed discussing their answers with their peers and making jokes about the information being asked. Because of this, it may be beneficial in further research to have the participants take the survey alone. Additionally, it may be beneficial for the survey to be condensed or simplified in some fashion. Some of the participants complained to the researcher after their participation that they did not know how to answer some of the questions.

The rate of Body Dysmorphic Disorder is increasing in today's population. Those conflicted with BDD tend to have higher rates of depression associated with suicide and suicidal thoughts. Also, because of shame and privacy most people conflicted with BDD do not report their symptoms leading to the low rate of diagnosis and mistreatment of the disorder (Grace 2017).

Those diagnosed with BDD tend to have higher rates of depression associated with suicide and suicidal thoughts. Also, because of shame and a desire for privacy, most people diagnosed with BDD do not report their symptoms. This surely contributes to the low rate of diagnosis and mistreatment of the disorder (Grace 2017). There have been a couple of treatment options that have been investigated. For example, one study examined the effect of schema therapy on those diagnosed with Body Dysmorphic Disorder. It was found that schema therapy generally increases one's positive thoughts about his/her body (Debhaneh 2018). However, no such other research has been found. If one of the underlying causes of BDD is because of maladaptive

schemas, then schema therapy would make sense as a treatment option. However, if the other explanation of BDD, abnormal visual pathway processes, is the underlying cause of BDD then schema therapy may not be the best option for the patients. In contrast, if both of the explanations mentioned are underlying causes of Body Dysmorphia symptoms then perhaps therapy and a type of medication would be the best choice of treatment. Currently, no research has been conducted to identify medication that could be used to help counteract BDD symptoms. Further research needs to be conducted on the treatment of Body Dysmorphic Disorder.

References

Dehbaneh M.A. & Bahrainian S.A. (2018). The comparison between early maladaptive schemas and dysfunctional attitudes and coping strategies in people with Body Dysmorphic Disorder and healthy people in a study population in Tehran. *World Family Medicine/Middle East Journal of Family Medicine, 16(1).*

Nieuwoudt J.E., et al. (2015). Symptoms of Muscle Dysmorphia, Body Bysmorphic Disorder, and Eating Disorders in a nonclinical population of adult male weightlifters in Australia. *Journal of Strength and Conditioning Research, 29.* 1406-1416.

McLean C.P., et al. (2011). Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *Journal of Psychiatric Research, 45.* 1027-1034.

Grace S.A., et al. (2017). The neurobiology of Body Dysmorphic Disorder: a systematic review and theoretical model. *Neuroscience and Biobehavioral Reviews, 82.* 83-97.

Taqui A.M., et al. (2008). Body Dysmorphic Disorder: gender differences and prevalence in a Pakistani medical student population. *BMC Psychiatry, 8(20).* 1-10.

Bray, Sean. (2015). Gender Dysphoria, Body Dysmorphia, and the problematic of body modification. *Journal of Speculative Philosophy, 29(3).* 424-435.

Ramsawh H.J., et al. (2011). Age of onset, clinical characteristics, and 15-year course of anxiety disorders in a prospective, longitudinal, observational study. *Journal of Affective Disorders, 132.* 260-264.

Menees, Lauren, et al. (2013). Critical comments about the body and Muscle Dysmorphia symptoms in collegiate men. *International Journal of Men's Health, 12(1).* 17-28.

Pondehnezhadan, Ayeh & Fard, Reza J. (2018). Effectiveness of schema therapy on body-image, self-concept, maladaptive schemas in patients with body dysmorphic disorder. *World Family Medicine/Middle East Journal of Family Medicine, 16(1).*

Phillips, Katherine A., et al. (2014). Psychometric evaluation of the Yale-Brown Obsessive-Compulsive Scale modified for Body Dysmorphic Disorder (BDD-YBOCS). *J Clin Psychiatry, 62(2).* 87-91.