



**ERGO** **Volume 8**  
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# Contents

<i>Journal Staff</i>	vi
<i>Research Reviewers</i>	vii
<i>Acknowledgments</i>	viii
<i>Letter from the Editor</i>	ix

## Botany

Edge Effect on Mycorrhizal Infection Occurrence in <i>Gutierrezia sarothrae</i> [Asteraceae] <i>Shannon M. Call</i>	2
Interaction of Fungal Endophytes and Gall-Forming Aphids on Cottonwood Trees <i>Julia B. Hull</i>	9
Survey for Helenalin in Utah Asteraceae Species <i>Taylor Nelson</i>	20

## Computer Science

Advancements in Anatomical 3D Segmentations: <i>In Silico Modeling of the Human Rectal Vault</i> <i>Tina Van Riper &amp; John Ho</i>	30
--	----

## Health Promotion and Human Performance

The Efficacy of Motivational Imagery Intervention in Distance Athletes <i>Jenna Deelstra</i>	42
--	----

## Medical Laboratory Sciences

Effects of Glucose on Swarming Motility of <i>Proteus Mirabilis</i> <i>Karlee Emal &amp; Scott Chamberlain</i>	54
--	----

Variations of Glucose Metabolism in Group O and Non-Group O Blood Types <i>Daniel Savage, Paulette Padilla, Britton Odle, &amp; Callie Odle</i>	61
Cholesterol and Triglyceride Validation <i>Lexie Yarema, Ashley Park, &amp; Ronnie Erickson</i>	68
Alpha-1 Antitrypsin and the Diagnosis of Exercise-Induced Bronchoconstriction <i>Richard R. John, Megan E. Hirschi, &amp; Justin D. Anderson</i>	73
Comparison of Tooth Soap® and Colgate® on General Oral Health <i>Anja Greenhalgh, Jolette Neeley, &amp; Ron Bettridge</i>	79
Prevalence of <i>Escherichia coli</i> O157:H7 in Grain-Fed Cattle and the Implications to Public Health <i>Michelle Kimball &amp; Ruchi Kasajoo</i>	86

## Microbiology

Microbial Screening of Potable Water Sources in Guatemala: <i>A Potential Source of Disease Transmission</i> <i>Emma Bentley</i>	98
--	----

## Nursing

An Evaluation of Motivators for Colonoscopy Screening Compliance <i>Holly M. Wright</i>	116
---	-----

## Political Science

Service Learning for Undergraduates in Local Elections: <i>College Poll Workers in the 2012 General Election</i> <i>Stuart Jensen</i>	126
---	-----

## Travel Abstracts

- |   |     |
|---|-----|
| Adult Role Models for Youth<br><i>Ashley Davis</i>  | 146 |
| Caching Behavior of the Specialist Woodrat<br><i>Neotoma stephensi</i><br><i>Ashley Van Leuven &amp; James Abbot</i>  | 141 |
| Establishing Two-Point Discrimination of<br>Cranial Nerve V<br><i>Kristie Williamson &amp; Jessica Adler</i>  | 142 |
| Food, Comfort and a Bit of Home: <i>Maude Porter</i><br><i>and the Ogden Canteen, 1942-1946</i><br><i>Lorrie Rands</i>  | 143 |
| Hook-up Culture: <i>A Qualitative Analysis of Sexual</i><br><i>Scripts Within Gender and Religious Identities</i><br><i>Sarah C. Dursteler</i>  | 144 |
| Novel Gene Sequence for Invertebrate Tachykinin<br><i>Chelsie Thomas</i>  | 145 |
| Peer-Assisted Learning in an Orthopedic Evaluation Flipped<br>Classroom Course: <i>The Effectiveness of Peer-Assisted Learning</i><br><i>with Podcast Remediation</i><br><i>Crystal Yamasaki, Adrian Eads, Jennifer Ostrowski, &amp; Brent Marshall</i> | 146 |
| Plant Secondary Metabolites as Inhibitors of<br>Drug-Resistant Bacteria Causing Wound Infections<br>in U.S. Military Personnel<br><i>Alisha Ryan &amp; Shiyu Li</i>   | 147 |

The Serotonin Transporter Gene Polymorphism as a Predictor of Novelty Seeking, Smoking Behavior, and Cognitive Assessment of Risk <i>Houda Nizam &amp; Desirae D. Wood</i>	148
Species Differences in the Restructuring of Syringeal Muscle After Denervation <i>Kyle Spainhower, Carly Milligan, &amp; Linsey Christensen</i>	150
Sustainable Transportation: <i>An Analysis of Bicycle Infrastructure Surrounding Weber State University</i> <i>Hannah Rice</i>	151
Traditional versus Experiential Teaching Methods on Student Learning <i>Stephanie Porter</i>	152
Understanding the Relationship Between Sleep Cycles and Autistic Symptomology <i>Trevor Hicks-Collins</i>	153
Urban Rainwater Harvesting Implementation: <i>Institutional and Human-Related Opportunities and Constraints</i> <i>Stephanie A. Mitts &amp; Steve Burian</i>	154

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## Letter from the Editor

Dear Reader,

It is my privilege to present the eighth volume of *Ergo: Weber State University Undergraduate Research Journal*. Through the combined effort of students and faculty, the following articles have been written and selected for this year's publication. In my second and final year as editor-in-chief of this unique journal, I have learned a great deal about a variety of topics, and I look forward to applying my experience to the next chapter of my career. It is my hope that you will learn something new and share your knowledge, and this journal, with someone else. By sharing this journal, you acknowledge these students, their hard work, and their dedication to research.

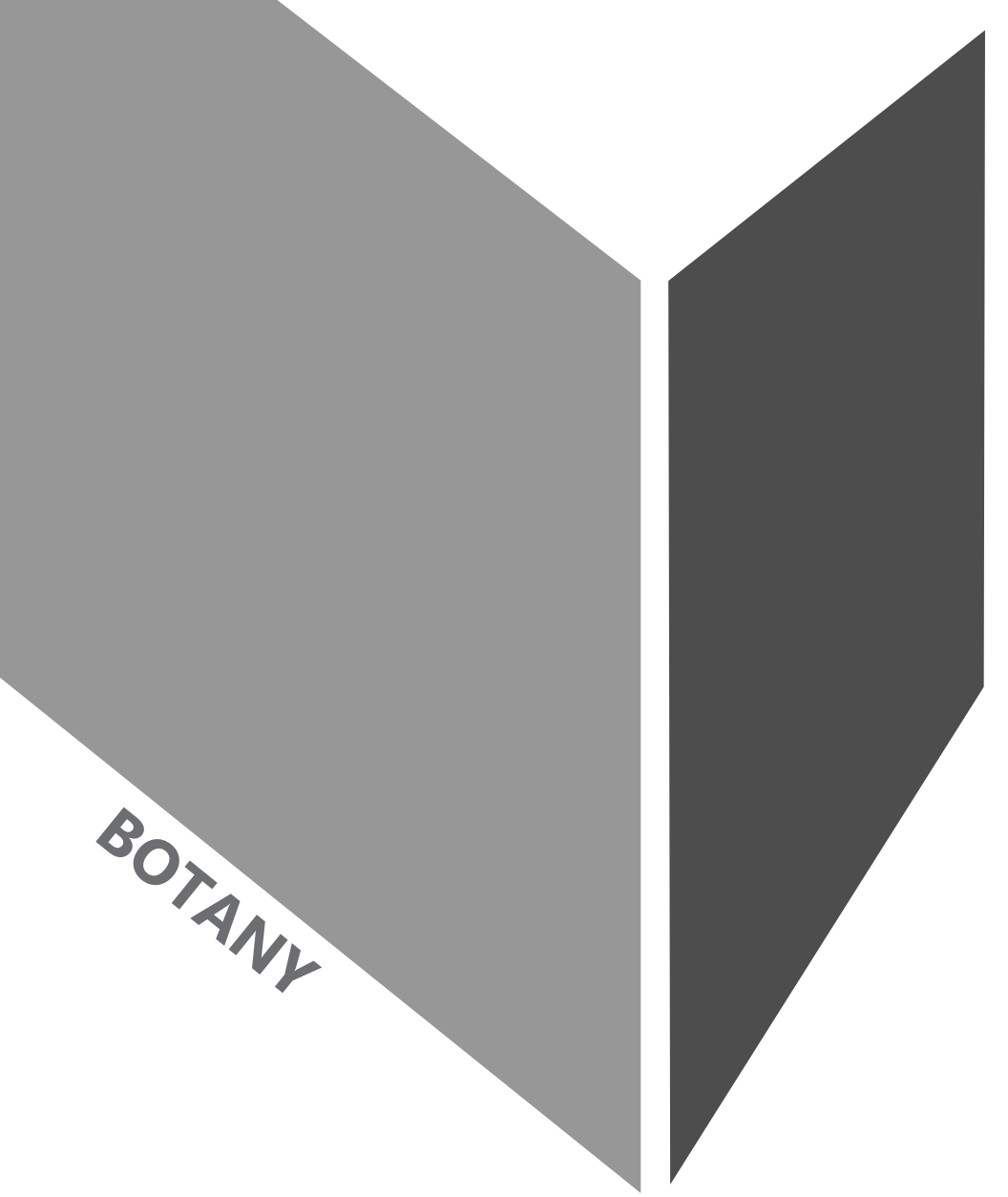
I would like to acknowledge the following people for their contribution to this year's *Ergo*: Erin Bryner and Dr. John Cavitt, you both are always willing to answer my questions and provide valuable input; Tess Woodward, your creativity and dedication is inspiring; and Randi Clawson, your ability to handle multiple projects at once has been invaluable. I would also like to thank the students who submitted their hard work—yours is the greatest contribution to this journal. Finally, I must express my gratitude to the faculty and student reviewers who donate their time and expertise to provide feedback on every submission.

A handwritten signature in black ink, reading "Kerry McShane". The signature is fluid and cursive, with the first name "Kerry" being more prominent than the last name "McShane".

Kerry McShane  
Editor-in-Chief

“Research is formalized curiosity. It is poking and prying with a purpose.”

— Zora Neale Hurston



**BOTANY**

# Edge Effect on Mycorrhizal Infection Occurrence in *Gutierrezia sarothrae* [Asteraceae]

Shannon M. Call

Faculty Mentor: Ron Deckert

## Abstract

Mycorrhizas have a mutualistic relationship between fungi and vascular plants. Mycorrhizal associations are important for plants as they provide minerals, especially phosphorous, and water, while the plant provides carbohydrates for the fungi. Trail edges affect plant community diversity by altering water runoff, soil moisture or encouraging invasive species growth. Because trail edges impact plant communities, I hypothesize mycorrhizal colonization frequency may decrease as distance to a trail edge decreases. To test this hypothesis, *Gutierrezia sarothrae* [Asteraceae], a woody shrub native to the western United States, was sampled from two similar sites and at three distances from hiking trails and mycorrhizal colonization frequency was determined. Plant samples were collected one meter, ten meters and twenty meters from the edge of a hiking trail. Roots were cleared, and stained with trypan blue. Statistical difference was determined using chi-square analysis. There was a significant difference in mycorrhizal associations between 1-meter to 10-meters ( $p < 0.01$ ) and 10-meters to 20-meters ( $p < 0.005$ ). There was no difference between roots at 1-meter and 20- meters ( $p > 0.9$ ). Mycorrhizal infection was highest in plants located 10-meters from the trail. After the study

*was completed, old aerial photographs were discovered showing the existence ten years previously of an old hiking trail which had since been abandoned, thus adding further support to the hypothesis. Results showed differential mycorrhizal colonization frequency, suggesting a negative response near trail edges. Understanding how mycorrhizas respond to disturbance can help managers understand the effects of recreational land use on plant communities.*

## Introduction

Roots provide numerous functions for vascular plants, including nutrient storage, anchorage and absorption and have developed various morphological adaptations due to environmental factors (Leake 1994). Water absorption is critical for all plants; feeder root function is involved in water acquisition. Vascular plants develop a symbiotic relationship with certain fungi called mycorrhizas. Evolutionarily mycorrhizal infections initially may not have been symbiotic; however, they have developed into a beneficial relationship for both vascular plants and the fungi (Willis 2013).

Mycorrhizal hyphae grow into roots of vascular plants in order to take advantage of the nutrients plants store, especially carbohydrates. Mycorrhizas help provide additional minerals and water for the plant due to their larger surface to volume ratio. Mycorrhizal associations occur in two types: intracellular associations in the form of arbuscular mycorrhizas and intercellular associations as ectomycorrhizas (Brundrett 2008). Arbuscular mycorrhizas penetrate the cells of roots, but not cellular organelles, while ectomycorrhizal hyphae surround cells; both forms are able to take advantage of nutrients provided by the plant. Because mycorrhizas provide such an important role in exchange, most plants form these associations.

For this study, *Gutierrezia sarothrae* was selected, as it is a common plant in the region. *G. sarothrae* provides a prime specimen for studying colonization rates because it forms mycorrhizal associations and has a large distribution (Rillig 1998). Disturbed areas experience increased erosion, loss of diversity and increased water run-off. This disturbance may make it difficult for plants to thrive near trail edges and plants may form a lower frequency of mycorrhizal associations. I hypothesize there will be a lower rate of mycorrhizal infection rates of plants closer to the trail and higher infection rate farther from a trail.

## Method

### *Site Description*

Samples of *G. sarothrae* were selected from two sites. The first site, site A, was located on the East bench in the Ogden, near Weber State University. This site was considered disturbed due to two trails running through the area. Four plants from each 1-meter, 10-meters and 20-meters were collected, totaling three groups and twelve plants.

The second site, site B, was similar in elevation, disturbance level and plant species present. This site was located directly east of 29th street in Ogden, Utah directly off the main trail approximately 400 meters from the trailhead. This site had one trail running from northeast to southwest. Four plants were selected at approximately 1-meter, 10-meters and 20-meters away from the main trail totaling three groups and twelve plants. Twenty-four plants from both sites combined were observed.

### *Sample Processing*

Samples were collected 24 hours before being analyzed and were refrigerated overnight with the entire plant and surrounding

soil still intact. Samples were then soaked in warm water to remove excess soil without disturbing the fine roots. Roots samples were stored overnight in a 2.5% potassium hydroxide solution. The next day roots were rinsed with distilled water three times, then soaked in a 1% hydrochloric acid solution for 15 minutes. Fresh trypan blue stain (0.05% trypan), a fungal specific stain, was freshly prepared and roots were left in the stain for several days. After staining, roots were rinsed with a de-stain solution made of acidified glycerol (500 ml glycerin, 450 ml of distilled water and 50 ml of 1% hydrochloric acid) and allowed to soak in the solution for three days. Fresh de-stain solution was added and roots were rinsed with de-stain solution several times per day, for three days. After de-staining, roots were mounted on microscope slides in lactoglycerol (1:2:1 lactic acid, glacial acetic acid: glycerol) and sealed with clear finger nail polish. A phase contrast microscope was used to observe root samples at 400x magnification using phase contrast.

Chi-square analysis was used to test for statistical difference between groups. From each site, 14.9 mm of roots were observed from 1-meter, 10-meter and 20-meter groups. Using the optical micrometer as a transect, mycorrhizas were counted only if hyphae intersected the transect. Infection condition was recorded as colonized or not. The occurrence of hyphae crossing the transect were observed and counted. In each group A and B, 1386 hyphal transect intersection points were collected. Data, defined as the occurrence of hyphal intersection, was combined from both groups, which totaled 4,158 intersection points.

## Results

I found a significantly higher occurrence in mycorrhizal infection rates on *G. sarothrae* as distance from a trail increased from 1-meter to 10-meter samples ( $p < 0.01$ ). A significantly higher difference was observed between 10-meter and

20-meters ( $p < 0.005$ ) from a trail. A significant difference was not observed between 1-meter and 20-meters ( $p > 0.9$ ). Overall, a significant difference between all three groups was observed ( $p < 0.005$ ) (Fig 1).

Most vascular plants form mycorrhizal associations, which increases plant success. The differences in size of *G. sarothrae* plants were obvious upon arrival at both sites. Plants located farther from the trails were smaller in size, less frequent and provided lower plant cover than plants near the trail. This may be due to several reasons. *G. sarothrae* may be able to take advantage of highly disturbed sites, such as along a trail. While mycorrhizal count was lower closer to trails, except group 1-meter versus 20-meters, *G. sarothrae* may have been able to collect needed nutrients due to lower competition from grasses and woody species or increased water run-off. Mycorrhizal colonization of plants located 20-meters from the trail may have been lower as a hiking trail previously ran through the sample site located on 29th street. This trail was not discovered until after the experiment was completed. The hypothesis that edge effect affects mycorrhizal colonization rates is supported because a statistical difference in mycorrhizal hyphae was not observed in plants between a current trail edge and those located on a past trail, located 20-meters from the current trail (Fig 2).

Also, it is important to note the large distribution of *G. sarothrae* (Ralphs 2004). The large distribution supports the notion of *G. sarothrae* may be a disturbance strategist species (Lane 1985). Based on the results of this experiment, I accept the hypothesis that lower mycorrhizal colonization frequency of roots occurs in plants located near trail edges.

## Conclusion

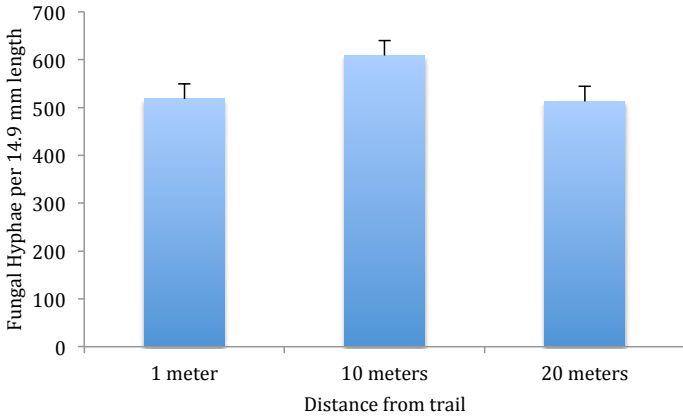
Studies need to be conducted in which plants along trails sites are kept separate so hyphal frequency can be determined per



plant. This will help reduce the chance of sampling errors, such as plants with an unusual mycorrhizal interaction. It would also be interesting to measure mycorrhizal colonization in multiple sites. Additional focus on how *G. sarothrae* responds to climate change, in addition to living in disturbed sites, could lead to a greater understanding of how mycorrhizas are not only affected by microclimate factors, but also climate change. Arbuscular mycorrhizas may often be disregarded or overlooked in comparison to large-scale edge effect studies; however, mycorrhizas play an important role in nutrient acquisition for plants and may have implication for how plants acquire nutrients in the future (Rillig 1998).

## References

- Brundrett, M. (2008). *ARBUSCULAR MYCORRHIZAS*. Retrieved from <http://mycorrhizas.info/vam.html>
- Lane, M. A. (1985). Taxonomy of *Gutierrezia* (Compositae: Astereae) in North America. *Systematic Botany*, 10(1), 9–28.
- Leake, J. R. (1994). The biology of myco-heterotrophic ('saprophytic') plants. *New Phytologist*, 127(2), 171–216.
- Ralphs, M. H. & Wiedmeier, R. D. (2004). Conditioning cattle to graze broom snakeweed (*Gutierrezia sarothrae*). *Journal of Animal Science*, 82(10), 3100–3106.
- Rillig, M. C., & Allen, M. F. (1998). Arbuscular mycorrhizae of *Gutierrezia sarothrae* and elevated carbon dioxide: evidence for shifts in C allocation to and within the mycobiont. *Soil Biology and Biochemistry*, 30(14), 2001–2008.
- Willis, A., Rodrigues, B. F., & Harris, P. J. C. (2013). The Ecology of Arbuscular Mycorrhizal Fungi. *Critical Reviews in Plant Science*, 32(1), 1–20.



**Figure 1.** Fungal hyphae count per each group from both sites combined. 518 mycorrhizal hyphae at 1 m, 609 hyphae at 10 m, and 513 hyphae at 20 m. A significant difference was observed between 1- and 10-meter samples ( $p < 0.01$ ). A significant difference was observed between 10- and 20-meter ( $p < 0.005$ ) samples. A significant difference was not observed between 1- and 20-meter ( $p < 0.9$ ) samples. Overall, a significant difference between all three groups was observed ( $p < 0.005$ ).



**Figure 2.** Site B location illustrates a trail in 2003 that is greatly diminished in 2011.

# Interaction of Fungal Endophytes and Gall-Forming Aphids on Cottonwood Trees

Julia B. Hull

Faculty Mentor: Ron Deckert

## Abstract

*Endophytes are fungi that live within aerial portions of plants for most or all of their life cycle without causing visible signs of disease. Gall-forming aphids, Pemphigus betae, are highly competitive over gall site selection, forming galls on the leaves of narrowleaf cottonwoods and their hybrids with Fremont cottonwoods. The favored gall location overlaps with areas of highest endophyte probability. I hypothesized that a negative correlation would exist between endophyte infection and aphid galling on leaves of backcross hybrid cottonwood trees as to avoid secondary metabolites created by the fungi. I tested my hypothesis by obtaining samples of six backcross hybrid cottonwood trees along the Weber River in Weber County, Utah from July 10–17, 2013. I took leaf samples of galled and ungalled leaves and the corresponding twigs from the current year and the previous year. Samples were surface sterilized, aseptically plated onto potato dextrose agar, and were incubated at room temperature for four weeks. Samples were scored every other day for endophytes. At the end of four weeks, data were collected and statistical analysis was performed via t-test and a Chi-squared test. My hypothesis was rejected. I found significantly more endophytes associated with galled*

leaves than in leaves without galls. Possibly, either the aphids are attracted to the areas with high endophyte infections, or the aphids themselves are introducing endophytes into the leaves.

## Introduction

### *Endophytes*

Endophytes are fungi that live within aerial portions of a plant host for all or most of their life cycle without causing visible signs of disease. Due to their inconspicuous nature, the presence of endophytes has only recently been discovered, and their role is only beginning to be understood. Endophytes are found in a variety of plant hosts and are not constrained by taxonomical distinctions of host plant (Saikkonen et al., 1998; Schulz & Boyle, 2005). Grass endophytes have been shown to increase drought tolerance (Archevaleta et al., 1989), deter insect and mammalian herbivory (Clay et al., 1993), and increase seed dispersal (Saikkonen et al., 1998).

### *Hybridization of Populus*

The hybrid system of *Populus angustifolia* X *P. fremontii* has been well documented (Rehill et al., 2005; Bailey et al., 2005). *P. angustifolia* (narrowleaf) and *P. fremontii* (Fremonts) will form F1 hybrids which may further hybridize with narrowleaf cottonwoods to form complex backcrosses (BC1, BC2, BC3, and BC4). Hybrid leaf morphologies reflect the proportion of Fremont genes to narrowleaf genes, as shown in Figure 1. The hybrids also vary in the production of secondary metabolites (Bailey et al., 2005; Rehill et al., 2005). Bailey et al. (2005) found that tannin levels in Fremonts and F1 have comparatively lower levels of tannins than the narrowleaf and backcross hybrids, leading to higher endophyte infection rate in F1 and Fremonts. Additionally, Paige and Capman (1993) noted that *Pemphigus betae* [Aphididae] prefer to colonize on backcross hybrids, with a few colonizing on F1 hybrids and do not use Fremonts as a host.

### *Aphids*

*Pemphigus betae* have a complex life cycle (Figure 2). Their primary host plant is a *Populus* backcross hybrids, with the secondary host on nearby *Rumex* and *Polygonium* species. In autumn, after they have mated, the aphids will form winged migrants and leave the secondary host plant roots, flying clumsily a few hundred meters to the hybrid tree where the females lay an overwintering egg. The migrant forms are responsible for tree selection. Just at budburst the following spring, the eggs hatch into stem mothers who form galls on the cottonwood leaves (Moran, 1993). Clones of the stem mother form over the next four to six weeks. Around the second week of July, the galls open and the aphids return to the secondary host for the remainder of the summer. Stem mothers prefer to form galls at the base of the petiole, where photosynthates are the highest, and where they have the greatest manipulation of plant hormones. They are highly competitive over the preferred location. Zucker (1982) observed that greater leaf area lead to more successful galls. Stem mothers, then, compete for large leaves and for placement upon that leaf. Moreover, survivorship of the aphid galls varies from tree to tree ranging 0–90% (Dickson & Whitham 1996) with extreme trees side by side. Furthermore, the same trees tend to be favored from year to year (Moran & Whitham 1990).

The objective of this study was to test the hypothesis that *Pemphigus betae* gall success rate in *P. angustifolia* X *P. fremontii* individuals will be affected by the presence of endophytes, by comparing the endophyte infection rates of leaves with and without galls and the associated woody tissue. I predict that a negative correlation between gall rates and endophyte infection exists in BC1 hybrids, so the aphids avoid secondary metabolites produced by the endophytes.

## Method

I sampled *Populus angustifolia* X *P. fremontii* BC1 hybrids along the Weber River, approximately 400 m from the mouth of Weber Canyon (elevation approx. 1400 m) in Weber County, Utah. Collections were made from July 10–17, 2013. Six trees were randomly selected from a 100 m stretch along the riverbank. Leaves were also selected randomly and categorized according to the amount of galling. Heavily-galled leaves had more than one gall, moderately galled leaves had only one gall, and leaves with no galls were categorized as low. The fourth or fifth leaf, depending on which leaf was larger, from the apex of the branch was sampled using the above categories since the aphids favor this leaf the most frequently due to lower phenolic levels (Rehill, 2005). Five leaves of each gall frequency were selected from each of the six trees, yielding 30 samples of each gall frequency.

Leaves were examined for endophyte infections in two locations. The first location, near the petiole, is the preferred location of the aphids, the primary (1°) location. The distal end of the leaf, the secondary (2°) location, was used as a control because low endophyte infection rates are expected here. Wood samples of the current year and previous year growth were also taken for every leaf sample. Figure 3 shows the locations of the sampling.

Samples were processed within 24 hours of collection by the following procedure. Leaves were treated with 70% ethyl alcohol for 60 s, 50% bleach for 60 s, and three washes in sterile distilled water. If the galls were unopened, I cut them open along the major axis of the gall using a flame-sterilized scalpel, ensuring that all surfaces came in contact with the sterilizing agents. Wood samples were cut into 1 cm segments, and then treated with 70% ethyl alcohol for 5 min followed by 5 min of 50% bleach and three washes in sterile distilled water. Each sample was placed on potato dextrose agar (PDA) and petri

dishes were sealed with Parafilm. The plates were incubated at room temperature ( $\approx 22^{\circ}\text{C}$ ) and were scored every two days. Endophytes were allowed to grow for four weeks before data analysis took place. Data were analyzed using Chi-squared tests and t-tests.

## Results

### *Leaf*

There was no difference ( $p=0.57$ ) in endophyte infection in moderately galled leaves ( $\bar{x}=0.83$ ) and highly galled leaves ( $\bar{x}=0.93$ ). Data from the moderately galled and the heavily galled samples were pooled and compared to the low galled samples ( $\bar{x}=0.27$ ). There were significantly more ( $p<0.05$ ) endophytes in the primary location with galls ( $\bar{x}=0.88$ ) than those without galls ( $\bar{x}=0.27$ ). There was no difference ( $p=1$ ) in galled and ungalled in the secondary location (Figure 4).

### *Wood*

The current year's wood showed no significance difference ( $p=0.85$ ) between galled and ungalled samples. Likewise, the one-year-old wood showed no significance ( $p=0.311$ ) between galled and un-galled samples. As expected, there were significantly more ( $p<0.05$ ) endophytes in the one year old wood than in the current year's wood (Figure 5).

## Discussion

I hypothesized that *Populus* BC1 hybrids with higher endophyte infection would have fewer galls. These data show the opposite. The presence of more endophytes in galled leaves may potentially be explained in at least two ways.

First, *P. betae* stem mothers may be introducing endophytic spores into the leaves. If this were the case, one may expect to see a significant difference of endophytes in the wood of

galled and ungalled samples. No difference was found in this study. However, endophytic infections of woody plants tend to be localized, so the possibility of spore introduction cannot be ruled out without further inquiry. Migrating stem mothers could be captured and tested for endophytic fungal spores. DNA comparison with known endophyte population versus fungi on stem mothers may answer this question.

The possible second explanation is that *P. betae* migrants and stem mothers are looking for trees and leaves with higher rates of endophytic infection. Since nearby trees have varying rates of gall support (Dickson & Whitham, 1996), one might identify trees with high and low rates of gall support. Because migrants tend to return to the same individual trees year after year (Moran & Whitham, 1990), one might capture incoming migrants and redirect them to the trees that tend to support fewer galls. The following summer, after the stem mothers have formed the galls, endophyte populations could be compared to that of the previous year. Changes in gall success or failure could inform us of the relationship of endophytes and stem mothers. The current study cannot compare gall rates with endophyte rates because I investigated individual leaf relationships—not that of the entire tree.

Endophytes may play an important role in the functioning of this ecosystem. Aphids have been shown to support increased populations of arthropods, fungi, and birds by providing increased nutriment for higher trophic levels (Dickson & Whitham, 1996). If the endophytes influence gall formation, then the endophytes are key factors in the *Populus*-associated community composition. Understanding the relationship between endophytes and the genetic basis behind them is crucial information for conservation managers and riparian restoration ecologists.

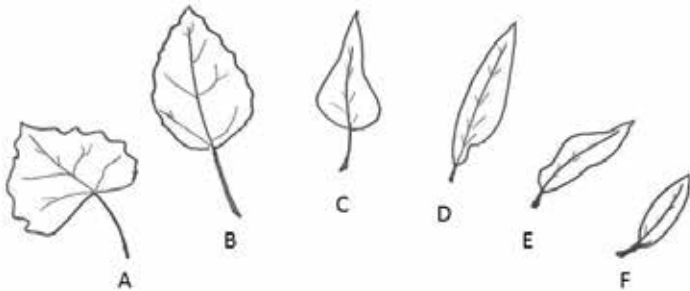


## References

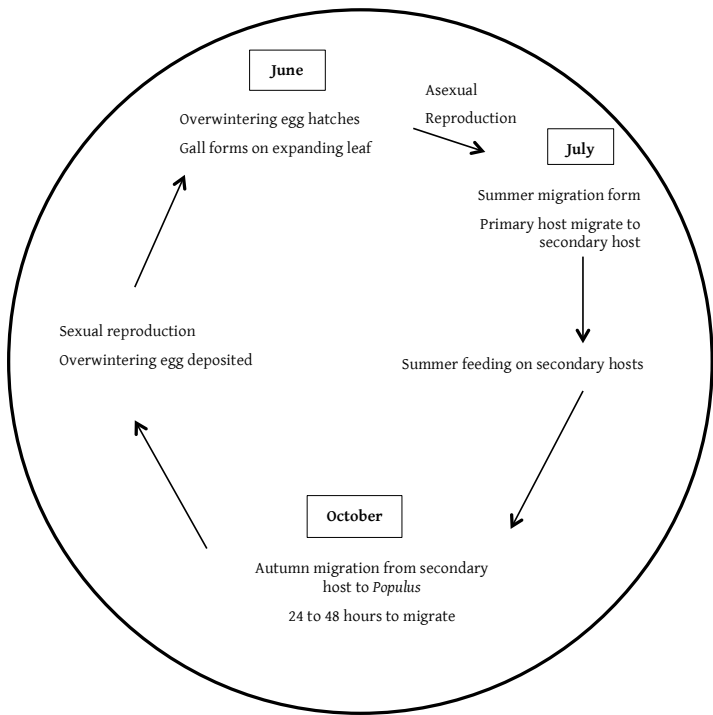
- Arahevaleta, M., Bacon, C. W., Hoveland, C. S., & Radcliff, D. E. (1989). Effect of tall fescue endophyte on plant response to environmental stress. *Agronomy Journal*, 81(1), 83–90.
- Bailey, J. K., Deckert R., Schweitzer J. A., Rehill B. J., Lindroth, R. L., Gehring, C., & Whitham, T. G. (2005). Host plant genetics affects hidden ecological players: links among *Populus*, condensed tannins, and fungal endophyte infection. *Canadian Journal of Botany*, 83(4), 356–361.
- Clay, K., Marks, S, and Cheplick, G. P. (1993). Effects of insect herbivory & fungal endophyte infection on competitive interactions among grasses. *Ecology*, 74(6), 1767–1777.
- Dickson, L. L. & Whitham, T. G. (1996). Genetically-based plant resistance traits affect arthropods, fungi and birds. *Oecologia*, 106(3), 400–406.
- Moran, N. A., & Whitham, T. G. (1990). Differential colonization of resistant and susceptible host plants: *Pemphigus* and *Populus*. *Ecology*, 71(3), 1059–1067.
- Paige, K. N., & Capman, W. C. (1993). The effects of host-plant genotype, hybridization, and environment on gall-aphid attack and survival in cottonwood: The importance of genetic studies and the utility of RFLPs. *Evolution*, 47(1), 36–45.
- Rehill, B., Clauss, A., Wieczorek, L., Whitman, T., & Lindroth, R. (2005). Foliar phenolic glycoside from *Populus Fremontii*, *Populus angustifolia*, and their hybrids. *Biochemical Systematics and Ecology*, 33(2), 125–134.
- Saikkonen, K., Faeth, S. H., Helander, M., & Sullivan T. J. (1998). Fungal endophytes: A continuum of interactions with host plants. *Annual Review of Ecology and Systematics*, 29, 319–343.
- Schulz, B., & Boyle, C. (2005). The endophytic continuum. *Mycological Research*, 109(6), 661–686.

Wilson, D., & Carroll, G. C. (1997). Avoidance of high-endophyte space by gall-forming insects. *Ecology*, 78(7), 2153–2163.

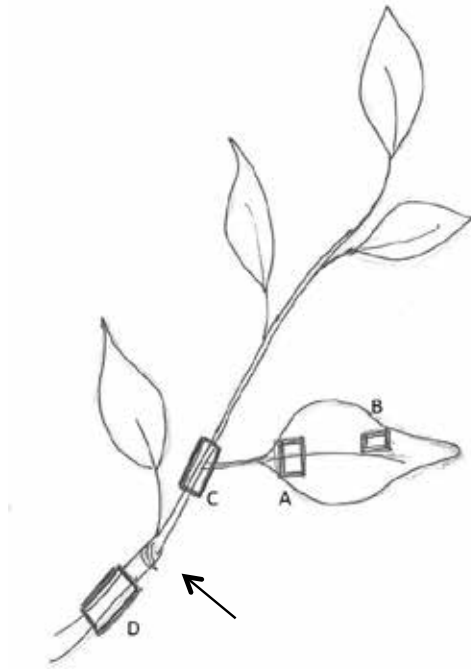
Zucker, W. V. (1982). How aphids choose leaves: the roles of phenolics in host selection by a galling aphid. *Ecology*, 63(4), 972–981.



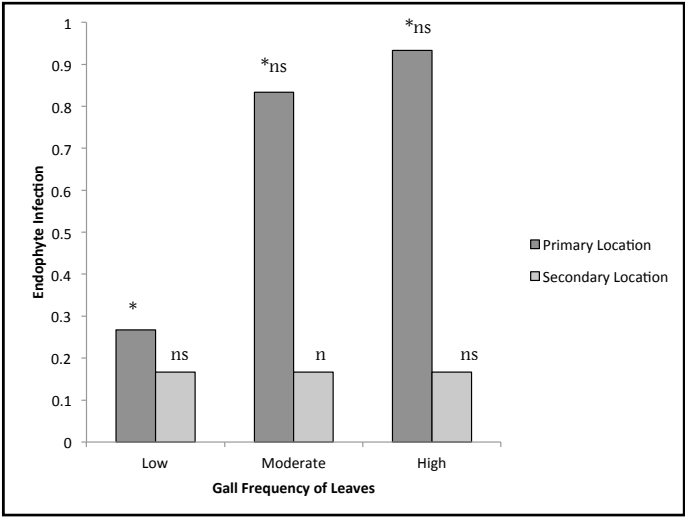
**Figure 1.** The hybridization of Fremont and narrowleaf Cottonwood trees. A) Fremont; 0% Narrowleaf genes B) F1 hybrid; 50% narrowleaf genes C) BC1 hybrid; 75% narrowleaf genes D) BC2 hybrid; 88% narrowleaf genes E) BC3 hybrid; 98% narrowleaf genes F) narrowleaf; 100% narrowleaf genes.



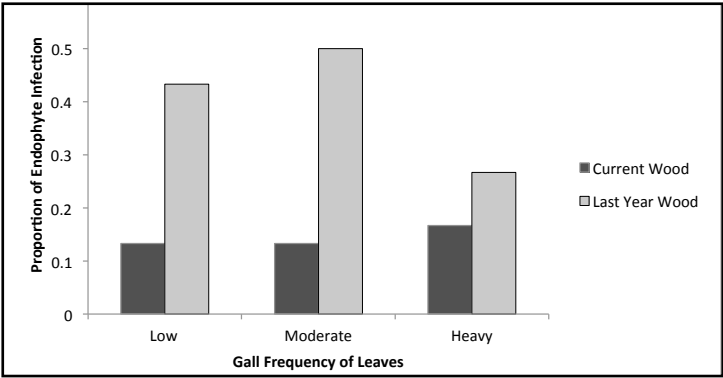
**Figure 2.** The life cycle of *Pemphigus betae*.



**Figure 3.** Sampling locations. The fourth or fifth leaf from the apex was chosen for the leaf sampling, depending on which leaf was bigger. A) The primary location. This is where aphids prefer to form galls. B) The secondary location; used as a control for endophyte infections within the leaf. C) Wood from the current year's growth. D) Wood from growth of the last year's growth (i.e. one-year-old wood). The arrow marks the bud scale scar, indicating the beginning of the current year's growth.



**Figure 4.** Comparison of proportions of endophyte infections of leaves according to the gall frequency. There was a significant difference between the galled and ungalled leaves in the primary location. The secondary location showed no variation in the endophyte frequency across the treatment groups. \* indicates a significant difference. ns indicates no significant difference.



**Figure 5 .** Comparison of endophyte infection in wood samples. There was no significant difference in the endophyte infections in relation to gall presence in either year’s wood. There were, however, significantly more endophytes in the older wood as was expected.

# Survey for Helenalin in Utah Asteraceae Species

Taylor Nelson

Faculty Mentor: Sue Harely

## Abstract

*Arnica*, a salve containing a mixture of sesquiterpenes extracted from flowers of *Arnica montana*, is a common form of complementary medicine used to treat bruises and sore muscles. Due to *Arnica*'s popularity, *A. montana* is becoming scarce in Europe (its native range) and is sometimes substituted commercially with *A. chamissonis*. Therefore, this study focused on finding other sources of helenalin, the presumed active component, among the Asteraceae, the plant family that contains *A. montana*. *A. chamissonis*, *A. cordifolia*, *A. latifolia*, *A. longifolia*, *A. mollis*, and *Helianthella uniflora* were collected from Alta, UT and *Helenium autumnale* from Salt Lake City, UT, at elevations of approximately 10,500 ft and 4,200 ft, respectively. Sesquiterpenes were extracted from the flowers by dipping them in methylene chloride and separated by thin-layer chromatography. Helenalin and helenalin-like compounds were identified by their reaction with vanillin and comparison to a pure helenalin standard. Flowers from six of the seven species contained helenalin, with *Hele. autumnale* containing the highest concentration and *Heli. uniflora* containing none. Therefore, six of the seven species tested in this study have the potential to be used commercially to prepare arnica salves and thus allow the *A. montana* population to re-establish itself.

## Introduction

Arnica, a salve containing a mixture of sesquiterpenes extracted from flowers of *Arnica montana*, is a common form of complementary medicine used to treat bruises and sore muscles. It is known to inhibit the transcription factor, NF- $\kappa$ B (Siedle et al., 2004). By blocking NF- $\kappa$ B, helenalin inhibits inflammation and pain associated with the inflammatory response of the body. NF- $\kappa$ B controls the expression of genes for hundreds of different functions, including the immune system and inflammation (Pahl, 1999). Included in these genes are the genes for inflammatory cytokines, which are produced when the transcription factor NF- $\kappa$ B receives signals that an inflammatory response is necessary (Pahl, 1999). By inhibiting NF- $\kappa$ B, the genes for the response to inflammation are not expressed and production of pro-inflammatory agents ceases.

Arnica, like other natural remedies for common ailments, has been increasing in popularity for many years now. However, due to its popularity, *A. montana* is now becoming a scarce species in Europe (its native range) (Cassells, Walsh, Belin, Cambornac, Robin, & Lubrano, 1999). In past studies, *A. chamissonis* has been shown to be an effective substitute for *A. montana* due to its helenalin content (Cassells et al., 1999). Thus, it is possible that other species belonging to the *Arnica* genus, and even other genera in the same family, may be suitable substitutes for *A. montana*. There are several species of *Arnica* that are native to Utah, including *A. chamissonis*, *A. cordifolia*, *A. longifolia*, *A. latifolia*, and *A. mollis*. In addition, *Helianthella uniflora* and *Helenium autumnale* are closely related to species of *Arnica*. Flowers were collected from all seven species and tested for the presence of helenalin.

## Methods

### *Flower Collection*

Flowers of *A. chamissonis*, *A. latifolia*, *A. longifolia*, *A. cordifolia*, *A. mollis/diversifolia*, and *Hele. uniflora* were collected near Alta, UT, on July 19, 2012 and August 1, 2012 at elevations from 8,000 to 11,000 ft above sea level. Flowers of *Hele. autumnale* were collected from a bog near 3900 South and 900 East in Salt Lake City on August 1, 2012. Flowers were cut 3–4 cm below the involucre and placed in plastic bags according to species. Upon returning from the field, the bags were stored at 4°C.

### *Flower Extraction*

Within two days of each collection date, the sesquiterpenes were extracted from the flowers by dipping them in methylene chloride (Spring et al., 1995). The extracts were concentrated to six flower heads per ml of solvent by evaporation of the methylene chloride and stored at 4°C.

### *Thin-Layer Chromatography (TLC)*

The sesquiterpenes in the flower extracts were separated by TLC using 20 cm x 20 cm silica gel-coated glass plates with a fluorescent indicator. Each TLC plate was spotted with 15 µL of each flower extract and three control spots of pure helenalin in ethanol. The chromatographs were run in an unlined chamber in 6:1, chloroform: acetone (Picman, Ranieri, Towers, & Lam, 1980). A vanillin-based spray reagent (Picman t 1980) was prepared one to four minutes before each TLC finished and was applied as soon as the chloroform/acetone solvent had evaporated from the TLC plate.

After application of the spray reagent to the TLC plate, the plates were allowed to dry for approximately five minutes and were then placed in a 70°C oven. After ten minutes, the plates were removed and the position and color of the spots on the plate were analyzed and recorded.



## Results

### *Flower Harvesting*

Flowers of *Arnica* and related species bloom around the end of July and the beginning of August. Therefore, two trips to the mountains near Alta, UT, were made to ensure that sufficient flowers of each species were collected. Different habitats and population densities were observed among the species collected from the two sites on the two occasions. *A. longifolia* was the most densely populated species where it occurred and preferred riparian habitat. *A. chamissonis* and *A. latifolia* had the least densely populated species in a given area. It was impossible to tell if one of the species was *A. mollis* or *A. diversifolia*. This difficulty perhaps indicates that the population was a hybrid of the two species as *Arnica* species are known to interbreed.

### *Thin-Layer Chromatography (TLC)*

Helenalin turns pink when reacted with vanillin and has an  $R_f$  value of roughly 0.42 when chromatographed in chloroform:acetone. The  $R_f$  value was found to vary depending on the concentration of the helenalin. For example, a sample of helenalin with 25% more helenalin than normally used had an  $R_f$  value of 0.47 (Figure 1).

The vanillin reagent was very effective at exposing the helenalin-containing extracts as long as the spray reagent was prepared fresh before each use. If the spray reagent was prepared more than four minutes prior to treatment of the plate with the spray reagent, then the helenalin could not be visualized.

Of the seven species of tested flowers, *A. chamissonis*, *A. cordifolia*, *A. mollis/diversifolia*, *A. latifolia*, *A. longifolia*, and *Hele. autumnale* all tested positive for helenalin, with *Hele. autumnale* containing the most and *A. latifolia* the least. The only species tested which did not contain helenalin was *Heli. uniflora*.

In congruence with the difficulty in differentiating between *A. mollis* and *A. diversifolia*, Figure 2 demonstrates the usefulness of chemical makeup in deriving taxonomic origin of the plant. Lane 7 of Figure 2 is labeled as an extract from *A. latifolia*, but when this chemical profile is compared with other chemical profiles of *A. latifolia* (as in lane 8) it does not match up.

## Discussion

The TLC results showed that all of the sampled flowers within the *Arnica* genus produced helenalin to varying degrees. According to this study, certain species (*A. chamissonis* and *A. longifolia*) have more potential to be a successful substitute for *A. montana* as medicines. Given that color intensity on the TLC plate is a representation of chemical concentration, *Hele. autumnale* may be an even better source of helenalin than any of the tested *Arnica* species. However, due to differences in its chemical profile compared to *Arnica* species (Figure 2), clinical studies using extracts of *Hele. autumnale* in place of *A. montana* would be recommended because of possible side effects from the other chemicals.

Although many *Arnica* species look very similar, according to the chemical profiles of each species which I studied, chemotaxonomy may be the best and quickest solution to the taxonomic ambiguities within the group. An example of this is seen in Figure 2. While, the extracts of *A. latifolia* in lanes 7 and 8 do not match up, lane 7 does match up nicely with lane 1 of Figure 1. This suggests that this particular extract of *A. latifolia* which I collected may actually be *A. mollis/diversifolia*. Therefore, while TLC is a functional method for determining helenalin presence within a given species, it may also be a supplement to traditional taxonomy where anatomical ambiguities are encountered.

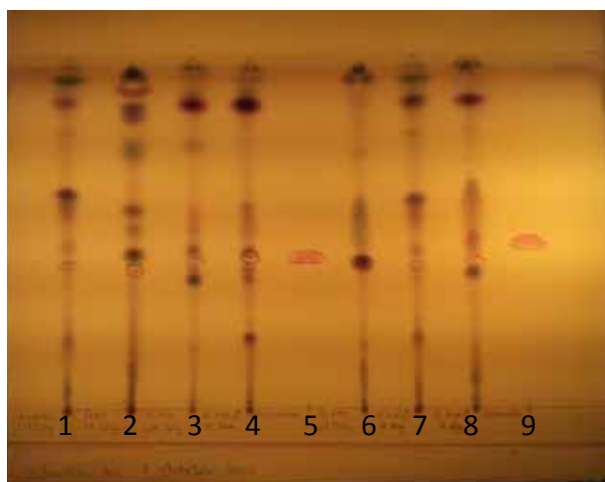
## Acknowledgments

I would like to thank Dr. Sue Harley of the Weber State University Department of Botany for her guidance, direction, and counsel in the preparation, plant growth, flower harvesting, chemical analysis, and writing processes. I am also grateful to Blake Wellard, a graduate student at the University of Utah, for his help in locating, identifying, and harvesting specific flowers. In addition, I would like to thank Maura Olivos and Alta Ski Resort for not only allowing me to harvest flowers for analysis from within the resort, but also aiding me in doing so. Dr. Stephen Clark spent time attempting to identify some of the more questionable *Arnica* specimens which were collected, and I would like to thank him for doing so. Finally, special thanks to the Office of Undergraduate Research at Weber State University for the funding to perform this research.

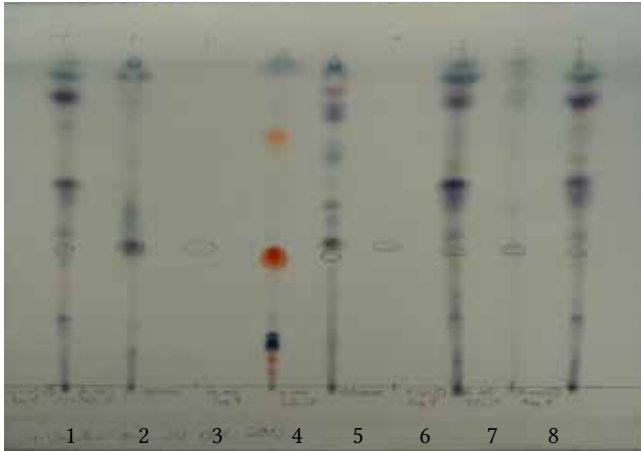
## References

- Cassells, A., Walsh, C., Belin, M., Cambornac, M., Robin, J., & Lubrano, C. (1999). Establishment of a plantation from micropropagated *Arnica chamissonis* a pharmaceutical substitute for the endangered *A. montana*. *Plant Cell, Tissue and Organ Culture*, 56(2), 139–144. <http://dx.doi.org/10.1023/A:1006224215503>
- Pahl, H. L. (1999). Activators and target genes of Rel/NF- $\kappa$ B transcription factors. *Oncogene*, 18(49), 6853–6866. <http://www.stockton-press.co.uk/onc>
- Picman, A. K., Ranieri, R. L., Towers, G. H. N., & Lam, J. (1980). Visualization reagents for sesquiterpene lactones and polyacetylenes on thin-layer chromatograms. *Journal of Chromatography A*, 189(2), 187–198. <http://www.sciencedirect.com/science/article/pii/S0021967300815186>

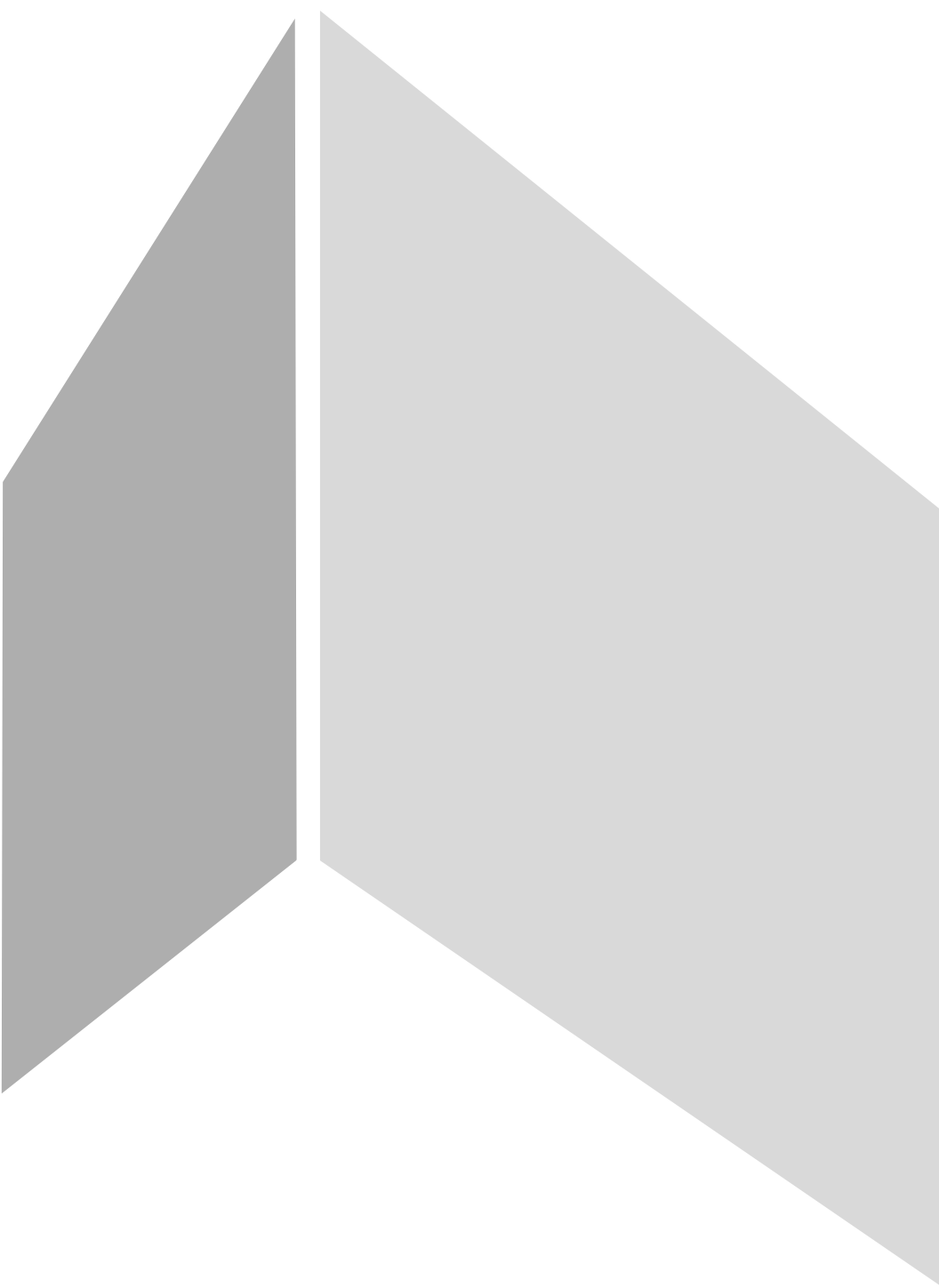
- Siedle, B., Garcia-Pineros, A. J., Murillo, R., Schulte-Monting, J., Castro, V., Rungeler, P.,...Merfort, I. (2004). Quantitative structure-activity relationship of sesquiterpene lactones as inhibitors of the transcription factor NF- $\kappa$ B. *Journal of Medicinal Chemistry*; 47(2), 6042-6054. <http://pubs.acs.org.hal.weber.edu:2200/doi/pdfplus/10.1021/jm049937r>
- Spring, O., Buschmann, H., Vogler, B., Schilling, E. E., Spraul, M., & Hoffmann, M. (1995). Sesquiterpene lactone chemistry of *Zaluzania grayana* from on-line LC-NMR measurements. *Phytochemistry*, 39(3), 609-612. <http://www.sciencedirect.com/science/article/pii/003194229500084K>

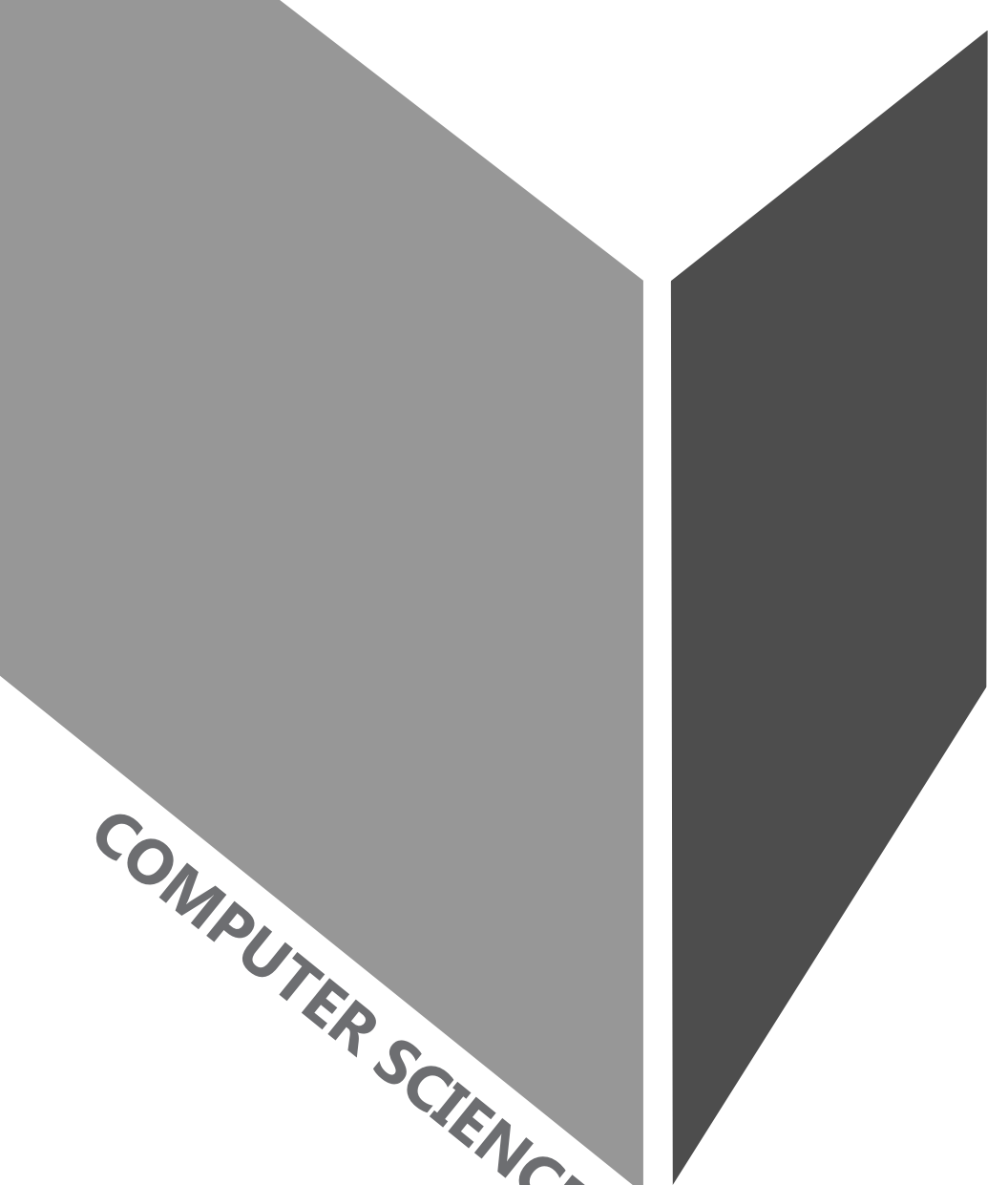


**Figure 1.** TLC analysis of seven species within the Asteraceae. Helenalin was detected with a vanillin reagent. Lane 1 is spotted with an extract of *Arnica mollis/diversifolia*, lane 2 is *Helianthella uniflora*, lane 3 is *Arnica chamissonis*, lane 4 is a different collection of *A. chamissonis*, lane 5 is a pure helenalin control, lane 6 is *Arnica cordifolia*, lane 7 is *Arnica mollis*, lane 8 is *Arnica longifolia*, and lane 9 is another pure helenalin control. The pure helenalin control in lane 9 had 25% more helenalin and exhibited an  $R_f$  value of 0.47 vs. and  $R_f$  value of 0.42 for the control in lane 5.



**Figure 2.** A TLC plate sprayed with a vanillin reagent. Lane 1 is spotted with an extract of *Arnica mollis*, lane 2 is *Arnica cordifolia*, lane 3 is a pure helenalin control, lane 4 is *Helenium autumnale*, lane 5 is *Helianthella uniflora*, lane 6 is another pure helenalin control, lane 7 is *Arnica latifolia*, lane 8 is an extract of *A. latifolia* from a different collection, and lane 9 is an extract of *A. mollis* from a different collection.





**COMPUTER SCIENCE**

# Advancements in Anatomical 3D Segmentations: *In Silico* Modeling of the Human Rectal Vault

Tina Van Riper & John Ho

Faculty Mentors: Yong Zhang & Brian Rague

## Abstract

Over 100 million Americans are patients of U.S. hospitals every year. With so many people in need of treatment, there is an even greater need for improved medical devices for health care practitioners to adequately heal and comfort their patients. This project aims at building an *in silico* model by segmenting anatomical structures of the lower gastrointestinal tract by extracting data from Magnetic Resonance Imaging (MRI) data sets. This model is then combined with biological mechanics data to be exported to manufacturing software for 3D printing or to engineering software for finite element analysis (FEA). Through the use of cutting-edge technology, the exported models, with their corresponding biological data, can be included to produce kinetic and static biological simulations. Further analysis utilizing these *in silico* models provides a necessary deeper understanding of the human lower gastrointestinal tract for medical device companies to improve current medical device designs. An equally urgent need for this type of research is to provide a means for doctors to accurately be able to diagnose and treat their patients by providing 3D simulations of their patient's anatomy and anomalies. With this type of technology in the hands of healthcare



*practitioners, they will be able to provide computer-aided diagnoses and state-of-the-art, personalized treatment, and they will be able to take advantage of the benefits of efficient data management.*

## Introduction

A finite element model is an abstraction of a more complicated physical system (Bathe, 1996; Hughes, 2000). Such methods can be employed in anthropology and biology to study morphological variation in the skeleton related to function (Boccaccio et al., 2011; Holzapfel, Gasser, & Stadler, 2002; Huiskes & Chao, 1983; Liu et al., 2006; Panagiotopoulou, 2009; Richmond et al., 2005; Tada, Stegaroiu, Kitamura, Miyakawa, & Kusakari, 2003). However, it is extremely challenging to abstract complex biological structures using finite element models, due to the highly irregular, complex geometric shapes of organic systems (Duncan & Ayache, 2000). With the help of advanced biomedical imaging techniques, it is now possible to capture virtually any structure in 3D space. Image processing can then be applied to the image sequences to reconstruct detailed, water-tight 3D surfaces to be used as a finite element template. The image processing includes organ segmentation, surface reconstruction, surface smoothing, and model simplification.

This project comprises two steps: (1) to build and deliver an in silico model by combining human MRI data and biological data that is suitable for input into a Dassault Systemes Abaqus Unified FEA product, and (2) to produce an in vitro physical model made of transparent polymer material that mimics human rectal and anal compliance. Data extracted from Human MRI slices has been reconstructed into 3D images using appropriate software by tracing/drawing appropriate tissue outlines in the digital data set. Biological elements included are the colon below the sigmoido-rectal junction, the rectal vault, the valves of Houston, and the anal canal including the interior anal sphincter. The MRI images used for this project

also contain an invasive fecal incontinence catheter within the rectal vault. Peristaltic and haustral waveforms have been integrated into the biological simulations to obtain and display the kinematic gastrointestinal tract below the sigmoido-rectal junction as an active organ.

## Method

In this section, we propose standard operating procedure guidelines to create a segmentation of human anatomical structures, specifically the lower gastrointestinal region, for the purpose of creating a 3D model. In this project, students have played major roles in proposing the standard operating procedure guidelines on how to create a segmentation of human anatomical structures. Step-by-step software instructions on how to segment the sigmoid colon, rectum, anal canal, and internal sphincter have been investigated in detail. Students have also proposed strategies and techniques on how to create a surface model from these segmentations that can be exported to manufacturing software for 3D printing or engineering software for finite element analysis.

### *Medical Image Segmentation*

In the first step, students work with MRI series data of the human lower GI region from three patients provided by a collaborating clinic.

### Introduction to Seg3D segmentation software

Seg3D (v2.1.4) organizes the image processing work as projects. The main user interface (called workspace) is displayed, as shown in Figure 1. There are four sections to Seg3D's interface. The default workspace windows are known as the "One and Three" view. The benefits to this type of view are to see the 3D Volume and three different 2D views (sagittal, axial, and coronal) of the MRI in the workspace. Depending on the

user's viewing preference, the user may change the number of viewing windows on the workspace to better accommodate their needs. An advantage of having all four views on the workspace is that the user can quickly identify their location when referring to a Cartesian coordinate system.

#### Segmenting anatomical structures

Figure 2 shows an example sagittal view of an MRI slice. Parts of the colon have been saturated with a contrasting agent. The spine and tailbone are visible on the right side of the image. The rectum is also labeled and located near the middle of the image. In this particular picture, a catheter has been inserted into the rectum and its balloon has deformed the shape of the structure. The thin white line is where the contrasting agent is moving through the catheter's lumen.

Segmentation Level Set (Malladi, Sethian, & Vemuri, 1995; Sethian, 1999) is the preferred method for segmenting MRI data sets. In order for the Segmentation Level Set tool to work, "seeds" are added to the stack of images. In this case, we are referring to "seeds" as small, circular pixels shown as colored regions in Figure 2. Additional seeds towards the first and last MRI slices have also been added. For the Level Set method to work properly, it is critical to select the right parameters to avoid over-segmentation or under-segmentation. If an area of interest has been over- or under-segmented, the resulting segmented volume will not give an accurate portrayal of the patient's anatomy. Over-segmentation may result in an end product that is larger than the actual anatomical structure and suggests data that was never on the MRI.

#### Exporting the segmentations

Once the segmentation is completed, the resulting volume may be exported as a NRRD file. NRRD (nearly raw raster data) is a library and file format designed to support scientific

visualization and image processing involving N-dimensional raster data. As a side note, this type of model is purely for diagnostic purposes and cannot be used for any type of engineering analysis. For this reason, we need to convert or “mesh” this model into a calculated surface model also known as a tetrahedral surface mesh.

### *Model Surface Reconstruction and Meshing*

In the second step, several software tools are used to produce an in vitro physical model made of transparent polymer material that mimics human rectal and anal compliance. This step involves several operations, including building geometry, converting file format, surface smoothing and simplification, and model optimization.

#### **Building geometry**

SCIRun (v4.6) is used to convert a NRRD file into a tetrahedral volume. Python (v2.7.4) is used as the run time environment to create a tetrahedral volume from a NRRD file. The students generate python scripts with specific parameters to create the volume.

Once the tetrahedral volume is created, the geometry mesh is generated using the python scripts. This process is computationally intensive in that it takes up CPU power, and the computer may slow considerably if performing other tasks. The duration of this process may vary between one and four hours depending on the speed of the computer and the complexity of the geometry.

#### **Converting file formats**

After the meshing process has successfully completed, the output mesh files are converted to VTK format. VTK (Visualization Toolkit) provides a number of source and

writer objects to read and write popular data file formats. The main reason for using this format is to offer a consistent data representation scheme for a variety of dataset types and to provide a simple method to communicate data between software. When dealing with medical images, it is always a good idea to use the most widely used format.

Once the VTK file is created, ParaView (v4.6) can be used to convert it to STL format. STL file formats are native to the stereolithography CAD software, and used for rapid prototyping and computer-aided manufacturing (Chua, Leong, & Lim, 2010). The conversion is straightforward using this tool.

### Surface smoothing and simplification

The last stage is to smooth the mesh. The software tool we used to perform this task was MeshLab. MeshLab offers several options to allow for mesh smoothing. The two tools that worked the best for this particular project were the HC Laplacian Smooth and Taubin Smooth algorithms. Figure 3 shows a raw mesh and a smoothed mesh.

### Model optimization

As an optional stage, we also utilized the commercial software tool, Mimics 3-Matic, to optimize the model such that it is more suitable to export to a Finite Element Analysis (FEA) product for use in software such as Dassault Systemes Abaqus. The optimization includes scaling and registration of the anatomical data, reconstructing anatomy outside of the original scan field of view (FOV), and remeshing. Example results are shown in the results section.

## Results

We include in this section segmentation and meshing results based on sample MRI slices of the human lower GI tract. Figure 4 shows the completed color-coded segmentation results. The blue area represents the sigmoid colon and rectum, the green portion represents the internal sphincter, and the red represents the catheter's lumen as it exits the body. Due to the lack of information from the MRI images, parts of the anatomy have been truncated, and thus we are given an incomplete and unnatural view of the anatomical structure. We can fix this issue with reconstruction.

After the smoothing, reconstructing, and remeshing steps are completed, the volume is ready to be exported (Figure 5). They can be exported into Computer-aided Design (CAD) software, which can execute computer simulated analysis to improve medical device design. With FEA software tools such as Abaqus, engineers are able to select areas of interest and load conditions such as stress to the lining of the rectum and to stimulate the structure's reaction to those conditions.

## Conclusion

This type of research has the potential to reshape healthcare through new scientific knowledge and ultimately provides the necessary information to improve current medical tools by creating more accurate static and kinetic models/simulations of human anatomy. Technological advancements in the medical field are essential for the medical community to continue to be provided with ever-improving medical devices and tools for patients and healthcare practitioners. Not only that, but through the use of advanced, cutting-edge technology, health care practitioners can be aided in improving diagnosis accuracy, patient treatment, and monitoring to shorten recovery times. In conclusion, continued support for medical technology research projects ultimately saves lives.

## Acknowledgment

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## References

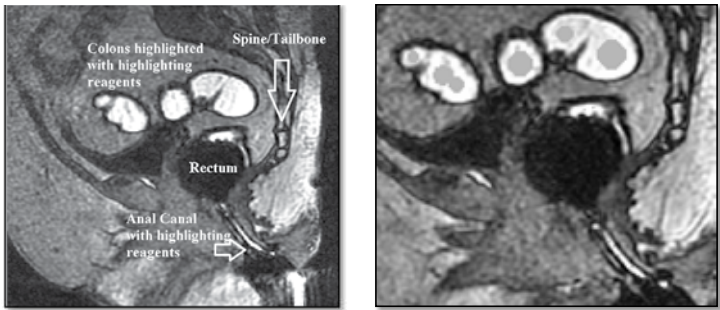
- Bathe, K. J. (1996). *Finite Element Procedures* (Vol. 2). Englewood Cliffs, NJ: Prentice Hall.
- Boccaccio, A., Ballini, A., Pappalettere, C., Tullo, D., Cantore, S., & Desiate, A. (2011). Finite element method (FEM), mechanobiology and biomimetic scaffolds in bone tissue engineering. *International Journal of Biological Sciences*, 7(1), 112.
- Chua, C. K., Leong, K. F., & Lim, C. C. S. (2010). *Rapid Prototyping: Principles and Applications*. Hackensack, NJ: World Scientific.
- Duncan, J. S., & Ayache, N. (2000). Medical image analysis: Progress over two decades and the challenges ahead. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 22(1), 85–106.
- Holzapfel, G. A., Gasser, T. C., & Stadler, M. (2002). A structural model for the viscoelastic behavior of arterial walls: continuum formulation and finite element analysis. *European Journal of Mechanics-A/Solids*, 21(3), 441–463.
- Hughes, T. J. (2000). *The Finite Element Method: Linear Static and Dynamic Finite Element Analysis*. Mineola, NY: Dover Publications.
- Huiskes, R., & Chao, E. (1983). A survey of finite element analysis in orthopedic biomechanics: the first decade. *Journal of Biomechanics*, 16(6), 385–409.
- Liu, W. K., Liu, Y., Farrell, D., Zhang, L., Wang, X. S., Fukui, Y., . . . Lee, J. (2006). Immersed finite element method and its applications to biological systems. *Computer Methods in Applied Mechanics and Engineering*, 195(13), 1722–1749.

- Malladi, R., Sethian, J. A., & Vemuri, B. C. (1995). Shape modeling with front propagation: A level set approach. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 17(2), 158–175.
- Panagiotopoulou, O. (2009). Finite element analysis (FEA): Applying an engineering method to functional morphology in anthropology and human biology. *Annals of Human Biology*, 36(5), 609–623.
- Richmond, B. G., Wright, B. W., Grosse, I., Dechow, P. C., Ross, C. F., Spencer, M. A., & Strait, D. S. (2005). Finite element analysis in functional morphology. *The Anatomical Record Part A: Discoveries in Molecular, Cellular, and Evolutionary Biology*, 283(2), 259–274.
- Sethian, J. A. (1999). *Level Set Methods and Fast Marching Methods: Evolving Interfaces in Computational Geometry, Fluid Mechanics, Computer Vision, and Materials Science* (Vol. 3). New York, NY: Cambridge University Press.
- Tada, S., Stegaroiu, R., Kitamura, E., Miyakawa, O., & Kusakari, H. (2003). Influence of implant design and bone quality on stress/strain distribution in bone around implants: a 3-dimensional finite element analysis. *The International Journal of Oral & Maxillofacial Implants*, 18(3), 357.

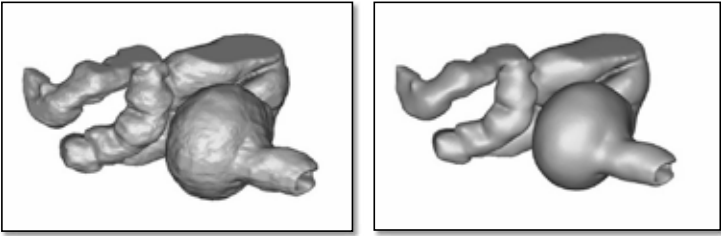




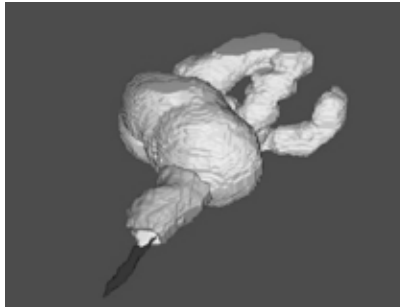
**Figure 1.** Example of Seg3D interface



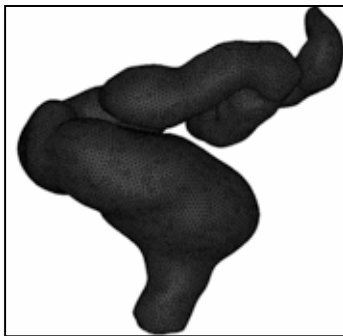
**Figure 2.** (Left) Sagittal view of MRI slice; (Right) Seeds added to Areas of Interest



**Figure 3.** Comparison between raw mesh (Left) and smoothed mesh (Right)



**Figure 4.** Completed Segmentation Result



**Figure 5.** Completed Tetrahedral Mesh



**HEALTH PROMOTION &  
HUMAN PERFORMANCE**

# The Efficacy of Motivational Imagery Intervention in Distance Athletes

Jenna Deelstra

Faculty Mentor: Jordan Utley

## Abstract

*The purpose of the present study is to explore the impact of facilitative mental imagery training on distance runners. Female (n=20) and male (n=11) athletes, separated by skill level classifications: Collegiate (n=19) and recreational (n=12) completed the Motivational Imagery Ability Measure for Sport (MIAMS) before and after mental imagery training. Included in the training were relaxation and breathing techniques followed by guided imagery related to distance-running training and competition. Multiple t-tests examining the effects of the imagery training on MIAMS survey responses between collegiate and recreational athletes for motivational general-mastery imagery (MG-M) and arousal imagery (MG-A) were employed. Significance was found in the emotional response evoked and the ease of producing imagery between the two groups. More research must be done to better understand the effects of mental imagery training on distance athletes.*

## Introduction

Mental imagery is the processing of perceptual information in the absence of external stimuli (White & Hardy, 1998). Mental imagery often relies upon memories and previous experiences

to create an image (Calmels, Holmes, Berthoumieux, & Singer, 2004). However, images of the mind can be so strong that a picture or a detailed story, not actually experienced, may become perceived as a memory and recalled at will (Matthews, Ridgeway, & Holmes, 2012). Researchers in many fields have studied this mental ability, but it is through the study of the mind and body as a whole, that the greatest physical and cognitive potentials of an individual may be discovered. A way to explore those abilities is through studying the psychology of sport (Moran, 1996).

The field of sport psychology has been interested in mental imagery since the mid-1950s (Kornspan, 2013). It has been suggested that mental imagery is a skill because imagery abilities can improve with practice (Rodgers, Hall, & Buckholz, 1991). Nordin and Cumming (2008) compared imagery abilities of aesthetic sport performers and dancers. Aesthetic sport performers included gymnasts and ice skaters. Dancers engaged in significantly more movement imagery when compared to aesthetic athletes. "A possible explanation for this finding could be that imagery is more overtly used and encouraged by dance teachers than by sport coaches" (Nordin & Cumming, 2008). Higher-level dancers showed more frequent use of imagery than lower-level dancers. Though this study was conducted with dancers and gymnasts, the results can benefit the sporting community in general.

While in the process of creating the Sport Imagery Ability Questionnaire, Williams and Cumming (2011) found that many individuals have strengths and weaknesses in creating mental images. An athlete may be able to emotionally recreate an image, but find it difficult to create a mental picture of the scene. Skill imagery, or the ability to feel and recreate a specific movement mentally, was found to be easier for most athletes than mastery, goal, or strategy imagery. It was concluded that an athlete's ability to create a single form of imagery should not be mistaken as the extent of the athlete's capabilities.

Paivio (1985) instituted a framework for identifying functions that mental skills may provide during practice and competition. He described imagery as having two dimensions: general and specific. The Sport Imagery Questionnaire (SIQ; Hall, Mack, Paivio, & Hausenblas, 1998) further distinguished five different functions of imagery: cognitive specific (CS; images of sport skills), cognitive general (CG; images of strategies or routines), motivational specific (MS; images related to goals), motivational general-arousal (MG-A; images associated with anxiety, stress, and excitement), and motivational general-mastery (MG-M; images of mental toughness and self-confidence). From those five, athletes reported using MG-A and MG-M most often (Mortiz, Hall, Martin, & Vadoa, 1996).

MG-A imagery can influence an athlete's emotional response to a situation in several ways by inducing anxiety, nerves, excitement, worry, and intimidation. These feelings can overwhelm an athlete learning to properly use imagery. Using arousal imagery to image a competitive situation alone can lead to higher levels of anxiety. By imaging the scene with calm, focused thoughts, the athlete may have a lower level of anxiety (Vadoa, Hall, & Moritz, 1997).

MG-M imagery has been found to reduce anxiety in high anxiety environments, such as in sport settings (Coelho et al., 2012). It has also been shown to increase sport confidence in athletes (Gregg & Hall, 2006). High levels of sport confidence are often positively correlated with high levels of performance (Hammond, Gregg, Hrycaiko, Mactavish, & Leslie-Toogood, 2012). Vadoa et al. (1997) believe that an imagery training intervention focused on decreasing competitive anxiety and increasing sport-confidence may be beneficial to sport performance.

Coelho et al. (2012) found that MG-M and MG-A imagery could influence how athletes handle stress. A pre- and post-test containing a competitive anxiety scale and a perceived stress scale were used. The athletes engaged in relaxation, behavior

modeling, and imagery videos three times a week for nine weeks. It was concluded that imagery training significantly reduced anxiety and increased confidence.

Research has shown that there may be a positive correlation between mental imagery use and positive self-talk (Neck & Manz, 1992). Guided imagery integrates positive self-talk, relaxation techniques, and vivid imagery into a routine that can be used daily. Guided imagery audio scripts were created for golfers in a competitive setting. It was found to be beneficial for stress reduction, exercise assimilation, confidence and self-efficacy, and sport performance (Hammond et al., 2012).

The purpose of this study is to explore the effectiveness of facilitative imagery training on the MG-M and MG-A imagery abilities of both collegiate and recreational athletes. It is predicted that collegiate athletes will score higher than recreational athletes on the MIAMS survey before and after the training intervention. Consistent with current theories, it is expected that collegiate and recreational athletes will score significantly higher on the post-training survey than on the pre-training survey.

## Method

### *Participants*

Study participants (n=31) were recruited from collegiate distance runners at WSU and students involved in an intermediate-level jogging course. Both groups engaged in at least three hours of running each week before and during participation in the study. See descriptive statistics in Table 1.

### *Procedures*

The study consisted of three phases over a 21-day period: baseline or pre-training survey, training intervention, and

post-training survey. Upon completion of the pre-training survey, participants were given a printed outline consisting of each workout, what day it was to be completed, the length of each workout, and a place to log the day/time each workout was completed.

### *Measures*

The MIAMS (Gregg & Hall, 2006) is a questionnaire developed to measure an athlete's ability to form a mental image and their emotional experience created by the image. The questions are directed at the athlete's mastery of both MG-M and MG-A. The participant reads each scenario and rates the ease on a subscale (1-not at all easy to form to 7-very easy to form) and emotion (1-no emotion to 7-very strong emotion). The MIAMS has been shown to have acceptable internal consistency ( $\alpha > .70$ ). For ease of scoring the MIAMS results, all questions addressing strength of emotional response were labeled as x.1 and ease of forming the image, x.2.

### *Imagery Training*

Athlete's Audio is a company that distributes performance-enhancing scripts through mobile devices. Included in the scripts are tactical breathing and progressive relaxation techniques in conjunction with self-talk, mental imagery, emotion control, and recovery.

## **Results**

Data were analyzed using multiple paired-samples t-tests. Significance between the recreational and collegiate groups was found in the post-imagery intervention survey. Results were significant at the  $< .05$  level. There was a significant difference post-intervention for collegiate athletes for the score MG-M emotion question 3.1 (strength of emotional response), pre-intervention ( $M=4.17$ ,  $SD=1.95$ ), and post-intervention ( $M=5.22$   $SD=1.35$ );  $t(17)=2.64$ ,  $p=.017$ . The effect of intervention



was also significant for recreational athletes; pre-intervention ( $M=4.27$   $SD=1.42$ ) and post-intervention ( $M=5.82$   $SD=1.25$ );  $t(10)=2.92$ ,  $p=.015$ . This shows that both collegiate and recreational participants had an increased ability to emotionally respond to images related to motivational mastery.

There was a significant difference post-intervention for collegiate athletes for the score MG-M ease 3.2 (ease of forming image);  $t(17)=2.36$ ,  $p=.030$  pre ( $M=4.28$ ,  $SD=2.05$ ) and post ( $M=5.44$   $SD=1.50$ ). The effect of intervention was also significant for recreational athletes; pre ( $M=4.27$   $SD=1.35$ ) and post ( $M=5.91$   $SD=1.22$ );  $t(10)=2.70$ ,  $p=.022$ . This shows that all athletes found it much easier, post-intervention, to image the motivational-mastery scene.

There was a significant difference post-intervention for collegiate athletes for the score MG-A emotion 6.1:  $t(16)=3.52$ ,  $p=.015$ , pre ( $M=4.53$ ,  $SD=1.01$ ) and post ( $M=5.53$   $SD=1.42$ ). The effect of intervention was not significant for any of the 24 pairs of club or recreational athletes. This shows that collegiate athletes responded emotionally to the scene designed to measure motivational-arousal.

There was a significant difference post-intervention for collegiate athletes for the score MG-M ease 8.2, pre ( $M=5.39$ ,  $SD=1.61$ ), and post ( $M=6.17$   $SD=1.10$ );  $t(17)=2.72$ ,  $p=.003$ . The affect of intervention was not significant for any of the 24 pairs of club or recreational athletes. This shows that collegiate athletes were able to image the mastery scene with greater ease. A summary of results can be seen in Table 2.

In order to accurately measure the participant's motivational imagery abilities, the means of emotion and ease for both MG-M and MG-A were calculated and a t-test was used to determine their significance. It was found that MG-M imagery post-intervention was significant for emotion in recreational

athletes, pre ( $M=5.22$   $SD=.68$ ) and post ( $M=5.94$   $SD=.69$ );  $t(11)=2.80$ ,  $p=.017$ . Collegiate athlete's responses showed to be even more significant pre ( $M=4.81$   $SD=1.79$ ) and post ( $M=5.56$   $SD=.87$ );  $t(17)=3.07$ ,  $p=.007$ .

There was a significant difference post-intervention for the score MG-M ease  $t(17)=2.68$ ,  $p=.016$ , Pre ( $M=5.19$ ,  $SD=1.26$ ), and post ( $M=6.00$   $SD=.822$ ). The effect of intervention was not significant for any of the 24 pairs of club or recreational athletes. This shows that collegiate athletes more easily imaged scenes related to motivational mastery than did recreational athletes. Both MG-A emotion and ease were not found to be significant at this level.

## Discussion

Results showed that an imagery intervention might improve imagery ability. This finding supports the theory of Vadao, Hall, and Moritz (1997) that MG-M and MG-A imagery training may be beneficial and improve an athlete's motivational imagery skills. As expected, both collegiate and recreational athletes increased in motivational imagery ability.

Our use of guided imagery containing relaxation and breathing techniques, positive self-talk, emotion control, and motivational images impacted imagery ability as was suggested by Coelho et al. (2012). It can be suggested to both coaches and athletes that imagery use containing these practices should be utilized in training and competition atmospheres. Because our athletes showed an improvement in imagery ability between pre- and post-surveys, it can be concluded that imagery is a skill and can increase with practice; this supports the findings of Rodgers et al. (1991).

## Conclusion

It was found that both collegiate and recreational athletes increased in MG-M imagery ability and were able to emotionally image mastery of a difficult competitive situation. Collegiate athletes proved to image motivational scenes more easily than recreational athletes. Guided imagery proved to be a useful tool in training athletes to use imagery on a daily basis. The ability to use mental imagery is a skill that can be fine-tuned with an imagery intervention and should be used daily. The results of the current study indicate that through MG-A and MG-A imagery use, athletes will have greater emotional self-regulation, sport confidence, and reduced psychological stress while using imagery.

More research must be done to investigate the impact of an MG-M and MG-A imagery intervention on sport performance. Results of distance athlete's use of all imagery types after an imagery intervention may prove intriguing and should be studied. Ways that athletes can integrate imagery training along with their physical training should be investigated and implemented.

## References

- Calmels, C., Holmes, P., Berthoumieux, C., & Singer, R. N. (2004). The development of movement imagery vividness through a structured intervention in softball. *The Journal of Sport Behavior*, 27(4), 307-322.
- Coelho, R. W., Keller, B., Kuczynski, K. M., Jr., E. R., Lima, M. C., Grebogg, D., et al. (2012). Use of multimodal imagery with precompetitive anxiety and stress of elite tennis players. *Perceptual and Motor Skills*, 2(114), 419-428.
- Gregg, M., & Hall, C. (2006). Measurement of motivational imagery abilities in sport. *Journal of Sport Sciences*, 24(9), 961-971.
- Hall, C. R., Mack, D. E., Paivio, A., & Hausenblas, H. A. (1998). Imagery use by athletes: Development of the sport imagery questionnaire. *International Journal of Sport Psychology*, 29(1), 73-89.

- Hall, C., Vadoa, E., & Moritz, S. (1997). The relationship between competitive anxiety and imagery use. *Journal of Applied Sport Psychology*, 9(2), 241–253.
- Hammond, T., Gregg, M., Hrycaiko, D., Mactavish, J., & Leslie-Toogood, A. (2012). The effects of motivational general-mastery imagery intervention on the imagery ability and sports confidence of inter-collegiate golfers. *Journal of Imagery Research in Sport and Physical Activity*, 7(1). doi: 10.1515/1932-0191.1066
- Kornspan, A. S. (2013). Alfred W. Hubbard and the sport psychology laboratory at the University of Illinois, 1950–1970. *The Sport Psychologist*, 27(3), 244–257.
- Matthews, A., Ridgeway, V., & Holmes, E. A. (2012). Feels like the real thing: Imagery is both more realistic and emotional than verbal thought. *Cognition and Emotion*, 27(2), 217–229.
- Moran, A. P. (1996). *The Psychology of Concentration in Sport Performers: A Cognitive Analysis*. East Sussex, United Kingdom: Psychology Press.
- Mortiz, S. E., Hall, C. R., Martin, K. A., & Vadocz, E. (1996). What are confident athletes imaging?: An examination of image content. *The Sport Psychologist*, 10(2), 171–179.
- Neck, C. P., & Manz, C. C. (1992). Thought self-leadership: The influence of self-talk and mental imagery on performance. *Journal of Organizational Behavior*, 13(7), 681–699.
- Nordin, S. M., & Cumming, J. (2008). Exploring common ground: Comparing the imagery of dancers and aesthetic sport performers. *Journal of Applied Sport Psychology*, 20(4), 375–391.
- Paivio, A. (1985). Cognitive and motivational functions of imagery in human performance. *Canadian Journal of Applied Sport Sciences*, 10(4), 22S–28S.
- Rodgers, W., Hall, C., & Buckholz, E. (1991). The effect of an imagery training program on imagery ability, imagery use, and figure skating performance. *Journal of Applied Sport Psychology*, 3(2), 109–125.
- White, A., & Hardy, L. (1998). An in-depth analysis of the uses of imagery by high-level slalom canoeists and artistic gymnasts. *The Sport Psychologist*, 12(4), 387–403.

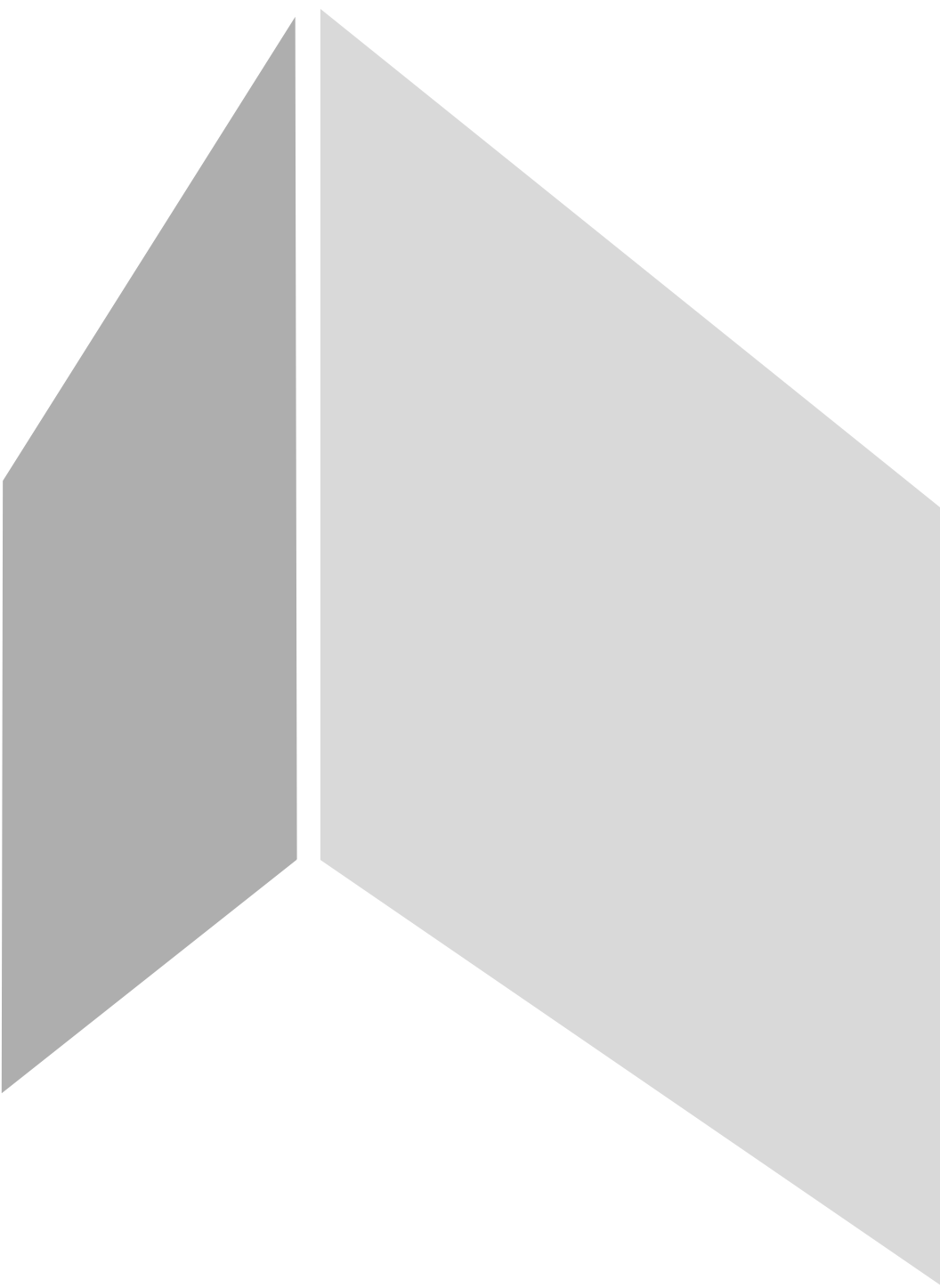
Williams, S. E., & Cumming, J. (2011). Measuring athlete imagery ability: The sport imagery ability questionnaire. *Journal of Sport & Exercise Physiology* , 33(3), 416–440. Retrieved from <http://www.athletesaudio.com>

**Table 1.** Gender, means, and standard deviations for age of athletes.

Gender	Level of Participation	# of Participants	Age Mean	SD
Male	Collegiate	7	22.43	2.88
Female	Collegiate	12	19.83	1.34
Male	Recreational	4	27.25	7.41
Female	Recreational	8	27.13	12.24

**Table 2.** T-test Summary Table

Question #	Rec.	Col.	M and SD		t	p*
			pre	post		
MG-M Emotion 3.1	X	X	M=4.27 SD=1.42	M= 5.82 SD=1.25	t(10)=2.92	.015
			M=4.17, SD=1.95	M=5.22 SD=1.35	t(17)=2.64	.017
MG-M Ease 3.2	X	X	M=4.27 SD=1.35	M=5.91 SD=1.22	t(10)=2.70	.022
			M=4.28, SD=2.05	M=5.44 SD=1.50	t(10)=2.92	.015
MG-A Emotion 6.1		X	M=4.53, SD=1.01	M=5.53 SD=1.42	t(16)=3.52	.015





**MEDICAL LABORATORY  
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# Effects of Glucose on Swarming Motility of *Proteus mirabilis*

Karlee Emal & Scott Chamberlain

Faculty Mentor: Scott Wright

## Abstract

Diabetes mellitus patients have an increased risk of urinary tract infections, presumably due to increased saccharides in their urine. *Proteus mirabilis* (*P. mirabilis*) is a common pathogen of the urinary tract and research has shown media supplemented with 4% glucose enhances *P. mirabilis* biofilm formation, suggesting glucose availability plays an important role in *P. mirabilis* uropathogenesis. Another feature of *P. mirabilis* is cyclic swarming motility. To investigate if glucose availability also enhances swarming motility, five clinically isolated strains were grown on sheep-blood agar with and without 4% glucose. Swarming motility was measured from the center inoculum outwards in millimeters (mm) every hour for 18 hours, repeated in triplicate. Silver stains performed on an organism from each plate assessed the morphology of the flagella. Multivariate linear regression analysis showed that in the presence of glucose, *P. mirabilis* swarmed a significantly less distance ( $p=5.57 \times 10^{-19}$ ) horizontally over an 18-hour period compared to strains on non-glucose media. This could be due to an increase in vertical colony growth during the consolidation phase of *P. mirabilis* in the presence of glucose. There were no distinguishable differences between morphology of flagella from each agar. It is unlikely the change in growth rate is due to changes in flagella morphology.



## Introduction

*P. mirabilis* is a motile gram negative rod-shaped bacteria, and is normal flora of the gastrointestinal tract. This organism causes complicated urinary tract infections (UTIs) resulting in cystitis, acute pyelonephritis and urinary stones. *P. mirabilis* utilize a number of different virulence strategies such as: adhesion, motility, biofilm formation, immune evasion, and nutrient acquisition. All of these virulence factors contribute to this organism's ability to colonize the urinary tract (Jacobsen, Stickler, Mobley, & Shirtliff, 2008).

Research has shown that the presence of glucose causes *P. mirabilis* to form larger biofilms. This may suggest a link between the presence of glucose in the urine of type 2 diabetes patients and their increased rate of catheter-acquired urinary tract infections (CAUTIs) (Hola, Peroutkova, & Ruzicka, 2012). Biofilm formation is a virulence factor that is of particular concern when a prosthetic device is present. Subsequently, other research has shown that non-catheterized patients with type 2 diabetes have a nearly 60% increased risk of UTIs as compared to non-catheterized patients without diabetes (Hirji, Guo, Andersson, Hammar, & Gomez-Camirero, 2012). These findings suggest glucose is enhancing the virulence of *P. mirabilis* in these individuals.

Swarming motility is a cyclic, multicellular behavior allowing for the rapid migration of cells, which is accomplished by rafts of cells growing in unison. These rafts are formed by the bacteria linking together via helical connections with their flagella (Jones, Young, Mahenthiralingam, & Stickler, 2004). *P. mirabilis* on laboratory culture plates displays swarming colonies with striking patterns characterized by circular symmetry and regularly spaced concentric terraces. These terraces develop as a result of periodic events during colony growth, most notably the cyclic repetition of alternating phases: swarming (active migration) and consolidation (growth without movement of the colony perimeter) (Rauprich et al., 1996).

It was hypothesized that glucose would increase the rate of at least one of the phases of swarming motility of *P. mirabilis*, indicating more biological activity. To test this, *P. mirabilis* samples were cultured both with and without glucose supplementation.

## Method

Five *P. mirabilis* clinical isolates from confirmed UTIs were obtained from Ogden Clinic and Ogden Regional Medical Center in Utah. Bacterial suspensions were made in Columbia broth from active growing cultures with a 0.1 optical density measured by a Siemens turbidity meter. Sheep blood agar (SBA) plates and SBA plates supplemented with 4.0% glucose (SBGA) were center inoculated with 1.0  $\mu$ L of the bacterial suspension. Plates were incubated agar side down with no lid at 37°C with 5.0% CO<sub>2</sub> for 18 hours. An incubation time of 18 hours was selected to allow all measurements to be read before swarming reached the edge of the plate.

To avoid desiccation of the agar, containers of sterile water were placed inside the incubator. Growth was recorded during incubation through time-lapse photography at one-minute intervals using a GoPro Hero 3HD camera. Growth rate was measured in millimeters per hour using the camera footage. Each strain was cultured on both types of media in triplicate. A multivariate linear regression analysis was performed averaging the growth at each hour for each agar type across all strains.

After the 18-hour incubation, glass slides of the organism were prepared for microscopic flagella assessment using a Presque Isle Cultures K13 flagella stain kit, consistent with manufacturer's instructions, and read using standard light microscopy. A multivariate linear regression was performed to assess if media had a significant effect on growth rates controlling for inter-strain variability (Figure 1). The data was analyzed using Microsoft Office Excel 2010.

## Results

The comparison of *P. mirabilis* growth between glucose and non-glucose agars was assessed using a multivariate linear regression analysis accounting for deviations among strains. The analysis averaged the growth in mm across all strain's triplicate results every hour for 18 hours, while controlling for inter-strain variability. The analysis indicates glucose has a statistically significant affect on growth rates averaged over time (Figure 1). The data shows on average *P. mirabilis* swarms 5.86 mm less horizontally in the presence of glucose ( $p=5.57 \times 10^{-19}$ ). This results in concentric terraces with smaller diameters (Figure 2).

Visual assessment of silver stained flagella revealed no quantitative or morphological differences among agars. All specimens had a wide arrangement of pleomorphic rods ranging from no flagella to many flagella per bacterium.

## Discussion

It was hypothesized glucose availability would affect the swarming motility of *P. mirabilis*. Results of this research show glucose availability allows *P. mirabilis* to swarm shorter distances in between consolidation phases. When in the consolidation phase *Proteus* cells are interconnected through their flagella, replicate, and stack on top of one another. This could be advantageous when colonizing the urinary tract, as it would provide an enhanced defense against the body's ability to clear the organism.

Previous research shows in the presence of glucose *P. mirabilis* forms higher biofilms. The evidence from this research shows in the presence of glucose *P. mirabilis* swarms less distance horizontally than when glucose is present. This could be caused by an increased vertical growth in the presence of glucose, rather than a horizontal one.

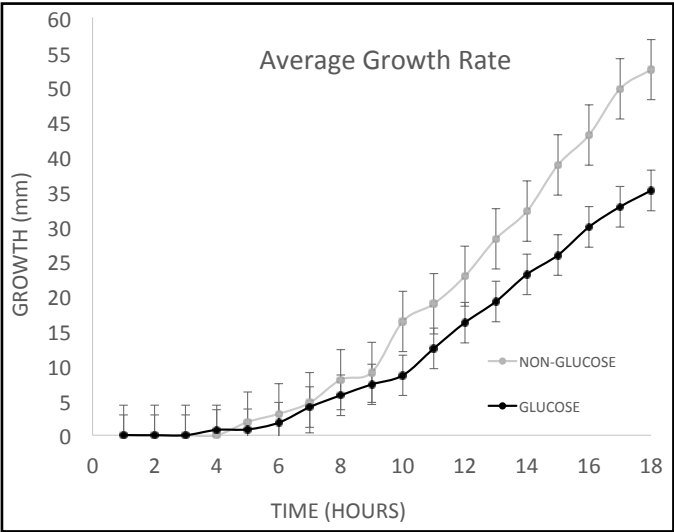
A comparison could not be made between the count or arrangement of flagella among agars due to the random and pleomorphic assortment of flagella. It is unlikely that any of the changes seen in growth rates due to glucose availability are caused by changes in flagella count or arrangement. To better assess this, scanning electron microscopy images of the active growing swarm fronts from each agar could be compared.

Glucose availability has a statistically significant affect on the swarming motility of *P. mirabilis*. This effect could be a contributing factor to the observed increase of complicated UTIs seen in patients with type 2 diabetes.

## References

- Hirji, I., Guo, Z., Andersson, S. W., Hammar, N., & Gomez-Camirero, A. (2012). Incidence of urinary tract infection among patients with type 2 diabetes in the UK General Practice Research Database (GPRD). *Journal of diabetes and its complications*, 8–11. doi:10.1016/j.jdiacomp.2012.06.008
- Hola, V., Peroutkova, T., & Ruzicka, F. (2012). Virulence factors in *Proteus* bacteria from biofilm communities of catheter-associated urinary tract infections. *FEMS immunology and medical microbiology*, 65(2), 343–9. doi:10.1111/j.1574-695X.2012.00976.x
- Jacobsen, S. M., Stickler, D. J., Mobley, H. L. T., & Shirtliff, M. E. (2008). Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clinical microbiology reviews*, 21(1), 26–59. doi:10.1128/CMR.00019-07
- Jones, B. V., Young, R., Mahenthiralingam, E., & Stickler, D. J. (2004). Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. doi:10.1128/IAI.72.7.3941

Rauprich, O., Matsushita, M., Weijer, C. J., Siegert, F., Esipov, S. E., & Shapiro, J. A. (1996). Periodic phenomena in *Proteus mirabilis* swarm colony development. Periodic Phenomena in *Proteus mirabilis* Swarm Colony Development, 178(22)



**Figure 1.** Growth rates of *P. mirabilis* on glucose and non-glucose media. The growth in mm for each strain of *P. mirabilis* was measured every hour over an 18-hour period, repeated in triplicate, and then averaged across strains. A multivariate linear regression was performed to assess if media had a significant affect on growth rates controlling for inter-strain variability, with media being statistically significant ( $p= 5.57\times10^{-19}$ ). Error bars represent standard error of the mean.



**Figure 2.** Visual representation of differences between agar types. Growth of the same strain of *P. mirabilis* at 18 hours shows differences in concentric terrace size between SBA and SBAG agar.

# Variations of Glucose Metabolism in Group O and Non-Group O Blood Types

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## Abstract

*Recent research indicates a significant difference between ABO blood types and the likelihood of developing various diseases such as pulmonary embolism, chronic heart disease, and pancreatic cancer. These studies indicate patients with type O blood have the lowest risk of developing these conditions. Two-hour glucose tolerance tests were used to evaluate the efficiency of glucose metabolism in 62 individuals (30 group O and 32 group non-O). Multivariate linear regression was used to detect statistically significant differences between group O and non-group O patients. There was no significant difference in the mean glucose metabolism between the two groups, but when stratified, group O had significantly more individuals that metabolized glucose to a high degree ( $>40\text{mg/dl}$ ) when compared to the non-O group. While further research is needed, the results of the study could have significant implications for the prevention of developing type 2 diabetes.*

## Introduction

Type 2 diabetes has become an increasingly prevalent public health issue worldwide. Specifically, in Utah, 6.0–7.4% of residents have been diagnosed with Type 2 diabetes

("Diagnosed Diabetes Prevalence," n.d.). Without proper treatment, Type 2 diabetes can quickly cause heart, skin, and kidney complications, which can lead to the patient's death. Although genetics and physical activity have been proven to have an effect on the risk of developing Type 2 diabetes, recent research has suggested ABO blood type may be another risk factor in the development of Type 2 diabetes, in addition to increasing the risk of developing other diseases. People with blood type O have consistently shown a lower risk of developing conditions such as pulmonary embolism, skin cancer, and coronary heart disease (Wolpin et al., 2010; Xie, Qureshi, Li, & Han, 2010; He et al., 2012).

The mechanism as to why blood types have such a varying effect on the developmental risk of diabetes is unclear. To investigate a possible difference in glucose metabolism by ABO blood group, 60 individuals were enrolled in a study to assess glucose metabolisms, using a two-hour glucose tolerance test. Blood types A, B, and AB were grouped together due to their similar blood surface antigens. The dissimilarity of type O made it necessary to test separately. A two-hour glucose tolerance test was used to evaluate glucose metabolism in group O and non-O blood types and then analyzed for significant differences. Categorically, statistically significant differences were found between group O and non-O blood types.

## Method

Advertisements for the glucose research project were posted on campus at Weber State University, and the researchers announced their need for healthy participants in many undergraduate college classes. Interested participants were asked to fill out a survey which included questions relevant to the study such as participant age, height, weight, race, and sex. Participants were also asked several questions about their medical history to inform the researchers if they had



been diagnosed or had any signs or symptoms associated with diabetes, hypoglycemia, thyroid disease, or any other metabolic disorder. After evaluating the surveys, participants were invited to arrive fasting on any one of four days of testing.

Patients were asked to fast, which entailed abstaining from food and drink, except water or black coffee, for eight hours before the test was administered. Students had one serum separator and one K<sub>3</sub>EDTA tube drawn for their baseline fasting glucose and ABO type. Participants were given one 10-oz., non-carbonated, 50-g glucose tolerance beverage. They were instructed to finish the entire drink within five minutes of starting it and were observed to ensure compliance. A researcher recorded the time that each participant finished his or her drink, and issued instructions to return two hours after the finish time.

The researchers verified that the participants returned within two hours +/-10 minutes of ingesting the glucose beverage. Each participant then had his/her blood drawn a second time. One serum separator tube was drawn for the two hour glucose level. Patients were thanked and given a \$25 Visa gift card for their cooperation.

All serum separator tubes were spun down within two hours of the draw to ensure accurate glucose results. Serum separator and K<sub>3</sub>EDTA tubes were spun at around 1,300 RPM for 10 minutes and, if testing was not immediately performed, were refrigerated as soon as possible after centrifugation.

Glucose samples were taken to Ogden Clinic to be run on their Dimension RXL MAX chemistry analyzer, which uses a colorimetric assay method. Printouts were produced for both fasting and two-hour samples and were organized in a binder, which will be retained for two years. Researchers ran ABO blood types manually. Researchers performed a forward type, using commercial anti-A and anti-B anti-sera, and a reverse

type, using reagent A and B cells. Each forward and reverse reaction was verified for discrepancies, and any incongruences were resolved.

Using a Fischer exact test, sex, age, BMI, and race covariates were compared to the amount of glucose metabolized by each blood group. All of these covariates were found to have a  $p > 0.05$  which indicated that they did not have a confounding effect on the glucose metabolism. Glucose metabolism (mg/dl) levels were measured to compare type O blood group and type non-O blood group.

## Results

Sex, age, BMI, and race variables were determined from the surveys given to participants. These variables were used to compare ratios between type-O blood group and type non-O blood group to see if they would have a confounding effect on the amount of glucose metabolized.

T-test analysis of the two groups showed that type-O showed no significant difference from the mean of the type non-O blood group. In addition, each yielded a  $p = 0.38$  indicating that there was no statistically significant difference between their glucose metabolism continuously.

However, when glucose metabolism was stratified into  $>40$ ,  $0-40$ , and  $<0$  mg/dl and a Fischer exact test with a  $p = 0.03225$  was used, a statistical significance in the proportions between blood groups appeared. The Fischer exact test was further broken down into sex, age, BMI, and race against the categorical glucose metabolism. These covariates all gave  $p > 0.05$ , indicating that neither sex, nor age, nor BMI, nor race were causing the difference seen and that blood type was the source of the statistical difference.

## Discussion

When glucose metabolism was analyzed as a continuous variable, the results concluded that there was no significant difference between the load of glucose metabolized by type-O and type non-O. When the results were broken into categories, a statistical difference was found within the large amount of glucose-metabolized grouping. If there were a true difference that could be seen using a linear model, the difference would most likely have been very small. A very large sample size of each category of blood type would be needed to detect the small difference. The results of the study provided very equal sample sizes, and obtaining a larger sample size would project a near-normal distribution that could show a significant difference in the means of the glucose metabolism of the blood types.

Each participant produced a fasting glucose that was consistent with a true fasting state. While the participants filled out the health screen survey to the best of their knowledge, the possibility of unknown cases affecting the fasting glucose level or glucose metabolism in each participant was still present. Testing in particular of the thyroid function, which plays a role in metabolism, would be very effective in screening for factors other than blood type which could influence results.

The metabolism of glucose requires the correct function of hundreds of enzymes and chemical reactions. Multiple tolerance tests, rather than the single one that was used, would be most effective at determining the average glucose metabolism of a participant. While there may not be a large variance between each, repeating the tolerance test multiple times would help to establish a stronger case for clinical correlation. The statistical variance seen in the large category of glucose metabolized may indicate that there is a difference caused by the participant's blood type. A recommendation for a longitudinal study involving in-depth metabolism testing and large sample size could reveal a difference between the type-O and non-O glucose metabolism. When thoroughly researched, the possible discovery of a

difference could aid future medical personnel in determining risk for the development of type 2 diabetes.

## Acknowledgments

The researchers would like to give special recognition to Ogden Clinic for allowing the use of their chemistry analyzer. Thanks are given to this project's mentor Matthew Nicholaou and to Kent Criddle for ordering the supplies that were used in the study.

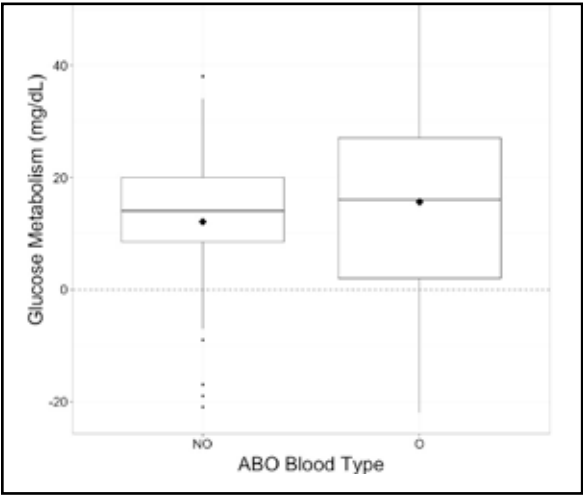
## References

- Centers for Disease Control. (n.d.) Diagnosed Diabetes Prevalence: Year=2004. Retrieved from [http://www.cdc.gov/diabetes/atlas/countydata/DMPREV/data\\_Utah.pdf](http://www.cdc.gov/diabetes/atlas/countydata/DMPREV/data_Utah.pdf)
- He, M., Wolpin, B., Rexrode, K., Manson, J. E., Rimm, E., Hu, F. B., & Qi, L. (2012). ABO blood group and risk of coronary heart disease in two prospective cohort studies. *Arteriosclerosis, thrombosis, and vascular biology*, 32(9), 2314–20. doi:10.1161/ATVBAHA.112.248757
- Qi, L., Cornelis, M. C., Kraft, P., Jensen, M., Van Dam, R. M., Sun, Q., Hu, F. B. (2010). Genetic variants in ABO blood group region, plasma soluble E-selectin levels and risk of type 2 diabetes. *Human molecular genetics*, 19(9), 1856–62. doi:10.1093/hmg/ddq057
- Wolpin, B. M., Kabrhel, C., Varraso, R., Kraft, P., Rimm, E. B., Goldhaber, S. Z.,...Fuchs, C.S. (2010). Prospective study of ABO blood type and the risk of pulmonary embolism in two large cohort studies. *Thrombosis and haemostasis*, 104(5), 962–71. doi:10.1160/TH10-05-0312
- Xie, J., Qureshi, A. A., Li, Y., & Han, J. (2010). ABO blood group and incidence of skin cancer. *PloS one*, 5(8), e11972. doi:10.1371/journal.pone.0011972

**Table 1.** Demographic Data of Covariates

		Type O Blood – N (%)	Type Non-O Blood – N (%)
Sex	male	13 (40.63)	13 (40.63)
	female	19 (59.38)	19 (59.38)
Age	<35	3 (9.38)	3 (9.38)
	>35	29 (90.63)	29 (90.63)
BMI	<25	20 (62.50)	18 (56.25)
	>25	12 (37.50)	14 (43.75)
Race	WnH	28 (87.50)	31 (96.88)
	Other	4 (12.50)	1 (3.13)

BMI = body mass index, WnH = White non-Hispanic, Other = all self-reported races not WnH



**Figure 1.** Glucose Metabolism by Blood Type. Box and whisker plots showing glucose metabolism (mg/dL) between individuals with Non-O blood type (NO) and O blood type (O). Glucose metabolism was determined by subtracting fasting glucose from the post two-hour glucose challenge levels. Black Diamonds represent the mean glucose level for each blood group. Black dots represent glucose values outside 1.5 times the interquartile range of glucose values for that group.

# Cholesterol and Triglyceride Validation

Lexie Yarema, Ashley Park, & Ronni Erickson

Faculty Mentor: Ryan Rowe

## Abstract

*Validation of medical laboratory equipment is required by Centers for Medicare and Medicaid Services, as per the Clinical Laboratory Improvement Act (CLIA) in order to ensure patient results are accurate (Centers for Disease Control and Prevention [CDC], 2004). This validation was performed on the ACE chemistry analyzer in the Medical Laboratory Sciences (MLS) Department at Weber State University (WSU). The two analytes validated were total cholesterol and triglycerides. Cholesterol oxidase and glycerol kinase were the two methods used for total cholesterol and triglycerides assays, respectively. The areas of focus for the validation involved precision, accuracy, minimum detection limit, linear range, and quality control (QC) range. Triglycerides are blood lipids necessary for transfer of adipose fat and blood glucose. Cholesterol is an important sterol for cell membrane permeability and fluidity, as well as synthesis of steroid hormones in the body (Olson, 2013). Therefore, the body has to maintain normal levels of these substances for proper function. If the levels of either one of these lipids are elevated, a wide variety of serious illnesses may occur. Proper and accurate evaluation of cholesterol and triglycerides is desired for prevention of these unwanted consequences.*

## Introduction

Coronary heart disease is the leading cause of death in the United States. Lipid buildup in coronary vessels results in hardening of the arteries, which in turn may lead to a decreased blood flow to the heart (CDC, 2010; CDC, 2012). Accurate evaluation of cholesterol and triglycerides is important to identify patients with increased risk. Evaluations are routinely performed on analyzers in the hospital laboratories, and are known as validation studies. A series of tests are performed on the analyzers to establish a range of values that would be considered acceptable for each of the analytes. Therefore, when a new analyzer is brought into the laboratory, a validation study needs to be completed.

## Method

The validation study included precision, minimum detection limit (MDL), QC range, linear range, and accuracy. Run-to-run and within-run analyses ensure that the precisions of the analyzer are within the acceptable limits. To perform run-to-run precision, two levels of controls were run 30 times each over a period of three weeks. The data was then used to find the mean, standard deviation (SD), and percent coefficient of variation (% CV). The results from calculations are used to determine the QC range (mean  $\pm$  2 SD) for future testing.

For the within-run precision, the same two levels of controls used in the run-to-run were analyzed randomly 30 times each in succession, after which the % CV was calculated to assess how precise the machine was functioning and to identify any carry over. MDL was calculated to determine the value of lowest concentration of analyte that can be distinguished from zero. This was performed by the assessment of values obtained from zero and non-zero calibrators run in triplicates. The obtained values were then subjected to statistical analysis in which mean, SD, and mean  $\pm$  3 SD were calculated and

graphed using  $y=mx+b$ . To calculate  $x$ , which is the MDL, the mean +3 SD of the zero calibrator was used as the  $y$  value.

Accuracy and linear range were defined by assessing several calibrator concentrations. Each concentration was run three times and the average value was compared to the target value established by the manufactures of the calibrators. The formula  $\% \text{ accuracy} = (1 - |(\text{mean target}) - \text{target}| / \text{target}) \times 100$  was used to calculate accuracy for each of the calibrator concentrations. Accuracy values above 90% were used in the determination of linear range.

## Results

The overall CV for triglycerides was below five (3.08), which indicated strong precision. For cholesterol the overall CV was also under five (2.98) indicating a strong precision as well (Chisala, 2013). The MDL values for both analytes indicated that the instrument is sensitive enough to include all of the lowest possible levels of cholesterol and triglyceride in humans. Linear range analysis revealed that the instrument is accurate from 20 mg/dl to 748 mg/dl for triglycerides and 135 mg/dl to 468 mg/dl for cholesterol. From the values obtained from this study, it was determined that the ACE analyzer did not show shifts, trends, or carry over, and the acceptable QC range was established.

## Conclusion and Discussion

Validation of qualitative analytical methods is an important matter to ensure quality results obtained from any analyzer used to assess human samples. This validation study assures accurate total cholesterol and triglyceride assay performance of the ACE analyzer in the MLS Department at WSU. The findings provide sufficient evidence to conclude that the ACE chemistry analyzer is suitable for total cholesterol and triglyceride assays,



with respect to the testing methods employed using the specific lot number of reagent and control material. Limitations in this study were time restriction, sample type analyzed, and age of reagent. With more time, more samples could have been run, which would increase accuracy. The data would have been collected over a broader time frame and more values could have been included. Serum/plasma was the only specimen type validated in this study. Reagents used in this study were older, possibly causing error in the results collected.

## Acknowledgments

The researchers would like to thank the MLS Department for access to the ACE chemistry analyzer. A special thanks to Kent Criddle for all his time and efforts helping with this project.

## References

- Centers for Disease Control and Prevention. (2004). Current CLIA Regulations. Retrieved from <http://www.cdc.gov/clia/regs/toc.aspx>
- Centers for Disease Control and Prevention. (2010). Cholesterol and Heart Disease. Retrieved from <http://www.cdc.gov/cholesterol/about.htm>
- Centers for Disease Control and Prevention. (2012). Cholesterol: *Understanding your risks*. Retrieved from <http://www.cdc.gov/cholesterol/>
- Chisala, C. (2013). CDC. Retrieved from <http://www.cdc.gov/dls/ila/cd/zambia/files/qualitycontrol-chisala.ppt>
- Olson, R. E. (2013). Discovery of the Lipoproteins, Their Role in Fat Transport and Their Significance as Risk Factors. Retrieved from <http://jn.nutrition.org>

**Table 1.**

Triglycerides			
		Level I	Level II
Run-to-run	QC range (mg/dl)	114-157	179-238
Within-run	% CV	2.02	4.14
	Overall	3.08	
	% CV		
MDL (mg/dl)	19.67		
Linear range (mg/dl)	20-748		

**Table 2.**

Cholesterol			
		Level I	Level II
Run-to-run	QC range (mg/dl)	94-117	273-332
Within-run	% CV	1.64	4.32
	Overall	2.98	
	% CV		
MDL (mg/dl)	7.62		
Linear range (mg/dl)	135-468		

# Alpha-1 Antitrypsin and the Diagnosis of Exercise-Induced Bronchoconstriction

Richard John, Megan Hirschi, & Justin Anderson

Faculty Mentors: Gary Nielson, Ryan Rowe,  
& Matthew Nicholaou

## Abstract

*Exercise-induced bronchoconstriction (EIB), generally known as exercise-induced asthma, is a commonly diagnosed pulmonary disease. While the symptoms are well documented, the pathogenesis is not fully understood and thus is commonly misdiagnosed for other pulmonary disorders. The objective of this study is to determine if a positive correlation exists between the diagnosis of EIB and genetic variations of alpha-1 antitrypsin (AAT). AAT is a protein produced by the liver that inhibits a number of enzymes, specifically affecting one found in the respiratory system known as human neutrophil elastase (HNE). Malformations of the AAT protein, due to variations of the individual's genotype, result in the inability to completely inhibit HNE. This leads to a chronic breakdown of host tissue resulting in respiratory problems which include wheezing, asthma, and emphysema. Participants who have been diagnosed with EIB and a control group were screened for the different phenotypes of AAT using isoelectric focusing. Results were analyzed to determine if there exists a correlation in the frequency of the various alleles for AAT among those diagnosed with EIB when compared to the control group. Results failed to show a definitive correlation between the different genotypes of AAT and the diagnosis of EIB.*

## Introduction

Alpha-1 antitrypsin deficiency (AATD) is a rare genetic disorder that causes severe complications of the hepatic and pulmonary systems. AATD is caused by a liver malformation of the protein AAT. This protein inhibits a number of enzymes, specifically affecting one found in the respiratory system known as HNE. HNE is secreted by white blood cells to destroy bacteria; however, it also breaks down host tissue, specifically the elastin that contributes to the elasticity of the lungs. When properly formed, AAT prevents HNE from damaging host tissue. The pulmonary system is specifically susceptible to the absence of the properly formed protein which can cause long-term terminal effects (Needham, 2004).

Most individuals exhibit the normal alleles or normal forms of the gene 'MM', however other versions of the alleles can be common among a normal population as well. These alleles: 'MS', 'MZ', 'SS', and 'SZ', cause differing amounts of malformation or improper distribution of AAT. The homozygous expression 'ZZ' although quite rare, is the most severe, well-documented and understood form of AATD. However, a true diagnosis of AATD may not occur for a substantial amount of time as its symptoms mimic many other respiratory disorders. This can be detrimental to the health of one suffering from AATD, as the effects of the disease are life-threatening and irreversible, and critical treatment to stop these effects would be postponed.

Fewer studies have been performed on the other heterozygous forms of AATD because the repercussions are less debilitating; however, they still present some malformation of the AAT protein and certain pulmonary defects could potentially present themselves. A study done in 2006 shows that a high percentage of patients with some genetic variation of the AAT gene experience different types of respiratory disorders including wheezing and asthma (Eden, Strange, Holladay, & Xie, 2006). Again these defects mimic many other respiratory

disorders such as EIB and thus are commonly misdiagnosed for those disorders.

AATD has been sufficiently studied and is well understood. The frequencies of different genotypes have been estimated and attributed to many parts of the world. (Luisetti & Seersholm, 2004) While EIB has also been studied, it is often suspected and diagnosed without the proper testing. For this study, a sample of people suffering from EIB donated blood to test for phenotypic variations of the alpha-1 gene using isoelectric focusing. The results of the study group were compared with a control group to determine if there is any difference in the occurrence or frequency of the different alleles for AAT among diagnosed EIB patients when compared to a healthy population. The expected results will demonstrate a positive correlation between EIB and phenotypic variations of AAT, which will demonstrate that many who have been diagnosed with EIB may actually be suffering from some form of AATD.

## Method

In order to obtain participants, a questionnaire was used to gather information concerning patient history and current respiratory defects. Questions were taken from a validated questionnaire (ATS-DLD 78). Amongst these questions was one regarding a previous diagnosis of EIB from a licensed health care provider. Those who indicated a previous diagnosis were placed in the EIB (study) group. Individuals with no previous diagnosis were placed in the control group. After receiving Institutional Review Board approval, the participants were contacted and capillary blood samples were collected using an AlphaTest Kit. This kit uses a lancet and special filter paper for collection and storage of samples until delivery for analysis. Dr. Edward J. Campbell, MD, director of the Alpha-1 Center, agreed to assist with testing by donating the kits and providing blinded results. Testing was performed using immunoassay,

phenotyping, and genotyping. This provided quantitative results for alpha-1 serum levels and qualitative results for alpha-1 phenotypes.

## Results

With a pre-set alpha level of 0.05, an epidemiology table was set up in order to run a Fishers Exact test to determine the p-value and the odds ratio. The analysis reported a p-value of 0.715 and an odds ratio of 1:48.

## Conclusion

Due to the odds ratio of 1:48 and a p-value of 0.715, the results fail to show any increased frequency of the various alleles of AAT when compared to the control group. These results indicate that there is no correlation between the diagnosis of EIB and the various alleles of AAT.

## Discussion

The failure to show an increased frequency of the various alleles of AAT among an EIB population could be due to the limited sample size ( $n=34$ ) of each group studied. Because the various alleles of AAT are fairly rare within a population, a bigger sample size is required for a more definitive conclusion. Another limitation with this study was the method in which the experimental group and control group were selected. A questionnaire designed by the American Thoracic Society was used to determine whether participants had been diagnosed with EIB. A more effective method to determine the experimental group would have been to have a licensed physician screen participants for EIB. These limitations could be dealt with in future studies to better determine if individuals who have been diagnosed with EIB show an increased frequency of various alleles of AAT and a possible misdiagnosis of EIB.

## References

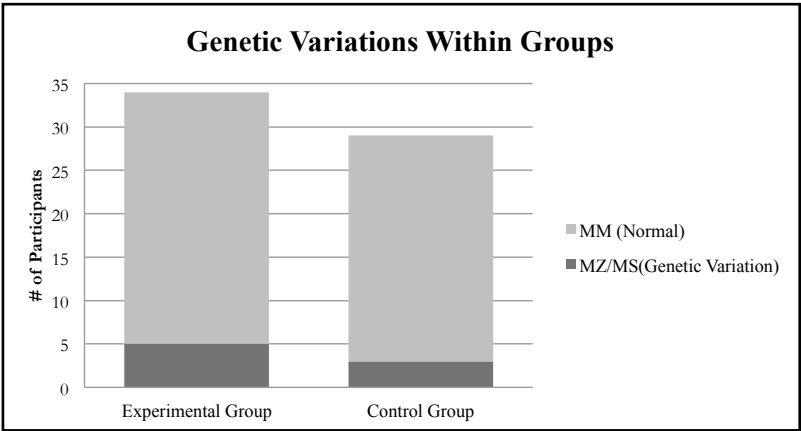
- Anderson, S., & Kippelen, P. (2005). Exercise-induced bronchoconstriction: pathogenesis. Retrieved from <http://www.springerlink.com/index/32R4848HQ36KX1U0.pdf>
- Eden, E., Strange, C., Holladay, B., & Xie, L. (2006). Asthma and allergy in alpha-1 antitrypsin deficiency, 100(8), 1384–91. doi:10.1016/j.rmed.2005.11.017
- Luisetti, M., & Seersholm, N. (2004). Epidemiology of alpha-1 antitrypsin deficiency. *Thorax*, 59(2), 164–169. doi:10.1136/thorax.2003.006494
- Needham, M. (2004). 1-antitrypsin deficiency \* 3: Clinical manifestations and natural history. *Thorax*, 59(5), 441–445. doi:10.1136/thx.2003.006510
- Rachelefsky, G., & Hogarth, D. K. (2008). Issues in the diagnosis of alpha 1-antitrypsin deficiency. *The Journal of Allergy and Clinical Immunology*, 121(4), 833–8. doi:10.1016/j.jaci.2007.12.1183
- Hallstrand, T. S., Curtis, J. R., Koepsell, T. D., Martin, D. P., Schoene, R. B., Sullivan, S., Yorioka, G. N., & Aitken, M. L. (2002). Effectiveness of screening examinations to detect unrecognized exercise-induced bronchoconstriction. *The Journal of Pediatrics*, 141(3), 343–349. doi:10.1067/mpd.2002.125729

**Table 1.** Number of participants that possess the different alleles of AAT. MS/MZ being carrier alleles; MM being the normal healthy allele. The experimental group being those who claimed to have been diagnosed with exercise-induced bronchoconstriction.

	MS/MZ	MM
Experimental Group	5	29
Control Group	3	26

**Table 2.** The Fisher’s Exact Test for Count Data

Fishers Exact Test for Count Data	
P-Value	0.715
Odds Ration	1:48



**Figure 1.** Occurrence of carrier alleles MS/MZ in the control and experimental population with the normal allele MM.



# Comparison of Tooth Soap® and Colgate® on General Oral Health

Anja Greenhalgh, Jolette Neeley, & Ron Bettridge

Faculty Mentors: Matthew Nicholaou & Shelly Costley

## Abstract

*Tooth Soap is an all-natural alternative to standard toothpaste that is marketed as being healthier and safer than fluoride toothpaste. It contains only natural oils, water, salt, and essential oils. To determine significant differences between Tooth Soap and Colgate on oral health, a two-group crossover study of 27 dental hygiene students was used. Oral health was assessed using salivary pH, plaque growth, and growth of *S. mutans*. These factors were measured over a five-week period with a total of three oral exams. After a preliminary exam, Group 1 began the study period using Tooth Soap for two weeks and Group 2 used Colgate during the same two weeks. An intermediate oral exam was given to both groups following the treatment. Both study groups then switched products for the final three weeks of the study, which was followed by a post-study oral exam. Participants were given a post-experiment survey concerning preference of Tooth Soap or Colgate and any other changes they may have noticed between products. Statistical analysis showed no significant difference between the two products, therefore, Tooth Soap may be an acceptable alternative to standard toothpaste. Survey results, however, showed a unanimous preference for Colgate based on taste comparison of the products.*

## Introduction

Dental caries remain the most common chronic disease of children aged 6–11 years and adolescents aged 12–19 years. Tooth decay is four times more common than asthma among adolescents aged 14–17 years. Dental caries also affect adults, with 9 out of 10 over the age of 20 having some degree of tooth-root decay (Centers for Disease Control and Prevention, 2009). The most important method of preventing plaque and tartar buildup from bacteria in the oral cavity is the mechanical action of using a toothbrush and flossing (Canadian Dental Hygienists Association, 2006). Even so, it is impossible to remove pathogenic bacteria and their products entirely from the mouth. The most common cause of dental caries is the bacterium *Streptococcus mutans* (Saini, Gupta, Mahajan, & Arora, 2003). This species of bacteria is able to alter the pH of the mouth, which makes the oral cavity more habitable to other caries related organisms like *Lactobacillus* species (Cvitkovitch, 2011). An increase in either bacteria has been shown to cause an increase in cavity risk and development (Badet & Thebaud, 2008; Saini et al., 2003). Most toothpaste contains fluoride as the primary active ingredient to minimize growth of caries-producing bacteria such as *S. mutans*. One reason fluoride works so effectively is because it is retained in leftover plaque after brushing the teeth (Otten, Busscher, Abbas, van der Mei, & van Hoogmoed, 2012). Despite the action of fluoride and other compounds present in most toothpaste, dental caries remain a common disease in the United States.

Tooth Soap is a new oral care product that is unevaluated by the FDA. It uses all-natural ingredients including coconut oil and other essential oils. It has become popular for manufacturers to produce all-natural or organic products to meet the growing demand of consumers seeking alternatives to products containing chemicals that are socially viewed to be more harsh or unnecessary, such as fluoride. The purpose of this research was to assess the impact Tooth Soap has on

general oral health when compared to conventional fluoride toothpaste as measured by changes in plaque growth, bacterial growth, and salivary pH.

## Method

A convenience sample of 27 junior students from the WSU dental hygiene department were enrolled and randomly assigned into two treatment groups. The study began in spring semester of 2013 and lasted for five weeks. Participants were seen at three time points during the study period: baseline, intermediate, and end. At each visit, variables used to assess oral health were collected including: salivary pH, plaque growth, and *S. mutans* colonization. Participants were given instruction on how to properly use the *S. mutans* collection kits and Tooth Soap. They were also asked to continue their current oral care habits throughout the study, with the exception of the change in toothpaste to account for individual variables such as diet, medications, etc. The two groups began the study with a preliminary oral exam on January 10 to assess a baseline quantification of salivary pH, plaque growth, and concentration of *S. mutans*. Group 1 began the study period using Tooth Soap for two weeks; Group 2 used Colgate Sensitive Enamel Protect during the same two weeks. Colgate Sensitive Enamel Protect was selected because it was readily available through the WSU dental hygiene department. An intermediate oral exam was given to Groups 1 and 2 on January 23, following the two-week treatment. Both study groups then switched products for the final three weeks of the study, which were followed by a post oral exam on February 13.

Plaque was quantified using the Plaque Free Index Score (PFI) with a two-tone liquid disclosing solution and counting four surfaces per tooth. *S. mutans* was measured on stimulated saliva using Dentocult SM strips from Orion Diagnostica. After collection, the SM strips were incubated for 48 hours in the

WSU microbiology lab before interpretation. SM kits were interpreted as growth being rated from 0–3; zero being less than 100,000 CFU/mL, two being between 100,000 and 1,000,000 CFU/mL, and three being greater than 1,000,000 CFU/mL. The salivary pH was measured using Hydrion pH paper read by one member on the research team to account for differing color perceptions. The dental hygiene students participating in the study collected the PFI scores, as they were already trained in this information collection method. Meanwhile, the members of this research group were responsible for the proper collection and interpretation of *S. mutans* and pH results. Participants were given surveys after completion of the final oral exam to document their compliance of use of each product, preference of either Tooth Soap or Colgate, and to express any other sentiments or concerns relevant to the study.

The data collected from this project underwent a mixed effects linear regression that accounted for longitudinal repeated measurements on an individual. This analysis also accounted for covariate factors such as treatment compliance, age, sex, and medications. Participants were instructed to continue their current oral care habits throughout the study with the exception of the change in toothpaste to account for individual variables. These variables also take into account brushing frequency, flossing frequency, rinsing, and diet difference among the study sample.

## Results

There was no statistically significant difference found between Tooth Soap and Colgate when comparing *S. mutans* and PFI scores. The only significant factor discovered was the difference between the baseline pH and both subsequent pH measurements. Colgate was associated with a 0.87 increase, with Tooth Soap associated with a 0.77 increase in regards to the pH. These values appear to be significant findings. Using Colgate

was associated with a 2.66 increase in PFI score, whereas Tooth Soap was associated with a 3.47 increase in PFI. Neither of these results was statistically significant. Colgate was associated with a 0.08 CFU increase in *S. mutans*, while Tooth Soap was associated with a 0.13 CFU increase in *S. mutans*. Neither of these results was significant. Survey statements showed that the taste of Tooth Soap was rated much less desirable on a 1 to 5 scale (1 being “Good”, 5 being “Bad”) when compared to Colgate. The average rating on taste of Tooth Soap was 4.74 on a scale of 1 to 5. Colgate’s taste was rated at an average of 1.44.

## Discussion

The assessments of oral health of patient samples yielded one significant result. The differences between the salivary pH values were later found to be the result of participants consuming an acid-containing beverage and food prior to the collection. Although there were no other statistically significant differences found in the variables measured between Tooth Soap and Colgate, the survey results showed a significantly decreased compliance of Tooth Soap use among the participants. Survey bias may also have been a concern due to all participants studying to be dental hygienists, many of whom had concerns over Tooth Soap lacking a fluoride ingredient.

Limitations to this study include the short duration that the study participants were using each product. Resources were not enough to prolong the study. Our population was also limited to almost all white female dental hygiene students, which had an effect on the extent of results that could be collected. A longer study period may yield new information, and because Tooth Soap is unevaluated by the FDA there is a wide availability of future research topics. This served as an introductory project to see if any significant findings would make an appearance, and to see if Tooth Soap would be an acceptable alternative to

fluoride-containing toothpaste, such as Colgate.

Through the course of research, though the taste was not found to be desirable, Tooth Soap was found to be an acceptable substitute for standard fluoride-containing toothpastes. Those who prefer “natural” toothpaste or have a problem with using fluoridated toothpaste may use this oral care system as an acceptable alternative to fluoridated toothpastes, as far as the results of this study have found. However, it must be noted that Tooth Soap has no fluoride. Fluoride has been shown to decrease the incidence of dental caries; therefore, the caries prevention potential of Tooth Soap was not evaluated.

## References

- Badet, C., & Thebaud, N. B. (2008). Ecology of lactobacilli in the oral cavity: a review of literature. *The open microbiology journal*, 2, 38–48. doi:10.2174/1874285800802010038
- Canadian Dental Hygienists Association. (2006). CDHA Position Paper on Tooth Brushing, 40(5), 1–14.
- Centers for Disease Control and Prevention. (2009). Hygiene-related Diseases Retrieved from [http://www.cdc.gov/healthywater/hygiene/disease/dental\\_caries.html](http://www.cdc.gov/healthywater/hygiene/disease/dental_caries.html)
- Cvitkovitch, D. (2011). Acid Tolerance Mechanisms Utilized by *Streptococcus mutans*. *Future Microbiol*, 5(3), 403–417. doi:10.2217/fmb.09.129
- Otten, M. P. T., Busscher, H. J., Abbas, F., van der Mei, H. C., & van Hoogmoed, C. G. (2012). Plaque-left-behind after brushing: intra-oral reservoir for antibacterial toothpaste ingredients. *Clinical oral investigations*, 16(5), 1435–42. doi:10.1007/s00784-011-0648-2
- Saini, S., Gupta, N., Mahajan, A., & Arora, D. R. (2003). Microbial flora in orodental infections. *Indian journal of medical microbiology*, 21(2), 111–4. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/17642993>

**Table 1.** Mean Changes of Outcome Variables by Colgate and Tooth Soap

Mean Changes of Outcome Variables by Colgate and ToothSoap			
		Mean Change (95% CI)	P-Value
PFI			
	Colgate	2.66 (-2.79, 8.11)	0.34
	ToothSoap	3.47 (-1.73, 8.67)	0.19
pH			
	Colgate	0.87 (0.62, 1.12)	< 0.001
	ToothSoap	0.77 (0.53, 1.00)	< 0.001
Strep Mutans			
	Colgate	0.08 (-0.21, 0.37)	0.60
	ToothSoap	0.13 (-0.15, 0.41)	0.37

Mean changes and p-values were obtained using mixed-effects multivariate linear regression and represent comparisons to the baseline patient visit values.

**Table 2.** Survey Results

Survey Results								
Age	Race	Sex	# of Visits	Medications	TSConsistency	CConsistency	TSTaste	CTaste
24	White	F	3	None	2.89	1.26	4.74	1.44

TSConsistency = Tooth Soap Consistency. CConsistency = Colgate Consistency. TSTaste = Tooth Soap Taste. CTaste = Colgate Taste. Results are based on a 1 to 5 scale (1 being Good, 5 being Bad).

This table shows the average results from the survey that was given to participants.

# Prevalence of *Escherichia coli* O157:H7 in Grain-Fed Cattle and the Implications to Public Health

Michelle Kimball & Ruchi Kasajoo

Faculty Mentor: Scott Wright

## Abstract

*Escherichia coli* O157:H7 has the ability to produce toxins and adhere to endothelial cells lining the digestive tract of humans, causing illness ranging from bloody diarrhea to the life threatening condition, hemolytic uremic syndrome. Cattle are known to be the primary reservoir of the bacteria, with transmission to humans occurring through direct contact, consumption of meat and milk products, and through manure contamination of water and field crops. There is concern that the practice of feeding grain to cattle leads to an increase of digestive acidity, which could possibly enhance the growth of *E. coli* O157:H7. The objective of this research was to determine if *E. coli* O157:H7 could be isolated from cattle raised in the northern Utah area and if the occurrence of the strain was more prevalent in grain-fed cattle. Four farms were studied: two that feed their cattle a grain-based diet, and two that feed a grass-based diet. The presence of the O157 and the H7 antigens were detected in two out of 107 grass-fed samples (1.8%), and six out of 59 grain-fed samples (10.2%). The prevalence of the two antigens, O157, and H7 were significantly different ( $p=0.02442$ ) between the two diet exposures, with an odds ratio of 5.88 (95% CI, 1.01–61.5) in grain-fed cattle versus



*grass-fed cattle. That is, grain-fed cattle had nearly six times the risk of having O157:H7 positive stool cultures compared to grass-fed cattle.*

## Introduction

*Escherichia coli* is a commensal bacterium that inhabits the gastrointestinal tract of healthy humans and animals (Shulman, Friedmann, & Sims, 2007). This bacterium was first discovered in 1884 by Theodor Escherich, a German bacteriologist and pediatrician. He noted it as a beneficial inhabitant of the intestinal tract that helps with the host's digestion and plays a key role in the inhibition of other virulent bacterial species by competitive exclusion. In 1945, researchers discovered genetically distinct strains of *E. coli* responsible for causing infant diarrhea (Kaper, Nataro, & Mobley, 2004). Then in 1975, the novel strain, O157:H7, was positively identified, and it was subsequently identified as a human pathogen in 1982 (Sanderson et al., 1995). By way of lateral gene transfer, *Escherichia coli* rapidly evolved and has acquired at least 1,387 genes that are absent in non-pathogenic strains (Gyles, 2007). These genes are responsible for the organism's virulence factors such as the ability to produce toxins and attach to endothelial cells lining the digestive tract (Caprioli, Morabito, Brugere, & Oswald, 2005; Vidovic & Korber, 2006). *Escherichia coli* O157:H7 has the ability to cause illness in humans, ranging from bloody diarrhea to the life threatening conditions hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Griffin & Tauxe, 1991). Although this pathogen can affect individuals of any age, children under the age of five are especially vulnerable, and it is the leading cause of kidney failure in children (Ochoa & Cleary, 2003; Griffin & Tauxe, 1991).

Because these bacteria have also acquired specific genetic capabilities to withstand the highly acidic environment of the human stomach, the infectious dose is very low (1–100 CFU) (Griffin et al., 1994; Paton et al., 1996). The extreme virulence of this pathogen along with the low infectious dose has caused the federal government to ban any presence of the bacteria in raw

ground beef (Food Safety and Inspection Service, 2001). If the pathogen is found, the offending product(s) must be immediately recalled from market inventories.

However, *Escherichia coli* O157:H7 is still getting into the food supply and is estimated to affect 73,000 people annually in the United States (Mead et al., 1999; Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005), resulting in approximately 200–500 fatalities and costing as much as \$726 million each year (Buzby, Roberts, Jordan Lin, & MacDonald, 1996). These numbers may actually be much higher because infections with *E. coli* O157:H7 can mimic other diseases, such as appendicitis, inflammatory bowel disease, or other forms of colitis, and may lead to a misdiagnosis. *Escherichia coli* O157:H7 can be transmitted through the contamination of fecal waste in or on food or water, person-to-person contact, animal contact, or environmental exposure. The most common source of human infections is through the consumption of ground beef contaminated with cattle feces (Mead et al., 1999).

Cattle are known to be the primary reservoir of the O157:H7 (Gyles, 2007; Rangel et al., 2005), with between 1 and 50% of the total United States cattle population shedding the pathogen through their manure at any given time (Olsen et al., 2002). Huge volumes of manure waste are generated from concentrated animal feeding operations in the United States, amounting to 100 times the volume of sewage sludge produced from human wastewater treatment plants in the United States (Gerba & Smith, 2005). Because these feeding operations are so large, the safe processing and sterilization of the waste has become a significant problem. Much of this manure waste is recycled and used as fertilizer for field crops, where it can contaminate vegetable food sources destined for human consumption. It has been demonstrated that the preventative measure of washing contaminated raw vegetables with chlorinated water before consuming does not significantly reduce the numbers of *E. coli* O157:H7 if it is present (Rangel et al., 2005).

Until recently, cattle were not known to suffer any ill effects from harboring this pathogen (Callaway, Carr, Edrington, Anderson, & Nisbet, 2009). Recent studies have now shown that cattle that persistently shed O157:H7 in their feces can become ill with a range of symptoms from mild gastrointestinal upset to severe intestinal pathology (Lowe et al., 2010). This new scientific data suggests that the organism continues to evolve and add to its pathogenic arsenal. This suggestion is also supported by the discovery of the emergence of even more virulent O157:H7 clones associated with recent human outbreaks. These clones are responsible for causing more than 50% of those infected to require hospitalization because of life-threatening complications (Eppinger et al. 2011) compared to an 18% hospitalization rate reported by the economic research service in 1996 (Food Safety and Inspection Service, 2001).

The relationship between man and cow extends back into history more than 8,000 years without incidence of O157:H7 illness. The sudden emergence of this organism as a pathogen about 30 years ago suggests that an equally sudden cause must be present that accounts for this abrupt change. Approximately 20 years prior to the first identified case of *E. coli* O157:H7, feedlot cattle operations were becoming the norm in beef production. These operations took advantage of the overabundance of cheap corn and began to produce beef from grain-based diets. Farmers discovered that cattle finished with a grain-based diet gained weight faster and were ready for slaughter sooner than the traditionally-raised cattle, leading to a substantial financial gain.

However, the physiological mechanism of the cattle's digestive tract is not able to properly digest the high starch content of grain-based diets. When they are forced to digest the grain, fermentation occurs in the gut, creating an acidic environment (Diez-Gonzalez, Callaway, Kizoulis, & Russell, 1998). Beneficial strains of *Escherichia coli*, which keep other microorganisms in check by competitive exclusion, are killed off by the acidic

environment, allowing only the most acid-resistant strains to survive and multiply (Diez-Gonzalez et al., 1998). The purpose of this study was to determine if the practice of feeding cattle a diet based on grain products rather than grass results in a higher incidence of cattle that harbor the pathogenic strain of *E. coli* O157:H7, and, consequently, pose a greater risk to human health than cattle fed a traditional diet of grass and hay.

## Method

### *Population*

Four farms in the northern Utah area were studied; farms A and B both raise certified grass-fed beef. These cattle are fed a 100% grass diet their entire lifetime. Farm D is considered a feedlot, in which cattle are fed a diet consisting of between 70–90% grain concentrations until slaughter. Cattle on feedlots typically arrive shortly after they have been weaned and remain there until full maturity, which takes approximately 12 months.

### *Collection*

Freshly dropped fecal samples were collected from the cattle and transported to Weber State University's clinical microbiology laboratory. The samples were then enriched with modified tryptic soy broth. The broth uses casein enzyme hydrolysate and papaic digest of soybean meal to provide carbonaceous nitrogenous compounds and other essential growth nutrients required to enhance the count of bacteria present, specifically *E. coli* O157:H7. The samples were then incubated at 37°C for 18 hours. After incubation, the samples were plated onto two selective plating media, SMAC and BBL CHROM agar O157 designed to identify potential O157:H7 strains and then incubated for 24 hours. *Escherichia coli* O157:H7 typically displays a sorbitol negative reaction on a SMAC plate and produces mauve colored colonies on a CHROM agar. Any

colonies that displayed these characteristics were considered suspect colonies and were transferred onto Sheep Blood Agar plates and incubated overnight at 37°C. After this final incubation, definitive confirmation was done using the Thermo Scientific Remel Wellcolex rapid latex agglutination test which uses latex beads coated with antigen specific antibodies for the O157 and H7 antigens causing agglutination with positive cultures.

### *Statistics*

The prevalence of O157:H7 from stool samples of grass-fed and grain-fed cattle was compared using Fisher's Exact Test for Count Data in the R statistical software package. The Odds Ratio for O157:H7 positive cultures in grain-fed vs. grass-fed cattle was determined from the Fisher maximum likelihood estimation.

## **Results**

Fifty-seven samples were processed from farm A (grass fed), with one of those samples testing positive for the O157 and H7 antigens. Out of the 52 samples from farm B (grass fed), one tested positive. The total number of samples taken from grass-fed cattle was 107, with two samples testing positive (1.8%). Fifty-nine samples from farm D (grain fed) yielded six positive results (10.2%). Thirty-five samples were tested from farm C, a grass-fed beef operation using minimal grain supplementation. There were no positive cultures from the 35 samples tested from farm C. (The results from farm C were excluded from the study due to the non-specific diet.)

## **Conclusion**

The data from this research showed a higher prevalence of *E. coli* O157:H7 in the manure samples tested from grain-fed cattle (odds ratio=5.88) (1.8% grass fed vs. 10.2% grain fed, p-value=0.02442).

These numbers are statistically significant (95% confidence intervals [CI]=1.01, 61.5) and show that cattle fed grain-based diets are nearly six times more likely to be colonized with *Escherichia coli* O157:H7 than cattle fed a traditional hay/grass-based diet.

*Escherichia coli* O157:H7 is a very hardy organism and can survive in the environment for extended periods of time. And coupled with the extreme illness caused by this bacteria and the very low infectious dose, it makes this organism especially dangerous. With the amount of manure produced from feedlot cattle in the United States that can seep into ground water, run off into nearby water sources, inadvertently contaminate meat products through the slaughter process, or be applied directly to field crops as fertilizer, it seems only prudent that greater oversight be implemented to reduce the potential harm to human health.

Additional research is needed to determine if there is an acceptable level of grain supplementation in cattle, but it is apparent that feeding cattle a grain-based diet, which is typical for feedlots across the United States, results in a higher prevalence of *E. coli* O157:H7. On September 1, 2012, there were more than 10.6 million head of cattle being fed grain on feedlots in the United States (USDA, 2012).

## References

- Buzby, J. C., Roberts, T., Jordan Lin, C. T., & MacDonald, J. M. (1996). Bacterial Foodborne Disease: Medical Costs and Productivity Losses. *Agricultural Economic Report No. (AER-741)*.
- Callaway, T. R., Carr, M. A., Edrington, T. S., Anderson, R. C., & Nisbet, D. J. (2009). Diet, *Escherichia coli* O157:H7, and cattle: a review after 10 years. *National Cattlemen's Beef Association*, 11(2), 67–80.
- Callaway, T. R., Elder, R. O., Keen, J. E., Anderson, R. C., & Nisbet, D. J. (2003). Forage Feeding to Reduce Preharvest *Escherichia coli* Populations in Cattle, a Review. *Journal of Dairy Science*, 86(3), 852–860.

- Caprioli, A., Morabito, S., Brugere, H., & Oswald, E. (2005). Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Veterinary Research*, 36(3), 289–311.
- Diez-Gonzalez, F., Callaway, T. R., Kizoulis, M. G., & Russell, J. J. (1998). Grain Feeding and the Dissemination of Acid-Resistant *Escherichia coli* from Cattle. *Science*, 281(5383), 1666–1668.
- Eppinger, M., Mammel, M., Leclerc, J., Ravel, J., & Cebula, T. (2011). Genomic Anatomy of *Escherichia coli* O157:H7 Outbreaks. *Proceeding of the National Academy of Sciences of the United States of America*, 108(50), 20142–20147.
- Food Safety and Inspection Service. (2001). *Risk Assessment of the Public Health Impact of Escherichia coli O157:H7 in Ground Beef*. Food Safety and Inspection Service.
- Gerba, C. P., & Smith, J. E. (2005). Sources of Pathogenic Microorganisms and their fate during Land Application of Wastes. *Journal of Environmental Quality*, 34(1), 42–48.
- Griffin, P. M., Bell, B. P., Cieslak, P. R., Tuttle, J., Barrett, T. J., Doyle, M. P., . . . Wells, J. G. (1994). Large Outbreak of *Escherichia coli* O157:H7 Infections in the Western United States, Recent Advances in Verocytotoxin-Producing *Escherichia coli* Infections. *Science*, 7–12.
- Griffin, P. M., & Tauxe, R. V. (1991). The Epidemiology of Infections Caused by *Escherichia coli* O157:H7, Other Enterohemorrhagic *E. coli*, and the Associated Hemolytic Uremic Syndrome. *Epidemiology Review*, 13(1), 60–98.
- Gyles, C. L. (2007). Shiga Toxin-Producing *Escherichia coli*: An Overview. *Journal of Animal Science*, 85(13 Suppl), E45–E62.
- Huntington, G. B. (1997). Starch Utilization by Ruminants: From Basics to the Bunk. *Journal of Animal Science*, 75(3), 852–867.
- Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. *Nature Reviews*, 2, 123–141.

- Lowe, R. M., Munns, K., Selinger, B. L., Kremenik, L., Baines, D., McAllister, T. A., & Sharma, R. (2010). Factors Influencing the Persistence of *Escherichia coli* O157:H7 lineages in Feces From Cattle Fed Grain Versus Grass Hay Diets. *NRC Research Press*, 56(8), 667–675.
- Manshadi, F. D., Gortares, P., Gerba, C. P., Karpiscak, M., & Rental, R. J. (2001). Role of Irrigation Water in Contamination of Domestic Fresh Vegetables. *American Society of Microbiology*, 101, 561.
- Mead, P. S., Slutsker, L., Dietz, V., McCraig, L. F., Bresee, J. S., Shapiro, C., . . . Tauxe, R. V. (1999). Food-Related Illness and Death in the United States. *Emerging Infectious Diseases*, 5(5), 607–625.
- Ochoa, T. J., & Cleary, T. G. (2003). Epidemiology and Spectrum of Disease of *Escherichia coli* O157:H7. *Current Opinion Infectious Disease*, 16(3), 259–263.
- Olsen, S. J., Miller, G., Breuer, T., Dennedy, M., Higgins, C., Walford, J., . . . Mead, P. (2002). A Waterborne Outbreak of *Escherichia coli* O157:H7 Infections and Hemolytic Uremic Syndrome: Implications for Rural Water Systems. *Emerging Infectious Diseases*, 8(4), 370–375.
- Paton, A. W., Ratcliff, R., Doyle, R. M., Seymour-Murray, J., Davos, D., Lanser, J. A., & Paton, J. C. (1996). Molecular Microbiological Investigation of an Outbreak of Hemolytic Uremic Syndrome Caused by Dry Fermented Sausage Contaminated with Shiga-Like Toxin-Producing *Escherichia coli*. *Journal of Clinical Microbiology*, 34(7), 1622–1627.
- Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982–2002. *Emerging Infectious Disease*, 11(4), 603–609.
- Sanderson, M. W., Gay, J. M., Hancock, D. D., Gay, C. C., Fox, L. K., & Besser, T. E. (1995). Sensitivity of Bacteriologic Culture for Detection of *Escherichia coli* O157:H7 in Bovine Feces. *Journal of Clinical Microbiology*, 33(10), 2616.
- Sargeant, J. M., Sanderson, M. W., Smith, R. A., & Griffin, D. D. (2003). *Escherichia coli* O157:H7 in Feedlot Cattle Feces and Water in Four Major Feeder-Cattle States in the USA. *Preventative Veterinary Medicine*, 61(2), 127–135.



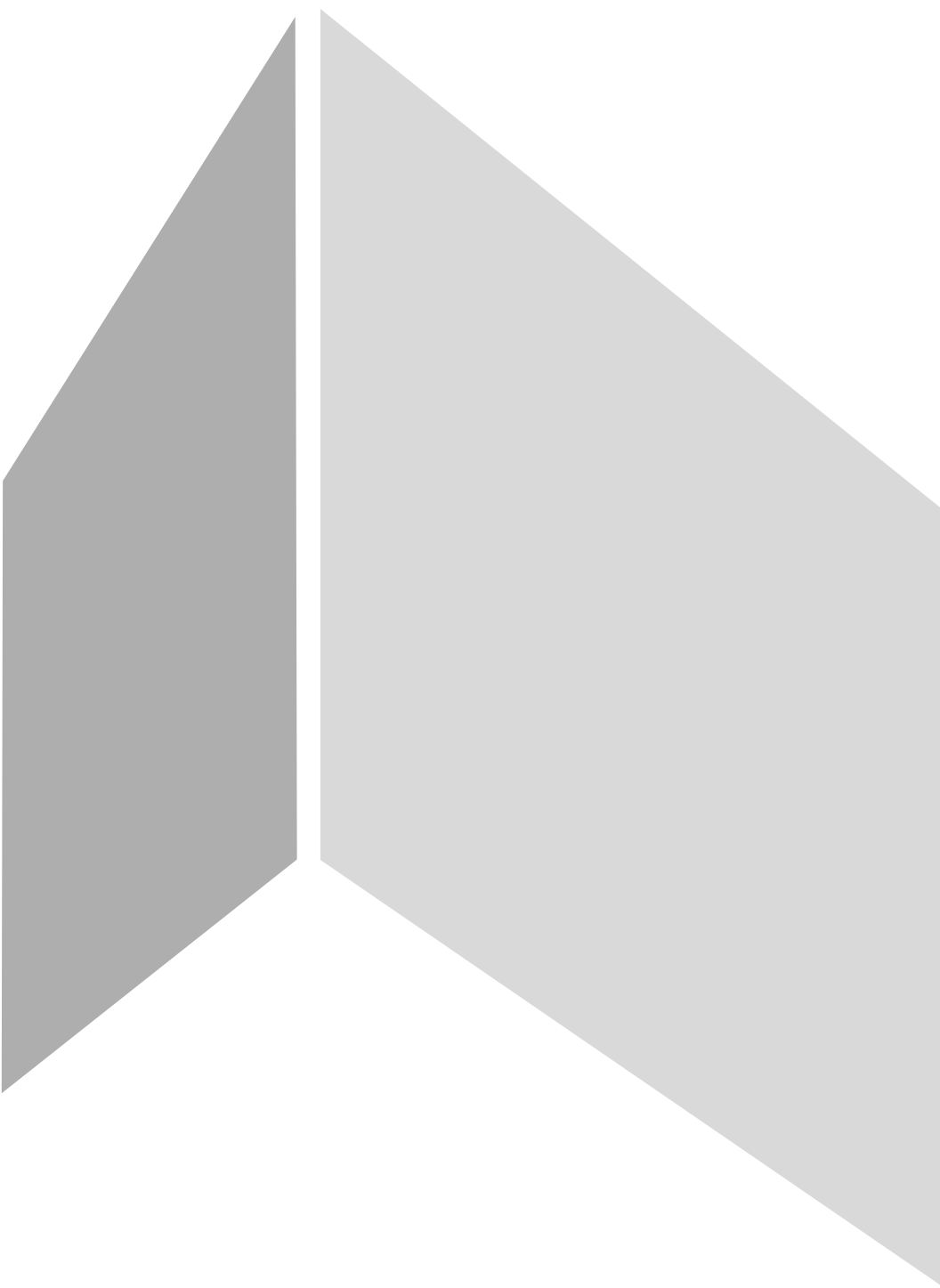
Shulman, S. T., Friedmann, H. C., & Sims, R. H. (2007). Theodor Escherich: The First Pediatric Infectious Diseases Physician? *Clinical Infectious Diseases*, 45(8), 1025–1029.

USDA. (2012). *Cattle on Feed*. National Agriculture Statistics Service.

Vidovic, S., & Korber, D. (2006). Prevalence of Escherichia coli O157:H7 in Saskatchewan Cattle: Characterization of Isolates by Using Random Amplified Polymorphic DNA PCR, Antibiotic Resistance Profiles, and Pathogenicity Determinants. *Applied and Environmental Microbiology*, 72(6), 4347–4355.

**Table 1.** Prevalence of positive and negative O157:H7 stool cultures in cows, organized by diet. A Fisher’s Exact Test was used to determine if there was any statistically significant difference in prevalence of positive cultures by diet. From that test a Odd’s Ratio was also calculated with a 95% confidence interval. These analysis were performed in the R statistica software package.

Diet	O157:H7	
	Negative	Positive
Grass (n=107)	105	2
Grain (n=59)	53	6
Odd's Ratio: 5.88 (Derived from Fisher's Exact Test)		
p-value: 0.02442		
95% Confidence Intervals: 1.01, 61.5		



**MICROBIOLOGY**

# Microbial Screening of Potable Water Sources in Guatemala: *A Potential Source of Disease Transmission*

Emma Bentley

Faulty Mentors: Craig Oberg & Karen Nakaoka

## Abstract

*Transmission of infectious diseases by fecally contaminated water represents a recurring health risk for the local population and has serious implications for travelers from developed nations. The presence of coliforms in water is an indicator of fecal contamination and, possibly, the presence of fecally-transmitted pathogens. In this study, the degree of fecal contamination in potable water, designated for either drinking or for washing or both, was determined by screening 27 samples collected in four different regions. Samples taken of water from a wide variety of potable water sources, both urban and rural, were plated on Total Coliform, Enterobacteriaceae, and E. coli/Coliform Petrifilms. Inoculated Petrifilms were incubated at ambient temperatures for 48 hr and then counted. Results showed that 92% of the water sources contained bacteria, 48% of samples contained coliforms, 15% contained E. coli, and 71% were positive for Enterobacteriaceae, indicative of Salmonella contamination. Coliform counts ranged from 0 to greater than  $1.5 \times 10^2$  per ml of potable water. These results indicate that potable water sources, particularly in rural areas, could be a significant source of fecal pathogen transmission to their consumers.*

## Introduction

Worldwide, more than 1.1 billion people do not have access to improved water, which includes a household connection, protected dug well, rain water collection, a protected spring, or borehole (World Health Organization [WHO] & United Nations Children's Fund [UNICEF], 2004). In 2007, it was noted that 90% of urban and 60% of rural populations had improved access to drinking water in Guatemala compared to that of conditions in 2002. Although there was improvement, it was still a major health concern (WHO & UNICEF, 2004). Water services in Guatemala are frequently interrupted, water pressure is insufficient, and tap water is often unsafe to drink (Vasquez, 2010).

In addition to complications delivering water services, in countries that experience heavy rains or wet seasons, flooding can allow sewage to contaminate drinking water. This provides a direct pathway for numerous pathogenic waterborne microorganisms to enter a community's water supply (McLennan, Peterson, & Rose, 2009). Guatemala City's rainy season is from July to October, with rainfall probability reaching over 70% and an average annual precipitation of 51.8 in. (Climatemps, 2013). In Guatemala, the risk is high for major infectious waterborne diseases, including bacterial diarrhea (Central Intelligence Agency [CIA], 2013).

Diarrhea is the second most common cause for morbidity and death in children under five years old (Benoit et al., 2013). The WHO reports that diarrheal disease will be the major factor in over 1.8 million deaths annually by 2015, for all ages and both sexes (WHO, 2013a). Traveler's diarrhea (TD) is the most common illness experienced by individuals traveling to other countries (Steffen, 1986; Sonnenburg et al., 2000). Studies show 45% of TD cases originate in developing countries and that bacterial pathogens are implicated in 80–90% of TD cases (Ouyang-Latimer et al., 2011). TD causes a disability period of 24 hr in most individuals (Steffen et al., 2011).

Guatemala, the most populous country in Central America with 14 million people and the highest growth rate in Latin America, is a predominantly poor country, struggling in the areas of health and development (CIA, 2013). Studies suggest that poverty level is linked to access to potable water (Ashraf, 2002). Although Guatemala is not the poorest country in Latin America, it does have the highest rate of malnutrition in both Central America and the western hemisphere, and the fourth highest rate in the world (WHO, 2013a). One of the challenges Guatemala faces is reducing the rural and indigenous poverty level below the current level of 56% (WHO & UNICEF, 2004). The deadly combination of widespread poverty and unclean water in Guatemala are evident in characteristics of the population. Diarrheal diseases have been linked to secondary health issues such as reduced cognitive functioning and malnutrition (Keush et al., 2006). Enteric diseases resulting in overt diarrhea also contribute long term to growth retardation and stunting [Moore et al., 2001; Moore et al, 2010; Victora, 2009; Checkley et al., 2008]. During 2005 to 2012, the World Health Organization (WHO) reported that 48% of children in Guatemala under the age of five were stunted in growth (WHO, 2013b).

Enterotoxigenic *E. coli* (ETEC) has been found to be the etiologic agent in 34% of TD cases, with enteroaggregative *E. coli* (EAEC) causing nearly 24% of the cases in Latin America (de la Cabada Bauche & DuPont, 2011). Diarrheal diseases in Guatemala can be attributed to numerous factors, including deficiency of potable water, poverty, lack of education, and consumption of fecally-contaminated water. In rural Guatemala, the responsibility to universalize access to basic needs such as drinking water and sanitation was decentralized to municipal governments in recent years (Speer & Vasquez, 2013). Currently, there is no standard for Guatemalan water including allowable bacteria enforced by the Guatemalan government. However, the standards from the US Environmental Protection Agency recommend a total coliform (fecal coliform and *E. coli*) count of

zero. The US EPA requires less than 5% of water samples to test positive for total coliform or a maximum of one sample if less than 40 samples are taken in a month (United States Environmental Protection Agency, 2003). Additionally, WHO recommends water intended for human consumption contain no fecal indicator organisms, including *E. coli* or thermo-tolerant bacteria, in water directly intended for drinking (WHO, 2006).

In this study, numerous potable water sources were screened for the presence of total bacteria, coliform, and Enterobacteriaceae bacteria. Typical water sources included hand-washing facilities, water used for general washing and cleaning, and water intended for drinking or cooking. The presence and concentration of coliform bacteria was used to determine the overall contamination of the water in order to assess the potential for disease transmission in potable water sources. This study employed 3M Petrifilm plates (AOAC INTERNATIONAL [Association of Analytical Communities] approved as an effective coliform screening tool) as an initial screening tool for fecal contamination (Vail et al., 2006). After screening potable water sources, bacteria were isolated to confirm their taxonomy and potential pathogenicity as human pathogens.

## Method

### *Sampling Areas*

Four areas, chosen to represent a variety of different environments, were selected in Guatemala for sample acquisition: Guatemala City, El Estor, Chulac, and Flores (Figure 1). Guatemala City is the national capital with a population of nearly one million people, while Chulac is a rural area isolated from most commercial ventures. Flores is a tourist town near the Tikal ruins with over 20,000 residents, and El Estor is a small town located in a predominately rural area.

### *Sampling Procedure*

Twenty-seven 6 ml water samples were taken of potable water from a wide variety of sources (Table 1). Approximately half of the samples were obtained in the Chulac area, the most isolated and rural of the four sampling areas. The rest of the samples were split between the other three cities. At the time of collection, samples were labeled with the exact location, type of water source, and the time. Samples were taken from a broad spectrum of water sources including commercially bottled water, public spigots, hospital taps, and hand-washing sinks. The focus was on collecting samples from water sources in actual use as sources of potable water. All samples were collected and stored in sterile 2 ml disposable plastic microtubes.

### *Sample Analysis*

One milliliter of each sample was plated onto Aerobic Count Plate Petrifilm, *E. coli*/Coliform Count Plate Petrifilm, and Enterobacteriaceae Count Plate Petrifilm. All Petrifilm plates were incubated at ambient temperature since no incubators were available in the test areas. Incubation required two readings to be taken, one at 24 hr and another at 48 hr. Interpretation of results was performed utilizing the specific Interpretation Guide provided by 3M for each type of Petrifilm used.

### *Harvesting Isolates from Petrifilm*

Three methods were used to harvest individual isolates from the Petrifilms for further characterization. The first method used Rodac plates, which were pressed down on the exposed surface to the Petrifilm media after the plastic top layer had been pulled back. Rodac plates containing Eosin Methylene Blue (EMB) agar (Hardy Diagnostics, Santa Maria, CA) were used to harvest samples from the *E. coli*/Coliform Count Plate Petrifilms. When obvious individual colonies were found on



the Petrifilms, the top was lifted up and the colony picked off with a sterile loop. Each isolate was then streaked on either EMB or Violet Red Bile (VRB) agar (Hardy Diagnostics, Santa Maria, CA). For several of the Petrifilms, the top cover was pulled back and the media scraped off using a sterile plastic spreader, which was then placed in a 9 ml dilution blank and vortexed to resuspend the media. One-tenth of a milliliter was then spread-plated on an EMB plate.

### *Characterization of Individual Isolates*

Samples were taken from 30 of the Petrifilms using one of the three methods previously described. Individual colonies were selected that showed typical lactose fermentation morphology (red-pink opaque colonies surrounded by a cloudy halo in the agar media) on either EMB or VRB agar and streaked again for isolation if the petri plate appeared to contain colonies with multiple morphologies. Pure cultures (individual colonies) were aseptically picked off and sub-cultured into 5 ml of Tryptic Soy Broth (TSB). Broth cultures were gram stained to confirm that the isolates were gram-negative short rods, indicative of coliforms or potential *Salmonella* strains.

## **Results**

Overall, 25 of 27 sources (93%) were positive for aerobic bacteria (Table 2). Thirteen of the 27 samples (48%) tested positive for coliforms (Tables 2 and 3). Four of 27 (15%) were positive for presumptive *E. coli* based on Petrifilm data (Table 3). Enterobacteriaceae were detected in 21 of the 27 samples (71%) indicative of possible *Salmonella* contamination (Table 3).

Prevalence of bacterial growth by Petrifilm Count Plate relating to the sampling regions of Guatemala City, Chulac, El Estor and Flores are provided in Tables 2 and 3. Chulac potable water samples had the highest incidence of aerobic bacteria growth with 18 of 18 sources (100%) containing bacteria growth. A

comparable incidence of bacterial contamination was found in El Estor and Flores, where 4 out of 4 samples (100%) were positive. Water samples from Guatemala City had the lowest incidence for bacterial contamination with only 3 out of 5 samples (60%) testing positive (Table 3).

Water from both El Estor and Flores had the highest number of coliforms with 3 out of 4 samples (75%) testing positive. Chulac had 10 out of 17 sources (56%) test positive for coliforms while water from Guatemala City showed no coliform growth. El Estor and Flores water samples were also highest for presumptive *E. coli* contamination with 1 out of 4 samples (25%) being positive. In Chulac, 3 out of 18 (17%) tested positive for presumptive *E. coli*, and Guatemala City water samples had no presumptive *E. coli* contamination. In regard to Enterobacteriaceae, El Estor and Flores had 4 out of 4 (100%) of water sources test positive, while Chulac had 15 of 18 sources (83%) test positive. Guatemala City water samples showed no Enterobacteriaceae growth.

Ranges for the overall bacterial concentrations in potable water samples testing positive are shown in Table 4. Aerobic bacteria growth, representing 93% of samples, ranged from 1 to greater than 10CFU/ml. Water sources testing positive for coliform growth, accounting for 48% of samples, ranged from 1 to  $1.6 \times 10$ CFU/ml. Presumptive *E. coli*, found in 14.8% of samples, ranged from 1 to  $1.2 \times 10$ CFU/ml depending on the water source. Finally, Enterobacteriaceae, found in 71% of samples, ranged from 1 to  $7.8 \times 10$ CFU/ml, again depending on the source water.

## Discussion and Conclusion

This study is one of the first to utilize Petrifilms to screen potable water sources for potential water contamination in Guatemala as possible indicators for waterborne disease transmission. In this survey, we gathered data in support of concluding that the rural regions in Guatemala continue to

suffer with contaminated water sources; while in urban areas of Guatemala, results indicate water there is less likely to carry disease-causing microorganisms (Table 2). Due to the high prevalence of travelers' diarrhea in developing countries, which continues to be a worldwide problem affecting millions of tourists every year (de la Cabada Bauche & DuPont, 2011), it is important to note that this study elucidates the potential for potable water contamination based on urban or rural areas that may be visited.

Because *E. coli* strains have been found to be common in Central America, and are isolated from 76% of travelers' diarrhea cases, it is highly likely that the coliform and presumptive *E. coli* bacterial counts observed in this study could be confirmed as *E. coli* (Ouyang-Latimer et al., 2011). This would be in harmony with history, as ETEC has been the most significant cause of traveler's diarrhea since the 1970s (Rowe, Taylor, & Bettelheim, 1970).

The accepted public health model is that safe water, adequate sanitation, and personal hygiene all contribute to preventing diarrhea and that deficiencies in these variables have been attributed to 88% of global diarrheal deaths (Divelbiss, Boccelli, Succop, & Oerther, 2012). Rural Guatemala continues to face obstacles that hinder the population's progression towards having and maintaining clean water. One obstacle is that Guatemala's public health expenditure is amongst the lowest in the Americas, at around 1% of GDP (WHO & UNICEF, 2004). Another challenge for Guatemala is that of poverty, since income levels are directly related to water sanitation and better housing conditions, including adequate sanitation and improved water supply (Divelbiss et al., 2012). As Guatemala continues to develop as a nation and improve their public health, the rural population may one day have water that exhibits the characteristics found in urban Guatemala.

One factor relating to the future success in the health of the Guatemalan people that may be underestimated is that of educational awareness. Although there is data on the effects of education and its relationship to overall health, little was observed concerning efforts in place to educate communities about water cleanliness, in addition to teaching communities methods to clean water and how to maintain it once clean. It has been observed that maternal academic skills are associated with improved management of childhood diarrhea (Webb, Ramakrishnan, Stein, Sellen, Merchant, & Martorell, 2009), further supporting the observation that if education is in place, there can be a positive impact on childhood health, which can follow through to adulthood.

Improvement of potable water sources, particularly in rural areas, in future years will result in a decrease in deaths due to factors associated with water-borne diseases. The WHO projects that by 2030, for all age groups and both sexes, 1.6 million deaths worldwide will occur annually from diarrheal disease (WHO, 2013a). While this is lower than is currently projected for 2015, continued monitoring of potable water to encourage improvement of these sources could further improve health in developing countries.

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## References

- Ashraf, H. (2002). Poverty linked to access to water, says new water league table. *Lancet*, 360(9349), 1950.
- Benoit, S. R., Lopez, B., Arvelo, W., Henao, O., Parsons, M. B., Reyes, L.,...Lindblade, K. (2013). Burden of laboratory-confirmed *Campylobacter* infections in Guatemala 2008-2012: Results from a facility-based surveillance system. *Journal of Epidemiology and Global Health*, 4(1), 51-59. dx.doi.org/10.1016/j.jegh.2013.10.001
- Central Intelligence Agency. (2013). The World Factbook. Central America and Caribbean: Guatemala. Retrieved from <https://www.cia.gov/library/publications/the-world-factbook/geos/gt.html>
- Checkley, W. Buckely, G., Gilman, R. H., Assis, A. M., Guerrant, R. L., Morris, S.S.,...Black, R. E. (2008). Multi-country analysis of the effects of diarrhoea on childhood stunting. *International Journal of Epidemiology*, 37(4), 816-830.
- ClimateMps. (2013). Rainfall/precipitation in Guatemala City, Guatemala. Retrieved from <http://www.guatemala.climateMps.com/precipitation.php>
- de la Cabada Bauche, J., & DuPont, H. L. (2011). New Developments in Traveler's Diarrhea. *Gastroenterology and Hepatology*, 7(2), 88-95.
- Divelbiss, D. W., Boccelli, D. L., Succop, P. A., & Oerther, D. B. (2012). Environmental health and household demographics impacting biosand filter maintenance and diarrhea in Guatemala: An application of structural equation modeling. *Environmental Science & Technology*, 47(3), 1638-1645. dx.doi.org/10.1021/es303624a
- Keush, G. T., Fontaine, O., Bhargava, A., Boschi-Pinto, C., Bhutta, Z.A., Gotuzzo, J.R., ... Laxminarayan, R. (2006). *Disease Control Priorities in Developing Countries*. Washington, DC: World Bank.

- McLennan, S. D., Peterson, L. A., & Rose, J. B. (2009). Comparison of point-of-use technologies for emergency disinfection of sewage-contaminated drinking water. *Applied Environmental Microbiology*, 75(22), 7283–7286.
- Moore, S. R., Lima, A. A., Conaway, M. R., Schorling, J. B., Soares, A. M., & Guerrant, R. L. (2001). Early childhood diarrhoea and helminthiasis associate with long-term linear growth faltering. *International Journal of Epidemiology* 30(6), 1457–1464.
- Moore, S. R., Lima, N. L., Soares, A.M., Oria, R. B., Pinkerton, R. C., Barrett, L. J.,...Lima, A. A. (2010). Prolonged episodes of acute diarrhea in reduce growth and increased risk of persistent diarrhea in children. *Gastroenterology*, 139(4), 1156–1164.
- Ouyang-Latimer, J., Jafri, S., VanTassel, A., Jiang, Z. D., Gurleen, K., Rodriguez, S.,...DuPont, H. L. (2011). In vitro antimicrobial susceptibility of bacterial enteropathogens isolated from international travelers to Mexico, Guatemala, and India from 2006 to 2008. *Antimicrobial Agents and Chemotherapy*, 55(2), 874–878.
- Rowe, B., Taylor, J., & Bettelheim, K. A. (1970). An investigation of traveller's diarrhea. *Lancet*, 1(7636), 1–5.
- Speer, J., & Vasquez, W. F. (2013). Opportunities and Challenges for Community Involvement in Public Service Provision in Rural Guatemala. (IFPRI 01250). Washington, DC: International Food Policy Research Institute.
- Steffen, R. (1986). Epidemiologic studies of travelers' diarrhea, severe gastrointestinal infections, and cholera. *Reviews of Infectious Diseases*, 8(Suppl. 2), S122–S130.
- Steffen, R., Tornieporth, N., Clemens, S. A., Chatterjee, S., Cavalcanti, A. M., Collard, F.,...von Sonnenberg, F. (2004). Epidemiology of traveler's diarrhea: details of a global survey. *Journal of Travel Medicine*, 11(4), 231–237.

- United States Environmental Protection Agency. Drinking Water Contaminants. (2003, June). Retrieved from <http://www.epa.gov/safewater/contaminants/index.cfm#1>
- Vail, J., Morgan, R., Merino, C. R., Gonzales, F., Miller, R., & Ram, J. L. (2003). Enumeration of waterborne *Escherichia coli* with Petrifilm Plates: comparison to standard methods. *Journal of Environmental Quality*, 32(1), 368–373.
- Vasquez, W. F. (2010). Municipal water services in Guatemala: exploring official perceptions. *Water Policy*, 13(3), 362–374. dx.doi.org/10.2166/wp.2010.211
- Victora, C. G. (2009). Nutrition in early life: a global priority. *Lancet*, 374(9696), 1123–1125.
- von Sonnenburg, F., Tornieporth, N., Waiyaki, P., Lowe, B., Peruski Jr., L. F., Dupont, H. L., ... Steffen, R. (2000). Risk and aetiology of diarrhoea at various tourist destinations. *Lancet*, 356(9224), 133–134.
- Webb, A. L., Ramakrishnan, R., Stein, A. D., Sellen, D. W., Merchant, M., & Martorell, R. (2009). Greater years of maternal schooling and higher scores on academic achievement tests are independently associated with improved management of child diarrhea by rural Guatemalan mothers. *Maternal and Child Health Journal*, 14(5), 799–806.
- World Health Organization. (2006). *Guidelines for Drinking-water Quality*. Geneva: WHO Press.
- World Health Organization. (2007). *Guatemala health and development. Country cooperation strategy at a glance*. Retrieved from [http://www.who.int/countryfocus/cooperation\\_strategy/ccsbrief\\_gtm\\_en.pdf](http://www.who.int/countryfocus/cooperation_strategy/ccsbrief_gtm_en.pdf)

World Health Organization. (2013a). Projection of number of deaths: WORLD by cause. Retrieved from <http://apps.who.int/gho/data/node.main-euro.PROJNUMWORLD?lang=en>

World Health Organization. (2013b). World Health Statistics 2013. Retrieved from [http://ecphf.uicp.cn:82/upload/2013-07/9789241564588\\_eng.pdf](http://ecphf.uicp.cn:82/upload/2013-07/9789241564588_eng.pdf)

World Health Organization, & United Nations Children's Fund. (2004). Meeting the MDG Drinking Water and Sanitation Target: A Mid-Term Assessment of Progress. Geneva: WHO Press.



**Table 1.** Water Sources Tested by City

City	Source #	Source Type
Guatemala City	1	Bathroom Sink
	23	Water Jug
	24	Bathroom Sink
	25	Bottled Water
	27	Bathroom Sink
Chulac	3	Pila Running
	4	Wash Basin
	5	Roadside Spring
	6	Bathroom Sink
	7	Bathroom Sink
	8	Bathroom Sink w/ Filter
	9	Pila Standing
	10	Pila Running
	11	Bathroom Sink
	12	Water Jug
	13	Water Jug
	14	Bottled Water
	15	Rain Catcher
	16	House Spigot
	17	Hand Sink
	18	Bathroom Sink
	19	Pila Running
	20	Groundwater
El Estor & Flores	2	Bathroom Sink
	21	Bathroom Sink
	22	Table Drink
	26	Bathroom Sink

**Table 2.** Average Colony Counts (CFU per ml) Based on Geographic Region and Source Type

City	Type	AE	TC	EC	EB
Guatemala City	Urban	$>10^3$	0.0	0.0	0.0
	Drinking	$>10^3$	0.0	0.0	0.0
	Washing	3.3	0.0	0.0	0.0
	Bottled	$>10^3$	0.0	0.0	0.0
	Tap	3.3	0.0	0.0	0.0
Chulac	Rural	1416	12	7.0	86
	Drinking	$>10^4$	0.3	0.0	25
	Washing	369	3.4	0.6	47
	Mixed Use	1114	23	15	136
	Bottled	$>10^4$	0.3	0.0	25
	Tap	749	15	9.0	99
El Estor & Flores	Urban	1444	7.3	0.8	64
	Drinking	$>10^3$	17	0.0	240

**Table 3.** Positive Results by Sample Area

Location	Samples	AE	AE %	TC	TC %	EC	EC %	EB	EB %
Guatemala City	5	3	60%	0	0%	0	0%	0	0%
Chulac	18	18	100%	10	56%	3	17%	15	83%
El Estor & Flores	4	4	100%	3	75%	1	25%	4	100%
Total	27	25	92.60%	13	48.15%	4	14.81%	27	71%

| AE: Aerobic | TC: Total Coliform | EC: *E. coli* | EB: Enterobacteriaceae|

**Table 4.** Bacterial Load Range for Positive Samples

Petrifilm™ Count Plate	Range of CFU per ml
Aerobic	1 to >10 <sup>4</sup>
Coliform	1 to 1.6 x 10 <sup>2</sup>
<i>E. coli</i>	1 to 1.2 x 10 <sup>2</sup>
Enterobacteriaceae	1 to 7.8 x 10 <sup>2</sup>

| AE: Aerobic | TC: Total Coliform | EC: *E. coli* | EB: Enterobacteriaceae |

**Figure 1.** Sampling Regions in Guatemala



**NURSING**

# An Evaluation of Motivators for Colonoscopy Screening Compliance

Holly M. Wright

Faculty Mentor: London Draper-Lowe

## Abstract

*Each year 150,000 people in the United States are diagnosed with colon cancer and more than 50,000 die from it (American Cancer Society [ACS], 2012). A Landmark study shows that colorectal cancer (CRC) is as much as 90% lower than expected rates when colonoscopy with polypectomy was performed (Winawer et al., 1993). Recent studies reveal that colonoscopy screenings have the potential to lower CRC by 65% (ACS, 2011). Current recommendations by the Centers for Disease Control and Prevention (CDC) are that all men and women should have a CRC screening by age 50 (Centers for Disease Control and Prevention [CDC], 2011). However, the ACS's 2011–2013 report on CRC states that only 53% of Americans who are age 50 comply with CRC screening guidelines (ACS, 2011). Willingness to complete a screening colonoscopy by those who are 50 and who have no family members affected by CRC has been found to be as low as 29% (compared to 66.9% of those with first-degree family members who have CRC) (Delgado-Plasencia, Lopez-Tomassetti-Fernandez, Hernandez-Morales, Torres-Monzon, & Gonzales-Hermoso, 2009). The current percentages of CRC screening rates are well below the ACS's goal of a 75% CRC screening compliance rate. The ACS hopes to achieve 75% by the year 2015 (ACS, 2011). There*

*is a need for improved compliance in colonoscopy screening in order to accomplish sufficient screening and to decrease rates of unnecessary deaths from CRC.*

*This study explores the reasons, motivations and influences that have prompted people to complete a colonoscopy screening procedure. A look into what is motivating people to comply offers increased knowledge and understanding of the effects of efforts made to promote colonoscopy screening.*

## **Method**

A study protocol was approved by the Ogden Regional Medical Center (ORMC) Institutional Review Board. A mixed-methods study using qualitative and quantitative features in a one-on-one interview was used. The subject was first given one qualitative, open-ended question which asked what motivated them to have a screening colonoscopy. The subject was instructed to look at and answer the quantitative questionnaire only after they had read and answered the qualitative question first. The interviews were held at ORMC.

### *Subjects*

Subjects were 18 or older and were either scheduled to undergo a screening colonoscopy or had already completed one. Subjects were drawn from the population of patients and their families (or other adults who accompanied patients) to ORMC and were primarily from northern Utah. All who came to the hospital to the Endoscopy suite were given the opportunity to participate. The study included 206 participants. The study began February 12<sup>t</sup>, 2013 and closed July 12<sup>t</sup>, 2013.

### *Procedure*

The method used was a survey in which the subject wrote his or her answers to the questions on the questionnaire. Questions were read to the patient, if needed, and translations services were offered. Since the principle investigator (PI) worked in Endoscopy, the PI was able to direct the surveys and oversee the process, as well as teach and assist other RNs and other staff members who assisted during the survey process. Each survey form was given a code number that was used for identification. The questionnaire was compiled by collaborating efforts of the PI, who has eight years of experience working with colonoscopy patients in a Gastroenterology (GI) lab, and gastrointestinal doctors at ORMC, including: Dr. Chad Gonzales, having over 10 years as an endoscopist, and Dr. Dennis Sobotka and Dr. Jeffery Poole, both having over 15 years of experience as endoscopists.

## **Results**

### *Qualitative Questionnaire*

The first open-ended question of the qualitative section of the survey showed that doctors are the greatest motivator for helping people to choose to comply with having a colonoscopy screening. Thirty-eight percent of participants volunteered that their doctor was the motivator who prompted them to have the procedure. At 25 %, the second greatest self-reported motivating factor that prompted individuals to have a colonoscopy was to prevent cancer and/or to maintain good health.

The third-most-common self-reported motivator given by participants was their age, with 22% reporting they had reached or passed the age of 50, or the age they had perceived as the recommended age to have a screening colonoscopy. At 17%, symptoms came in as the fourth self-reported reason that people were prompted to comply with a screening



colonoscopy. The majority of these people knew they needed a colonoscopy screening but did not pursue the test until they had symptoms. The highest reported symptoms were diarrhea, rectal bleeding, and abdominal pain. The fifth self-reported motivation for compliance was family history of colon cancer at 16%.

Interestingly, the sixth most reported motivator for screening was family members who were credited with encouraging subjects to have a colonoscopy screening by 11% of participants. Of those family members, 6% said that they were prompted by their spouses. Wives were credited at 4.85% with talking them into having the procedure, and slightly less than 1% credited their husbands for motivating them to comply with screening guidelines. Another 4.85% credited other family members including mothers, fathers, siblings, and sons and daughters with encouraging them to have the screening colonoscopy. The seventh motivator, at 4%, was reported as the subject's annual physical. The eighth, at 3.9%, was reported as family history of colon polyps. The ninth reported motivator, which had 3.4% of responses, was "knowing someone who had colon cancer" (a non-relative friend or acquaintance). The tenth motivator reported by subjects, at 3.4%, was television commercials that promoted colonoscopy screenings.

### *Quantitative Questionnaire*

By showing that 70% of subjects credited their doctor with having a role in motivating them to have a screening colonoscopy, the quantitative questionnaire validates the role of doctors in prompting patients to have a screening. Doctors were the highest chosen motivator to comply with a screening. When given the same multiple choice surveys, the second highest selection, at 39%, credited the media with prompting or motivating them to have the colonoscopy completed. Television was the highest media motivator at 34%.

Interestingly, family pressure or family influence was third and credited by 30% of subjects with motivating them to comply with colonoscopy screening guidelines by encouraging them to have the screening. The highest of those family members were spouses, who were credited with motivating 20% of all participants to have a colonoscopy screening. Ten percent of the family motivators were defined as wives, and 2% of the family motivators were defined as husbands. Family other than spouse, which combined mothers, fathers, siblings, children, aunts, uncles, and grandparents, totaled 11% of motivating colonoscopy screenings.

The fourth most chosen motivator was hearing a story of someone who had had colon cancer. Local sportsman and TV personality Doug Miller's story was the most frequent story mentioned, which motivated 12% of those who chose to have their screening colonoscopy. The second most common story of someone who had colon cancer that motivated individuals was that of a close family member, at 9%. Individuals motivated by hearing the story of a struggle with colon cancer of someone who was a friend, associate, or other non-family person was credited with 5% of prompting people to have a colonoscopy screening. Twenty-seven percent chose family history of colon cancer as a motivator, 24% gave family history of other cancers as a motivator for screening, and 24% chose fear of colon cancer as a motivator. Twelve percent credited symptoms for a reason to comply, and 8% chose personal history of other cancers as a motivation for screening. Nurses were credited by 4% of participants as motivators for colonoscopy screening compliance.

### *Combined Results*

Both the qualitative and the quantitative sections of the survey revealed that doctors were credited most by their patients as the reason they followed through with or chose to have a screening colonoscopy. Doctors, media (especially television),

family pressure, or encouragement, symptoms, family history of colon cancer, knowing of a story of a non-family member who had colon cancer, family history of cancer, personal history of cancer, and fear of colon cancer were among the top motivators in both the qualitative and quantitative sections of the study.

Spouses or significant others were notably credited with the majority of family influence towards a colonoscopy screening in both methods of the study. Wives were notably credited with encouraging their spouse much more than husbands were credited for encouraging their spouse to have the procedure, as shown by the data of both qualitative and quantitative sections of the study. Only 1% of participants reported Doug Miller's story as a motivator in the open-ended question of the study, but 12% gave his story credit for being a motivator for their scope in the multiple choice section. Of those who were asked directly if Doug Miller's story influenced their decision to have a colonoscopy, 52% said "Yes."

### *Demographics*

Ninety-four percent of the participants were White, 5% were Hispanic, 1% were Black, and less than 1% were American Indian. The general population of Ogden, Utah, is 75% White. Thirty percent of the population claim to have Hispanic or Latino origins, 2% are Black, and 1% are American Indian (Census Viewer, 2010). This shows there were a higher percentage of Caucasian/White people in the study than the percentage of Caucasian/White people in the surrounding population of the area. The average reported age of the participant's first colonoscopy was 52. The survey participants were 53% male and 47% female. The most recent census of 2010 for Ogden shows the area to be 51% male and 49% female (Census Viewer, 2010). Forty-eight percent of those asked said that they felt the cost of a colonoscopy caused a delay in having the procedure.

## Discussion

Limitations to this study include participants being limited to one hospital in the area and there is a possibility that questions in the quantitative section of the study may have promoted answers by suggesting them. There were over 40 categories of motivators mentioned by subjects in the qualitative section. A future study would include these in a comprehensive quantitative questionnaire. A new question for future studies would ask how the individual knew he or she needed to have a colonoscopy screening, and differentiation would be made between internal motivators and external motivators.

## References

- American Cancer Society. (2011). Colorectal Cancer Facts & Figures 2011–2013. Retrieved from <http://www.cancer.org/acs/groups/content/@epidemiologysurveillance/documents/document/acspc-028323.pdf>
- American Cancer Society. (2012). Five myths about colorectal cancer. Retrieved from <http://www.cancer.org/cancer/colonandrectumcancer/moreinformation/five-myths-about-colorectal-cancer>
- Census Viewer. (2010). Ogden Utah population: Census 2010 and 2000. Retrieved from <http://censusviewer.com/city/UT/Ogden>.
- Centers for Disease Control and Prevention. (2011). Colorectal cancer screening guidelines. Retrieved from [http://www.cdc.gov/cancer/colorectal/basic\\_info/screening/guidelines.htm](http://www.cdc.gov/cancer/colorectal/basic_info/screening/guidelines.htm)
- Delgado-Plasencia, L., Lopez-Tomassetti-Fernandez, E., Hernandez-Morales, A., Torres-Monzon, E., & Gonzales-Hermoso, F. (2009). Willingness to undergo colorectal cancer screening in first-degree relatives of hospitalized patients with colorectal cancer. *Journal of Medical Screening*, 16(1). <http://dx.doi.org/10.1258/jms.2009.008062>

Winawer, S. J., Zauber, A. G., Ho, M. N., O'Brien, M. J., Gottlieb, L. S., Sternberg, S. S.,...Stewart, E. T. (1993). Prevention of colorectal cancer by colonoscopic polypectomy. *The New England Journal of Medicine*, 329, 1977-1981. Retrieved from <http://www.nejm.org/doi/pdf/10.1056/NEJM199312303292701>

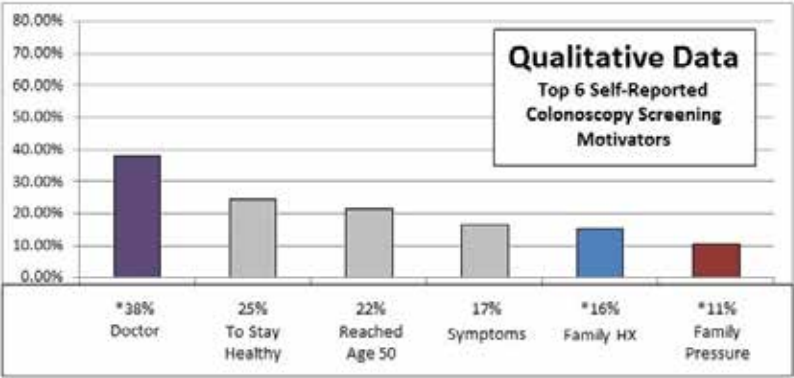
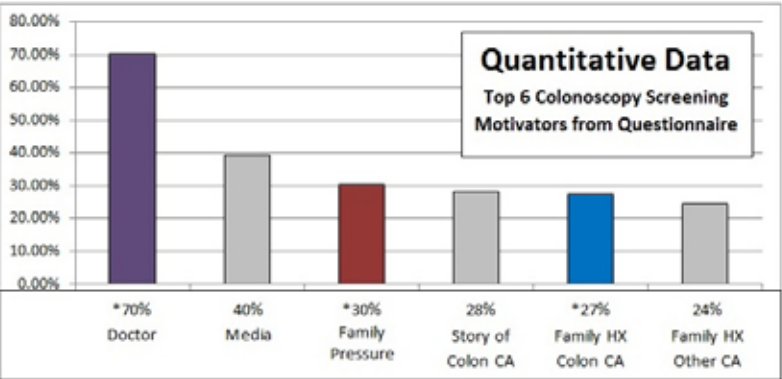
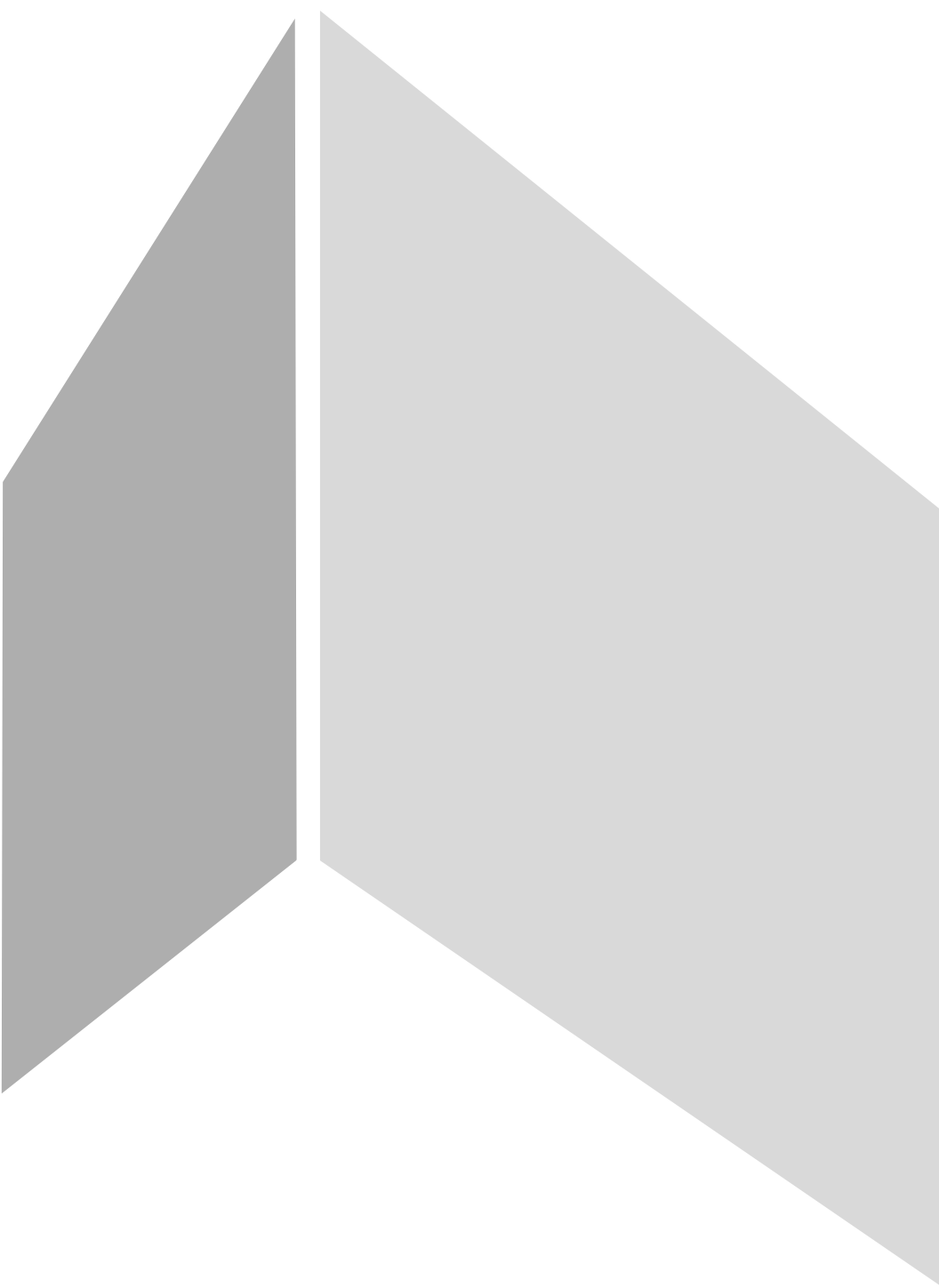


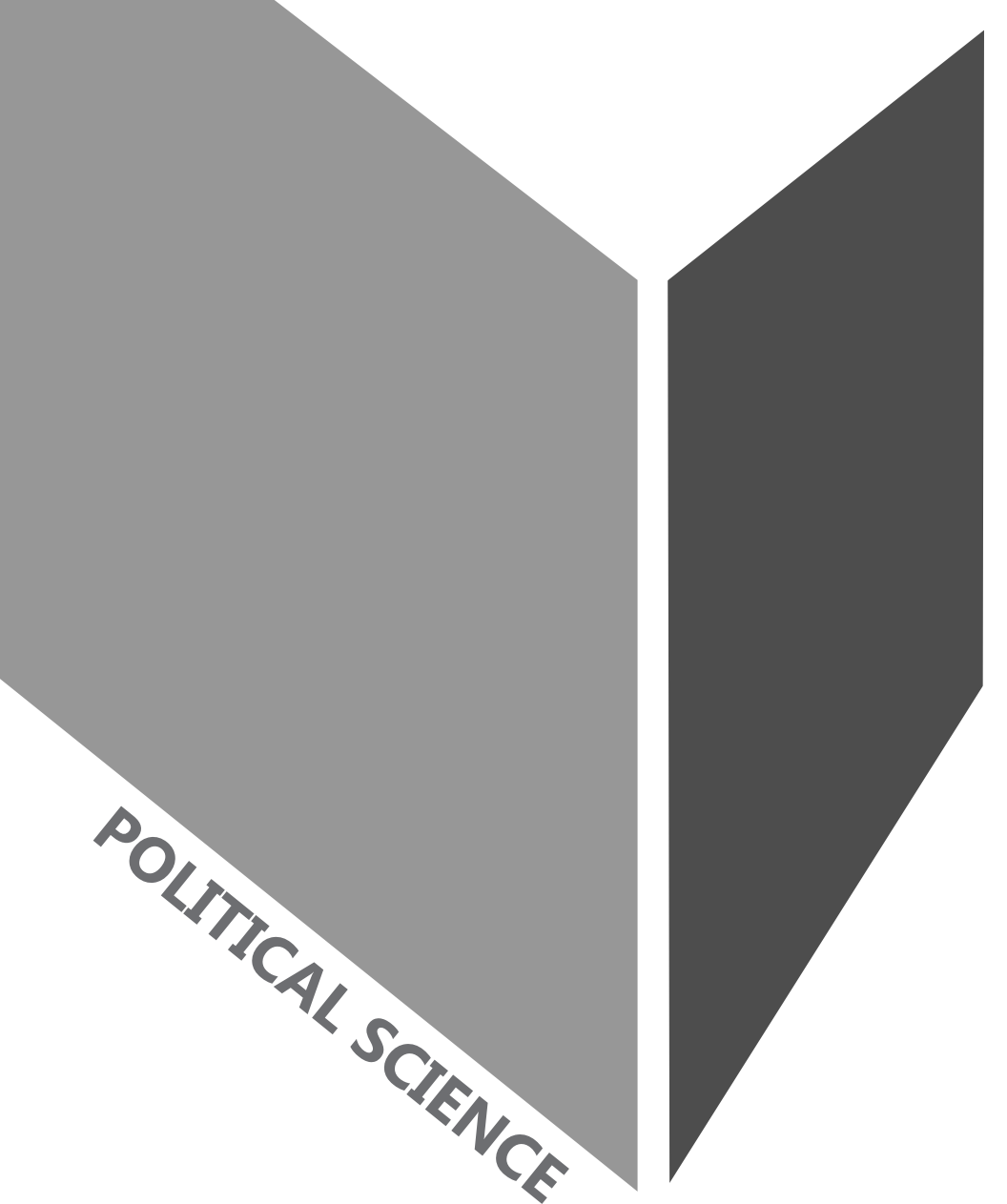
Figure 1. Qualitative Data



\*Motivators noted in the top 6 of both Qualitative and Quantitative Surveys  
Note: As participants were permitted to provide multiple responses, results will not equal 100%.

Figure 2. Quantitative Data





**POLITICAL SCIENCE**

# **Service Learning for Undergraduates in Local Elections: *College Poll Workers in the 2012 General Election***

Stuart Jensen

Faculty Mentor: Gary A. Johnson

## **Abstract**

*This study adds to the growing body of literature examining poll workers as street-level bureaucrats. Using an original survey instrument with a sample size of 449 traditional and college student poll workers in the 2012 general election, in two Utah counties, with 125 polling locations—we find college students bring important technological, cognitive, and organizational skills to local elections. We also find college students who participate in local election administration are likely to do so again. This study also quantifies poll workers' perceptions on voting processes, poll worker training, and areas for improving efficiency and fairness in local election administration.*

## **Introduction**

Election processes are inherently subject to errors and are historically subject to manipulation and fraud. These processes therefore require extraordinary integrity (especially for any computerized systems involved), as well as honesty and experience among people involved in administering elections.



In America, state legislatures administer all elections (United States Constitution Article I, Section IV). In this research, we examine the retention level of training, and the perceived quality and efficiency of the election processes in two Utah counties based on the experiences of poll workers. Further, college students were actively encouraged to participate in the election processes of these two counties, and we compare college student poll worker experiences with those of non-college-student poll workers. This study adds to the growing body of literature that poll workers are critical street-level bureaucrats (Lipsky, 1980). This study supports the evidence on the importance of poll workers (Alvarez, Atkeson, & Hall, 2013) as street-level bureaucrats. An important component of Lipsky's theory is the interactions of street-level bureaucrats with citizens. Here, we examined the experience of the poll workers themselves with voters in two separate counties in a presidential election year and found large variation in training, errors, physical facilities, troubleshooting, and technical expertise.

The results here support the finding that poll workers clearly affect voters' perceptions of the fairness and accuracy of elections. Indeed, à la Lipsky, the decisions of poll workers, the routines they establish, the training they undergo, the technology they use, and the devices and procedures they invent to deal with uncertainties and pressures to get the ballots cast and counted accurately on Election Day, effectively *become* the voting process.

This study has another purpose. We actively recruited and encouraged college students to serve as these street level bureaucrats in the 2012 general election in two Utah counties based on the notions that democracy requires each generation learn how to administer democratic governance, and that fair and accurate elections are essential to a democracy. This study, with IRB approval, recruited, trained, and surveyed Weber State University students who worked as poll workers in the 2012 general election.

According to the Weber County Elections Manual, there are specific requirements for poll workers regarding ethical behavior, professionalism, and the rights of voters. The manual states, “Poll Workers have the legal duty and responsibility to conduct the election according to the procedures set forth in this guide and the Utah Election Code” (Morrell, 2012). Weber and Davis Counties stress the importance of the poll workers to behave as representatives of the state. The counties instruct poll workers, “as a public official on Election Day, to conduct yourself appropriately. Always maintain a professional and politically neutral demeanor. Never discuss the election, candidates, politics, or religion with each other or the public at the polls” (Morrell, 2012). Both counties value ethical street-level bureaucrats. The counties stress the importance of respectful communication with voters and other poll workers. The counties inform poll workers that the highest priority is to “ensure that you and the other Poll Workers enforce these standards and the voter’s rights and report unresolved violations immediately to the Elections Office” (Morrell, 2012).

Poll workers in Utah have a multitude of duties on Election Day, ranging from setting up, compiling/recording information, breaking down voting machines, checking voter identification, and administering provisional ballots. These duties are integral to the election process and can affect the outcome of elections. A voter’s negative experience at the polls ultimately reflects negatively upon their view of government and the democratic process. Negative experiences usually happen due to common voting errors or procedural errors committed by poll workers.

## Methods

After the general election of 2012, we sent out a survey to all poll workers of Weber and Davis Counties. Both counties required poll workers to have an email address as their primary form of contact, but made exceptions in rare situations. These counties

implemented this policy in order to weed out poll workers who are not computer literate, as well as to provide for a quick way to disseminate information. This requirement made it easier to get the surveys to all poll workers. The poll workers' respective counties sent the survey in order to encourage a high response rate. This was successful, but poll workers may have tailored their responses to match what they might have thought the county wished to hear. In other words, they did not want to have a "gotcha" situation (Alvarez et al., 2013), i.e., an oversight designed to catch poll workers violating a regulation and discredit them. We made every effort to inform poll workers that we did not design this survey to evaluate their performance, but rather to effectively analyze the current election process and the affect students had on their locations.

Interestingly, about 73% of poll workers in Weber County are female and 82% for Davis. Current census data reveals that the female population in 2012 is 50.8% across the United States and 49.7% in Utah. We could not find a reason for the dramatic difference in poll workers in comparison to national and state averages. This may be because there is no glass ceiling in election administration. Both counties paid their female poll workers the same as their male counterparts. The average age of Weber County poll workers was 64. This makes our sample somewhat different demographically than the research conducted by Alvarez, Atkeson, and Hall (2013), in that our sample is more female and older. There is a significant over-representation of poll workers between the ages of 45 and 74. The 18–34 range is unusual in most American counties, but we actively recruited college students in our sample.

The majority of poll workers in our sample are experienced. This may be attributable to Utah's civic culture or the tendency for poll workers to be long-term residents of the community. Sixty-eight percent of the respondents in our sample have served at least once prior to the 2012 general election. We

see large variation in the experience levels between the two counties. Davis County has a much more experienced cohort. The average age of a poll worker in Davis County is 64 compared to 50 in Weber County. This may account for the satisfaction reported in the survey on the training process. Davis poll workers were much better prepared according to survey results than reported by Weber County poll workers, i.e., “the training provided was appropriate and I felt prepared” (84% for Davis compared to 36% for Weber).

All poll workers received the same survey with a subset of questions specifically engineered toward students. The goal was to analyze the impact students had on their precincts and election performance. Nineteen students responded from Weber County and 15 responded from Davis. Thirty-nine students were recruited and participated in the poll worker program, and thirty-four of these students responded to the poll worker survey. Since students were considerably younger than their veteran counterparts, we asked open-ended questions about their ability to use the voting technology (DREs) compared to older poll workers. This data is helpful to see differences between polling locations with students and those without. This data is important to analyze the efficiency of the student poll workers. We also asked poll workers about their ability to work with these students and their attitudes toward inexperienced students assisting in a presidential election. Compatibility and ability to cooperate is essential for future use of students and the connections between traditional and student poll workers. We also asked students about personal incentives to participate. Students do not have a natural tendency to become civically involved like their traditional coworkers. This may be because they do not know about opportunities, or they have not had the opportunity presented to them. Either way, offering this opportunity to the younger generation is important for the success of elections to come. As the older generation moves out of the election scene,

it is the younger generations that the public will count on to administer elections.

In general, older poll workers and more experienced poll workers believe college students are beneficial to the process. Fifty-five percent of Davis County poll workers in our data set either completely or somewhat agree that college students bring important skills and perspectives to administering elections. In Weber County, 63.2% of respondents agree with this statement. We asked all poll workers specifically about the training they received. Weber and Davis Counties conducted training differently this year because of information received after the 2012 primary election. Both counties conducted post-primary surveys to self-evaluate and improve their process. Weber found that poll managers desired the ability to move workers from one station to another when problems existed. Previously, Weber assigned and trained poll workers for one position and one position only; the poll worker was a receiving clerk, poll book clerk, provisional ballot clerk, or an encoding clerk. As a result, poll managers were limited in their on-site managerial powers. Weber County trained all poll workers for all positions. Davis maintained the same training it had administered prior to the primary.

Davis County held a mock election that simulated Election Day. Taking a voter, as one poll worker said, “door to door.” A trainer plays the role of a voter and they are taken from the point of standing in line through the entire voting process, including provisional ballots, incorrect address, machine difficulties, no identification, residency issues, and wrong precinct, with trainees performing hands on simulations of each of these contingencies. Davis County poll workers report that this is a useful training exercise, increasing their retention and knowledge of the procedures of each contingency. Each poll worker is given the opportunity to experience difficult issues before Election Day with a trainer available to teach solutions.

The most common complaint voiced by Weber County poll workers concerned the physical facilities and layout of the training stations. Many felt that the location was loud and distracting due to the large room used in Weber State University's Shepherd Union Building. This space has very high ceilings and wood floors. The eight poll worker training stations were located in the same room, in an open house configuration. This has the advantage of trainees being able to easily move from one station to another, but the disadvantage of loud noise levels and a chaotic atmosphere. Davis County poll workers were trained in a carpeted, quieter environment and respondents overwhelmingly felt the experience was positive and well organized. It appears the physical location of the training facilities was an important determinant of poll worker satisfaction with the process and their perception of preparedness for the general election. As is often the case at a university campus, visitors have a difficult time finding available parking. This was the most frequent criticism of Weber County poll workers about the training. Davis County used a county facility and had no parking issues. The questions in the survey about training are for comparative purposes between the two counties.

## Conclusion

It is somewhat remarkable that the most important street-level interaction between voters and the citizens who administer elections is so under-studied in the political science and public administration literature. Elections matter, and the ways in which we conduct elections in America and the variation in the quality of how those election processes are perceived by voters and poll workers is critical to democratic government.

Three important conclusions emerge from this study. First, this study highlights the importance of the poll worker training done by counties. The length, comprehensiveness, physical

facilities, and timing are important factors in poll worker retention of their training and confidence in their ability to administer on Election Day. Elections are chaotic and stressful for poll workers. By 7 a.m. when the precincts open, there is usually a line of voters. Comments in the qualitative section of our survey repeatedly mentioned that if the machines are not up and running and the poll workers are not prepared, things can deteriorate, leading to dissatisfied voters, long lines, and unpleasant atmosphere in the precinct. Respondents reported that feeling well prepared and supported by the county creates a much more comfortable and professional voting experience for the voter and the poll workers. We particularly note the role in Weber County of the “Rover.” Most of the college students served in this capacity. This allowed them scheduling flexibility, and they experienced several precincts and got to witness a variety of complications, communicating with the Elections Office to find solutions.

Second, college students bring important skills to elections. In our data, both traditional poll workers and the college students who serve as poll workers in Weber and Davis Counties strongly cite technical expertise with software and the electronic voting machines as important contributions to the election. Additionally, non-college poll workers noted the energy, cognitive skills, retention of training, and a general level of enthusiasm found in college students.

Third, getting college students involved with elections provides an experienced labor pool for future elections at time when counties have a difficult time recruiting and retaining poll workers. Our survey results indicate experienced poll workers are vital to the professional administration of elections. Experienced poll workers do not require as much training, and they retain more of the training that the county provides. This is consistent with other areas of civic engagement research such as voting, volunteering, and donating, where early engagement fosters lifelong habits. Respondents to the survey

overwhelmingly report that serving as a poll worker was a rewarding experience, and they are likely to serve again.

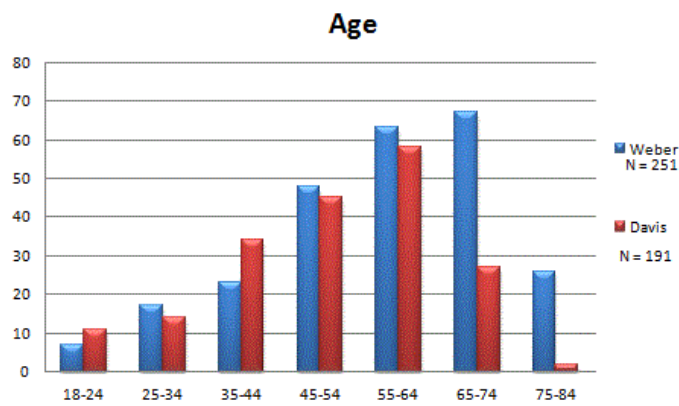
## References

- Alvarez, M., Atkeson, L. R., & Hall, T. E. (2013). *Evaluating Elections: A Handbook of Methods and Standards*. Cambridge University Press.
- Fischer, E. A. (2001). Voting Technologies in the United States: Overview and Issues for Congress. CRS Report for Congress. March. Washington, DC: Congressional Research Services, The Library of Congress.
- Knack, S., & Kropf, M. E. (1998). For Shame! The Effect of Community Cooperative Context on the Probability of Voting. *Political Psychology*, 19(3), 585–599.
- Lipsky, M. (1980). *Street Level Bureaucracy: Dilemmas of the Individual in Public Service*. New York, NY: Russell Sage Foundation.
- Mercuri, R. T., & Camp, L. J., (2004). The Code Of Elections. *Communications of the ACM*, 4(10), 53–57.
- Morrell, J. (2012). Weber County Elections Manual: 2012 General Election. Weber County Elections Department, Weber County, Utah.
- U.S. Bureau of the Census. (2013). Population Estimates Program (PEP). Retrieved from <http://www.census.gov/popest/estimates.html>

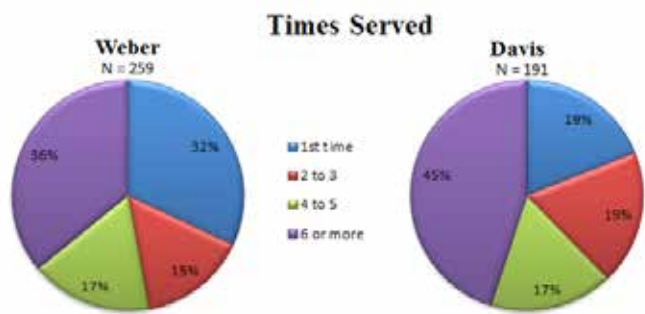


**Table 1.** Response Rate

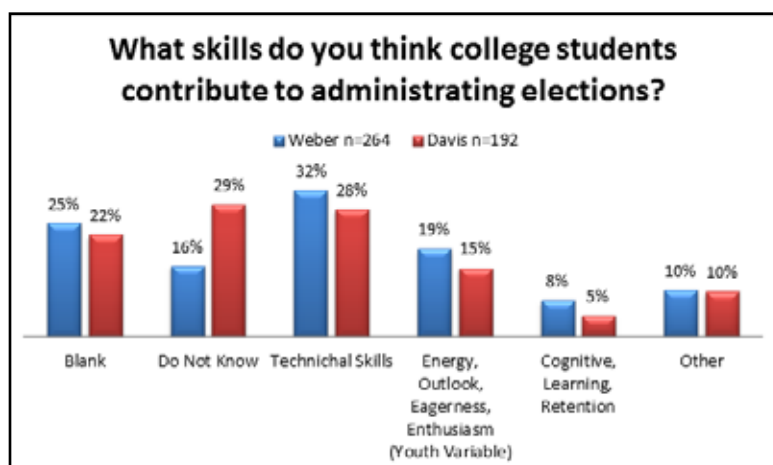
	Weber County	Davis County	Total
Total Number of Poll Workers	445	472	917
Non-College-Student Poll Workers Responded	275	204	479
College Students Responded	19	15	34



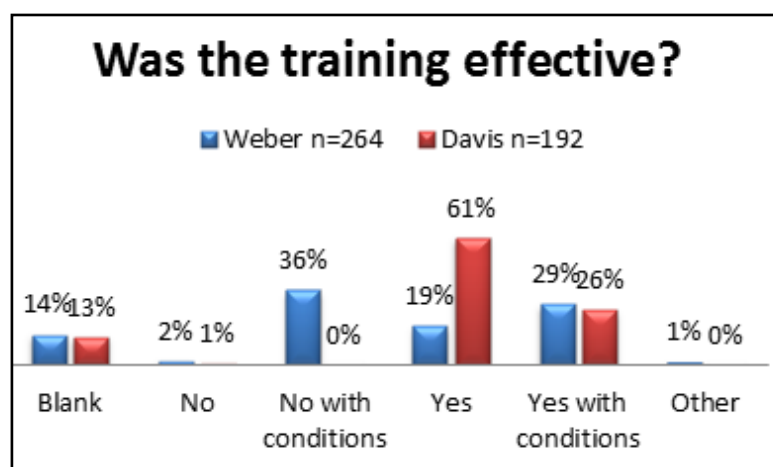
**Figure 1.** Age Difference



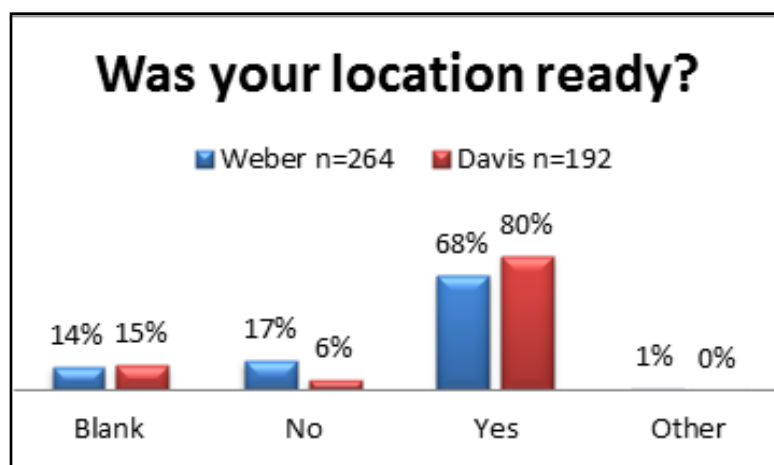
**Figure 2.** Experience



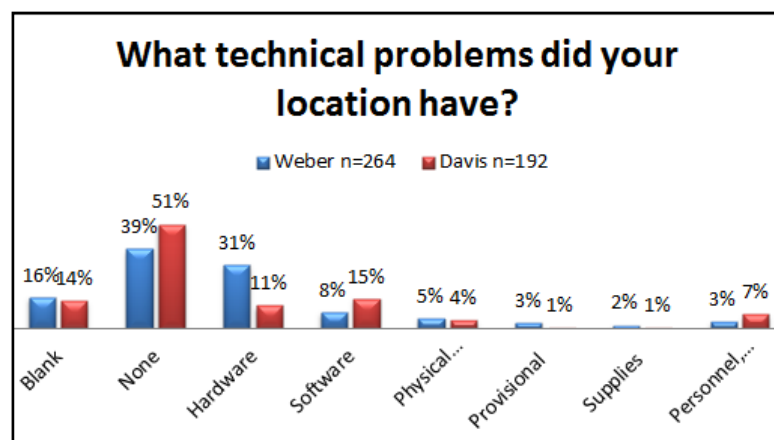
**Figure 3.** Benefits of College Students



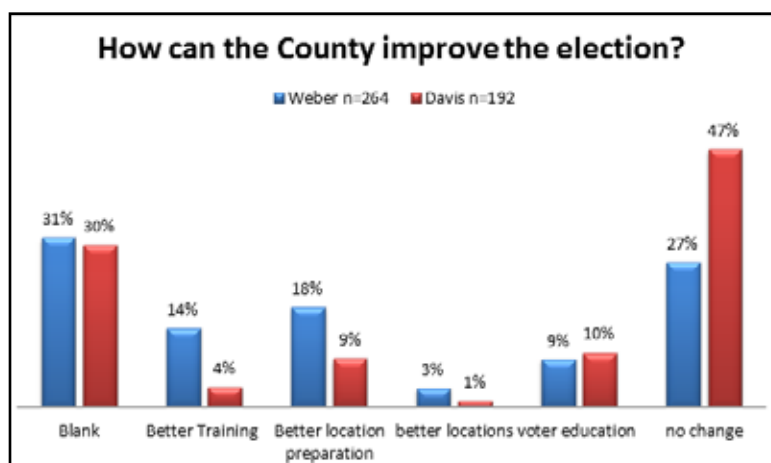
**Figure 4.** PW's View of Training



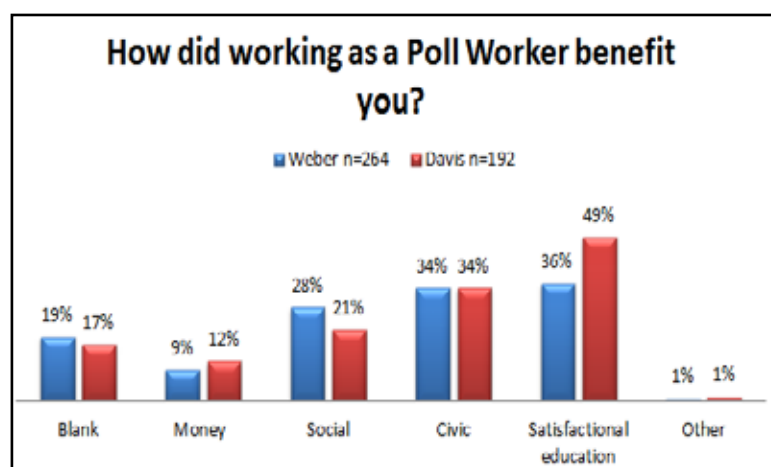
**Figure 5.** Location Preparation



**Figure 6.** Technical Problems



**Figure 7.** Qualitative Proposed Improvements



**Figure 8.** Qualitative PW Benefits

TRAVEL ABSTRACTS

# Adult Role Models for Youth

Ashley Davis

Faculty Mentor: Geri Conlin

National Association for Kinesiology in Higher Education Collaboration  
Conference and International Congress  
San Diego, California (January 8–11, 2014)

## Abstract

Students are more active when provided with a role model for fitness. (Senne et al., 2006) The investigation into graduate physical education students found students believed their role as a physical education teacher was to be a physically active role model, boost self-esteem, and present lessons to engage all students in class. (O'Bryant, O'Sullivan, & Raundesky, 2000) Physical educators who appear to be more physically fit act as a better role model. (Melville & Maddalozzo, 1988) A physical education teacher can demonstrate being an active role model through participation with their students. In this study we see the affects of a four-week physically active adult role model intervention on students' recognition of an adult role model of healthy activity. Findings suggest recommendations for secondary teachers in impacting their students in being more physically active.

# Caching Behavior of the Specialist Woodrat

## *Neotoma stephensi*

Ashley Van Leuven & James Abbot

Faculty Mentor: Michele Skopec

Society for Integrative & Comparative Biology

Austin, Texas (January 3–7, 2014)

## Abstract

Woodrats (genus *Neotoma*) are known for their prolific caching behaviors. *Neotoma stephensi*, a juniper specialist, frequently builds its middens in juniper trees and consumes up to 90% of its diet as juniper. Field evidence suggests that *N. stephensi* caches predominantly juniper in its middens. By contrast, the sympatric generalist *N. albigula* appears to cache a larger variety of both plants and non-plants in its middens. In order to determine if the two species have differences in their caching behavior, we compared them in a laboratory setting. Woodrats of both species were placed in cages that had two external compartments where different food and/or non-food items were placed for caching. When offered rabbit chow (food) in one compartment and jingle bells (non-food) in the second compartment, the generalists cached more food and non-food than the specialists; who solely cached food. When offered the choice between juniper in one compartment and rabbit chow in the second compartment, the specialists cached more juniper than rabbit chow and consumed more juniper than the generalists. However, the generalists still cached more juniper and rabbit chow than the specialists. Given that juniper was the specialists' preferred item to cache, we next offered the specialists jingle bells that covered juniper in one compartment; while the other compartment contained only jingle bells. The specialists showed a preference for the jingle bells that impeded their access to juniper. We conclude that the specialist, *N. stephensi*, displays a decreased caching behavior in terms of both quantity and variety of items cached compared to the sympatric generalist, *N. albigula*.

# Establishing Two-Point Discrimination of Cranial Nerve V

Kristie Williamson & Jessica Adler

Faculty Mentor: Jordan Hamson-Utley

NCUR 2013

La Cross, Illinois (April 11–13, 2013)

## Abstract

**Context:** Cranial Nerve V (CN V; Trigeminal nerve) is responsible for facial sensation. CN V has three separate branches which include ophthalmic, maxillary and mandibular. The ophthalmic nerve (V1) carries sensory information from the forehead; the maxillary nerve (V2) carries information from the cheek; and the mandibular nerve (V3) carries information from the chin. It is important to test all three areas of cranial nerve V to accurately assess full nerve function. Previous research has indicated that pressure threshold is not affected by age, however, normative values for two-point discrimination for CN V have not yet been established and may prove useful in concussion evaluation. **Objective:** Initial investigation aimed at determining gender-specific normative values for CN V two-point discrimination. **Design:** Prospective repeated-measures design. **Setting:** This study was performed in the athletic training facilities on the campus of a large Division I institution. **Patients or Other Participants:** Convenience sample of 106 healthy students enrolled at our institution (43 females, average age: 23.2; 63 males, average age: 22.1). Participants had no history of concussion within the last year, no facial scarring or plastic surgery and not a current smoker. **Interventions:** Measurement was taken using a two-point discriminator (Disk-Criminator) on the mid-forehead, mid-cheek, and mid-chin. Participants closed their eyes while each measurement was taken. Light pressure was applied (force equal to approximately 10-15 grams to produce blanching of the skin). Measurements were taken three times on each of three facial locations, sequentially.



## **Food, Comfort and a Bit of Home: Maude Porter and the Ogden Canteen, 1942–1946**

Lorrie Rands

Faculty Mentor: Kathryn MacKay

NCUR 2014

Lexington, Kentucky (April 3–5, 2014)

### **Abstract**

On March 25, 1942, the Weber County Red Cross opened a canteen to provide food and friendship to the soldiers passing through the Union Station in Ogden, Utah. The purpose of this paper is to provide the background as to how canteens were organized by the American Red Cross across the nation during World War II and to tell the history of the local Ogden canteen. Using canteen log books obtained from the Weber County Red Cross, scrapbooks related to Red Cross activities obtained from the Union Station Library in Ogden, other materials found at the National Archives in College Park, Maryland, and recently obtained oral histories, it is possible to piece together how the canteen was organized, to describe its day to day operations, and to come to know some of the women who volunteered at the canteen on a regular bases. The log books show how the Ogden canteen even became a model for volunteers throughout the country who wanted to open their own Red Cross canteen. The diary of the canteen committee chair, Mrs. Maude Porter, reveals how dedicated she and other volunteers were to this effort. The canteen was open seven days a week from 7:00 in the morning to whenever the last man was served. In its four years of operation, the canteen had 200 volunteers who served approximately 1.6 million service members. Ultimately this paper is about the women who organized and ran the canteen and about the impact they had on the soldiers they served.

# **Hook-up Culture: A Qualitative Analysis of Sexual Scripts Within Gender and Religious Identities**

Sarah C. Dursteler

Faculty Mentor: Colleen Packer

NCUR 2014

Lexington, Kentucky (April 3–5, 2014)

## **Abstract**

Dating scripts, sexual scripts, and the culture of sex outside intimate relationships has shifted to one of more ambiguity. Multiple changes in sexual scripts have occurred in the last 50 years with the diversification of types of relationships in which sex occurs, including, a greater acceptance of sex outside of relationships (Gagnon & Simon, 1987). This study is in response to the call for more research on how social identities influence perceptions of sexual interaction (Backstrom, Armstrong, and Puentes, 2012). The purpose of this study is to examine hook-up culture outside of traditional intimate relationships. This study explores the extent of the shift from traditional dating scripts to a culture of hooking up as perceived by males and females that identify with the predominant Latter-Day Saint (LDS) religious culture. Using the tenants of social exchange and scripting theories this study examined the attitudes of males and females. Transcripts from four same gender and religion focus groups and 100 open-ended survey responses provided data for analysis. The constant comparative method (Glaser and Strauss, 1967) provided the means by which to analyze data within emergent categories. One open-ended response question was reviewed to assess content theme analysis. The constant comparative method indicated distinct contrast of perceptions of sexual intimacy between males and females. Findings suggest that there are distinct differences between male and female and LDS and non-LDS attitudes about and perceptions of hook-up culture. These results are consistent with previous research indicating that hooking-up can be a functional strategy used to shift focus from traditional intimate relationships to more academic and professional goals.

# Novel Gene Sequence for Invertebrate Tachykinin

Chelsie Thomas

Faculty Mentor: Brian Chung

Experimental Biology

Boston, Massachusetts (April 17–21, 2013)

## Abstract

The invertebrate ribbon worm, *Paranemertes peregrina*, serves as a unique model in that it thrives in an environment exposed to drastic fluctuations in salinity during tidal interchange. Its ability to sustain homeostatic integrity is not well understood. With an aim to clarify this phenomenon, total RNA was isolated, and reverse transcription with polymerase chain reaction allowed us to serendipitously clone and elucidate a 488 base pair region of a gene coding for a Tachykinin Receptor (TKR), a subtype of G-protein coupled receptor (GPCR). This region shows 79% homology to the mouse TKR-2 mRNA sequence, and 44% homology to human Neuromedin-K receptor's amino acid sequence. Specifically, we have isolated a portion containing a cytosolic carboxy-terminus that has classically been associated with palmitoylation or otherwise hydrophobicity-enhancing interactions. This process facilitates the docking of cytosolic subunits to the membrane in the assembly of GPCRs, acting as a regulatory component. In vertebrate models, much of these proteins are conserved. Given that only a few of these genes have been reported for invertebrates, this suggests a critical need for investigation of the evolution of TKRs as they relate to stress response. Additionally, this receptor poses questions about its potential role in pain, with the prospect of revealing insight about the long-elusive pain perception in invertebrates.

# **Peer-Assisted Learning in an Orthopedic Evaluation Flipped Classroom Course:**

## ***The Effectiveness of Peer-Assisted Learning with Podcast Remediation***

Crystal Yamasaki, Adrian Eads, Jennifer Ostrowski,  
& Brent Marshall

Faculty Mentor: Jordan Hamson-Utley

NCUR 2013

La Cross, Illinois (April 11–13, 2013)

### **Abstract**

This scholarly research endeavor studied the effects of Masters peers teaching material to Undergraduate peers in a controlled flipped classroom environment in comparison to a standard flipped classroom taught by a scholastic instructor. The study also looked into the effects teaching Undergrad peers had on the Master peers doing the teaching. The study found that there is no significant difference by being taught by a Masters level peer student as by being taught by the scholastic instructor. Also found was that, by teaching, the master students did better on one section of the tested curriculum than the control group that did not teach Undergraduate peers. Funding for this research project is being sought at the Weber State University Office of Undergraduate Research. Funding will provide transportation and registration to the National Athletic Therapy Association Conference held in Las Vegas, Nevada in June 2013 where the abstract of this study has been accepted to be presented.

# Plant Secondary Metabolites as Inhibitors of Drug-Resistant Bacteria Causing Wound Infections in U.S. Military Personnel

Alisha Ryan, Shiyu Li, & Jason Fritzler

American Society for Microbiology 2013  
Denver, Colorado (May 17–22, 2013)

## Abstract

One of the major challenges facing U.S. military caregivers is the presence of multidrug resistant organisms in extremity wounds. The most frequently identified drug resistant strains of bacteria found in these wounds are *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Due to these organisms rapid increase in resistance to the commonly used drugs, it is crucial to discover and establish alternative methods for treating these microbial infections. Antibiotics are currently the most common treatment for infections by these pathogens, and there is little data on the evaluation of phytochemicals as potential chemotherapeutic agents that could take their place. We have screened 31 individual compounds from 10 major compound families to determine if plant-based phytochemicals could be explored further for use in treating bacterial infections in patients with military wounds. All compounds were tested to determine the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC). Of the 31 compounds tested, 21 (67.7%) inhibited at least one strain used in this study, with only 1 of the 21 (4.8%) inhibiting all strains. There were 10 (32.3%) of the compounds that displayed MIC values less than 100  $\mu\text{g/ml}$ . While there is much more research that needs to be done with each of these compounds, this work is a crucial first step in the drug discovery process. We believe that several of these may serve as a potential novel inhibitors of these drug-resistant bacteria.

# The Serotonin Transporter Gene Polymorphism as a Predictor of Novelty Seeking, Smoking Behavior, and Cognitive Assessment of Risk

Houda Nizam & Desirae D. Wood

Faculty Mentor: Matthew T. Schmolesky & Barb Trask

NCUR 2014

Lexington, Kentucky (April 3–5, 2014)

## Abstract

In previous research, a polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) has been associated with anxiety (Ebstein, 2006) and smoking behavior (Kremer et al., 2005). Other studies suggest that high novelty seeking (NS) personality trait scores predict marijuana use (Hale et al., 2003). In this current study, we test whether the 5-HTTLPR serotonin transporter gene polymorphism can be a predictor of NS, marijuana use, cigarette smoking, and cognitive risk assessment. Undergraduate students ( $n = 284$ ), aged 18 and above ( $M=21.5 \pm 6.0$ ), provided cheek cells for genotyping. Students completed three questionnaires in random order: the Cloninger Temperament and Character Inventory, a Physical Risk Frequency Inventory, and a Physical Risk Assessment Inventory to determine how often students engage in risky behavior and how dangerous they rate different behaviors (e.g. marijuana smoking, cigarette smoking). Genotyping was conducted using polymerase chain reaction to determine whether subjects were homozygous for the short version of the gene (S/S), homozygous for the long version (L/L) or heterozygous (S/L). Among the 77 genotyped participants, 21 were S/S, 35 were S/L and 21 were L/L. As predicted, we found a positive correlation between marijuana and cigarette smoking behavior ( $r=.39$ ;  $p<.05$ ) and negative correlations between smoking risk assessment and smoking behavior for both marijuana ( $r = -.37$ ,  $p<.05$ ) and cigarettes ( $r = -.13$ ,  $p<.05$ ). Interestingly, high novelty seekers rated marijuana smoking as less

dangerous than low novelty seekers. Moreover, religious activity was negatively correlated to smoking behavior and positively correlated to risk assessment. Supporting previous research, NS was positively correlated to cigarette smoking ( $r=.22$ ,  $p<.05$ ) and we found a similar relationship for NS and marijuana use ( $r=.19$ ,  $p<.01$ ). However, one way ANOVAs revealed no significant differences between the S/S, S/L, and L/L groups for NS (S/S =  $20.7 \pm 6.5$ ; S/L =  $21.0 \pm 7.3$ ; L/L =  $20.0 \pm 7.4$ ) or risky behavior (SS<sub>Cigarette</sub> =  $1.90 \pm 1.84$ ; SL<sub>Cigarette</sub> =  $2.0 \pm 2.0$ ; LL<sub>Cigarette</sub> =  $1.9 \pm 2.2$ ; SS<sub>Marijuana</sub> =  $1.9$ ; SL<sub>Marijuana</sub> =  $1.8 \pm 1.5$ ; LL<sub>Marijuana</sub> =  $1.6 \pm 1.6$ ). Further analysis and research remains necessary to understand more fully the role of the 5-HTTLPR polymorphism in modulating risky behavior, cognitive assessment, and personality traits.

# Species Differences in the Restructuring of Syringeal Muscle After Denervation

Kyle Spainhower, Carly Milligan, & Linsey Christensen  
Faculty Mentor: Ron Meyers & Franz Goller

Society for Integrative & Comparative Biology  
Austin, Texas (January 3–7, 2014)

## Abstract

The muscles of the avian vocal organ, the syrinx, are composed of fast and superfast muscle fibers, which enables them to rapidly control song features. Unilateral denervation of the syrinx results in the reduction of cross-sectional area and a shift to slower fiber types. Our previous denervation experiments on male Zebra Finches (*Taeniopygia guttata*) showed a 10% reduction of total muscle cross-sectional area and a 30% reduction of superfast fibers seven days after unilateral denervation of the syrinx. Decrease in cross-sectional area and superfast fiber percentage reached a maximum at eleven days at 72% and 70% of their original values. By 40 days, the cross-sectional area and fiber percentage of the denervated side had almost returned to their original values. Because this post-denervation recovery is unusually rapid in Zebra Finches, we repeated the experiment on male Yellow-headed Blackbirds (*X. xanthocephalus*) for comparison. Four birds were unilaterally denervated between three and 35 days. We quantified the cross-sectional area and the average fiber diameter for each bird, and the results differed greatly from those of the Zebra Finch experiments. By day seven, cross-sectional area was reduced to 85% of its original value, average diameter of both fiber types decreased, and each continued to decrease post-denervation. In contrast to the finches, cross-sectional area of the blackbird denervated syrinx was reduced to 40% of control by day 17. Results from the Yellow-headed Blackbirds are consistent with the results from work done on mammals and suggest that innervation of the syrinx in Zebra Finches may be unusual. Further studies will repeat the experiment at additional time intervals and increased sample size.



# **Sustainable Transportation: *An Analysis of Bicycle Infrastructure Surrounding Weber State University***

Hannah Rice

Faculty Mentor: Daniel Bedford

Association of American Geographers Annual Conference  
Los Angeles, California (April 9–13, 2013)

## **Abstract**

Today there are a number of universities working to promote sustainability initiatives as a way to improve their environmental credentials. In particular, transportation is one area where many universities can improve their environmental practices and image. Cycling is an active, environmentally friendly way of travel. However, the use of cycling as a mode of transportation is often underutilized due to many factors such as insufficient or unsafe cycling infrastructure, shortage of cycling amenities, and undesirable land use conditions, all of which work to inhibit a commuter from choosing cycling over the use of an automobile. This study examines bicycle commuting at Weber State University, a primarily undergraduate institution located in Ogden, Utah. A survey of road-surface conditions, signage, availability of cycling infrastructure, and quality of route content was conducted for all roads within a 1.5 mile radius surrounding the university, with the objective of producing a map of the most bicycle-friendly commuting routes to campus.

# Traditional versus Experiential Teaching Methods on Student Learning

Stephanie Porter

Faculty Mentor: Aaron Ashley

Association for Psychology Science  
Washington, DC (May 23–26, 2013)

## Abstract

The current study investigated the effect of two different teaching methods on students' learning of geology material. The first teaching method was a traditional method, using lectures. The second teaching method was an experiential method, using hands-on activities for learning. Participants were enrolled on one of seven weeklong summer camp sessions participating in a Geology Merit Badge class. Two classes were available for each weeklong session, one in the morning and one in the afternoon. Participants were exposed to only one type of teaching method based on class time. Teaching method alternated between morning and afternoon classes by week, so that in the first week the traditional teaching method was used in morning classes and the experiential method in afternoon classes, but the second week was switched, so that the experiential method was used in the morning and the traditional method in the afternoon.

Each participant completed a pre- and post-test, testing their knowledge of the geology principles taught in the Geology Merit Badge booklet, the scores of which were used in the analyses. Results suggest that both teaching methods were effective, but there was a significant interaction between teaching method and class time. This interaction was that traditional methods were more effective for morning classes, experiential methods for afternoon classes.

# Understanding the Relationship Between Sleep Cycles and Autistic Symptomology

Trevor Hicks-Collins

Faculty Mentor: Lauren Fowler

Posters on the Hill 2013

Washington, DC (April 13, 2013)

National Association for the Education of Young Children

Atlanta, Georgia (November 7–11, 2012)

## Abstract

Sleep concerns are common in children with autism spectrum disorder (ASD). Sleep problems in children with ASDs are markedly higher than in normal children and are reported in most children with ASD (Luci & Gregory, 2004). Although there have been limited studies that measure the sleep-wake cycles in children with ASDs, the literature on the relationship between sleep problems and the frequency of autistic symptomology is even more scarce. This study was designed to study the relationship between sleep problems and the frequency of autistic symptomology. The participants in this study were autistic but otherwise of normal health, between the ages of 5–8. The children wore Actigraph monitors after dinner each night until they woke in the morning for the period of one week. Actigraph monitor technology provides objective measures of sleep quality and quantity. The parents and teachers filled out the Child Behavior Checklist (Achenbach, 2001) to set a base line for the severity of each child's autistic symptomology. The parents also filled out a simple daily log formatted with 6 Likert scale questions to assess the child's daily behavior, and they kept a parent-report sleep log on their child. The aim was to ascertain if there is a relationship between ASD behavior changes and sleep quality and quantity. Results are expected to show that the amount and quality of sleep affects the frequency of autistic symptomology in children. Helping parents and teachers understand how important sleep is in affecting autistic symptomology can help modify behaviors.

# Urban Rainwater Harvesting

## Implementation: *Institutional and Human-Related Opportunities and Constraints*

Stephanie A. Mitts & Steve Burian  
Faculty Mentor: Carla Trentleman

NCUR 2014  
Lexington, Kentucky (April 3–5, 2014)

### Abstract

The recent development of rainwater harvesting (RWH) as a solution to stormwater management and water supply has led to many individual program implementations across the United States. RWH involves collecting stormwater runoff, storing it, and applying it for beneficial reuse or release at a controlled rate. Decreased need of freshwater withdrawals reduces hydrology-based energy consumption and protects ecosystems, potentially making RWH a more sustainable and efficient practice than centralized water supply. The goal of this research project was to compile and analyze the national trends for local government urban RWH program policy in order to organize a guide for startup programs. A survey was created and administered online to RWH managers (in both the private sector and the government) across the U.S to collect policy information. A total of 30 programs were contacted first over the phone, and were then asked to participate in the online survey. All were compliant and provided some amount of data, however, complete surveys were collected from 15. The survey contained questions pertaining to incentives, motivation of implementation, maintenance, and measurement of benefits. We found that the majority of programs first operated a pilot project before implementing a large-scale system. There is a popular occurrence of community outreach and education programs, as well as monetary incentives to ease implementation. Maintenance is generally the responsibility of the property owner. Most surveyed systems utilize the harvested water as non-potable, although some

deviation does occur. The survey findings indicate that RWH is easily customizable to project goals and environment while following a loosely structured formula for success.

