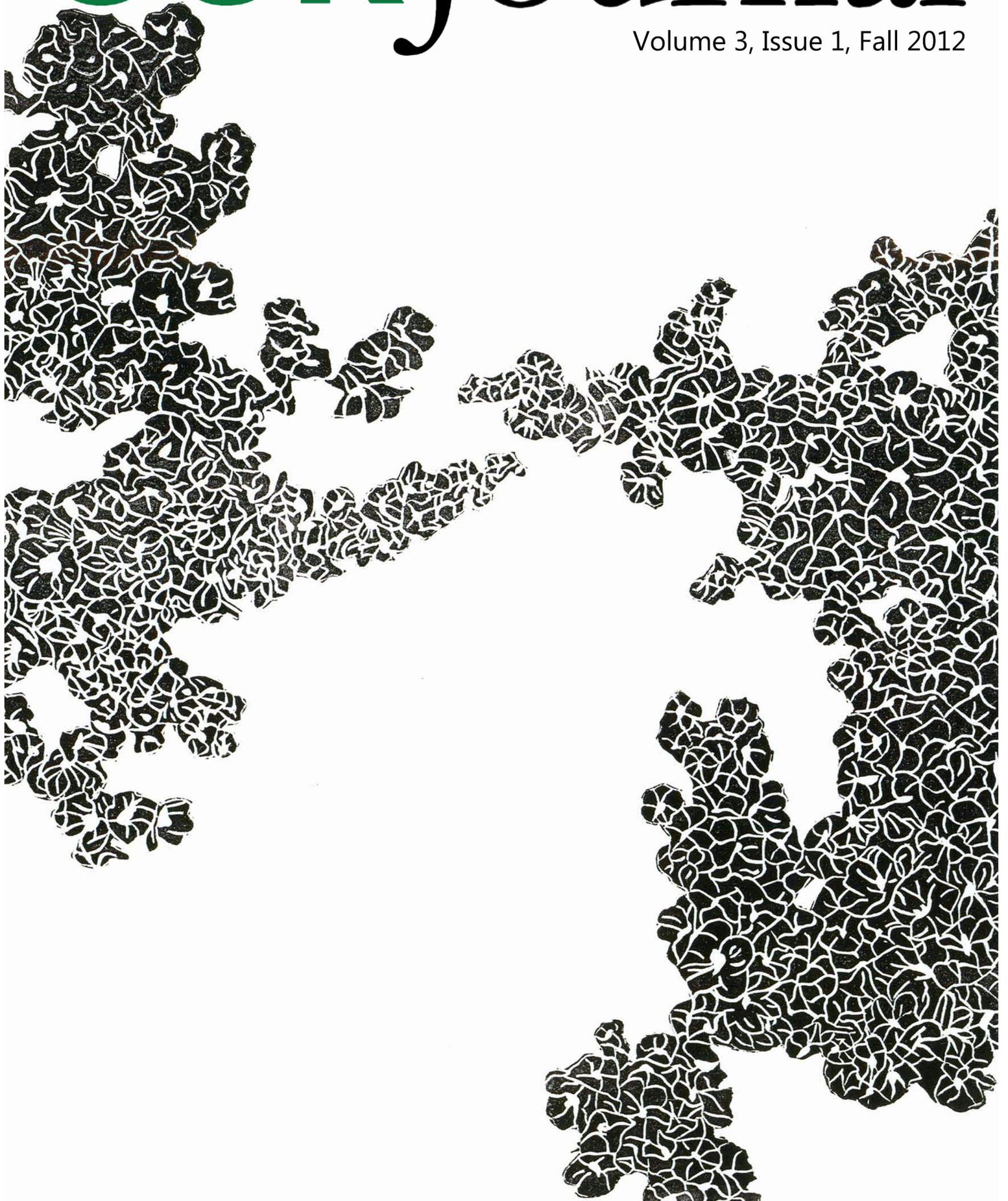


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Dedication

This issue of the *Oregon Undergraduate Research Journal* is dedicated to JQ Johnson with the fondest memories and deepest gratitude for his support of undergraduate research and of this journal.



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Editor's Letter

Kelsey Ward*, Environmental Science

Welcome to the third installment of *OUR Journal*! This issue marks the one-year anniversary of the first publication of the journal. When Lucy Gubbins and Drew Serres, founders of *OUR Journal*, first submitted a proposal for the journal, administrators were wary of their lofty goal of publishing the first issue in spring 2012. The *OUR Journal* editorial board, comprised entirely of University of Oregon undergraduates, proved any doubters very wrong; the first issue was indeed published in fall 2012 and immediately assumed a bi-annual publication cycle. This summer I assumed the role of executive editor, under the great guidance and support of former editor Lucy Gubbins. I am forever grateful for Lucy's fantastic mentorship and shining example of leadership and organization. It is hard to believe it has only been one year considering the momentous strides the journal has made!

In this issue, we are proud to feature the work of no less than 21 University of Oregon Undergraduate students who collaborated to produce four tremendous articles. Two articles, "Restoration Monitoring on the McKenzie River, Oregon" and "Assessing the Relationship Between Topography and Plant Diversity in Restored and Remnant Wet Prairies," are written by large teams of Environmental Leadership Program students who display inspiring collaboration and teamwork in their joint endeavors. Sierra Predovich's article, "Stomata Density of Orchids and Cloud Forest Humidity," is the first *OUR Journal* article that features research conducted abroad. Sierra conducted her research in the cloud forests of Costa Rica. Finally, Alexander Robinson's rigorous study on post-surgery dietary intake breaks *OUR Journal* into the exciting and fast-moving field of human physiology, a department which had never been featured in the journal.

The publication of this journal truly would not be possible without the support of many dedicated individuals. This issue marks the last contributions of our first group of outgoing editors who begin their post-undergraduate careers. Emily, Eva, Erin, and Lucy, your mark will forever be left upon the journal as the founding members who molded the journal into what it is today. Our new editors, Zeph and Meredith, jumped wholeheartedly into the editing and publication process, enthusiastically and so successfully rising to the high standards set by the fantastic group of outgoing editors. Alex and Vishesh, the third-year veterans of *OUR Journal*, have been incredible mentors and examples to the entire editorial board, including myself. The faculty advisors, Dr. Barbara Jenkins and Dr. Kevin Hatfield, gracefully advise the journal's trajectory. I am so appreciative of the trust and great support that Barbara and Kevin provide to the journal. Finally, this issue would not have been published without the tremendous technical support provided by the University of Oregon Libraries, and especially Karen Estlund and John Russell. Thank you!

*Kelsey Ward is a senior Environmental Science major at the University of Oregon and the executive editor of the *OUR Journal*. Direct correspondence to kelsey.j.ward@gmail.com.



Editorial: “A Legacy of Undergraduate Research”

David Frank*, Dean of the Robert D. Clark Honors College

Robert D. Clark, the founder of our honors college, which bears his name, was a strong advocate for the research mission of the University of Oregon. Clark won the prestigious Guggenheim scholarship for his research on Matthew Simpson, the minister who delivered the eulogy at Abraham Lincoln’s funeral. As dean of the College of Liberal Arts and then as president of the University of Oregon, Clark insisted on the centrality of research in a liberal arts education for undergraduate students.

This issue of the OUR Journal, with four articles by teams of undergraduate scientists, would have brought Clark joy. Clark was a professor of rhetoric who helped establish science on our campus. A special newsletter published in 2003, dedicated to the history of the sciences and the chemistry discipline at the University of Oregon, observed “Robert D. Clark, then Dean [of the College of Liberal Arts], had the vision and the courage to build up the sciences.” The intellectual source of this vision was Clark’s firm embrace of science as a critical way of knowing. He was, however, not a scientific fundamentalist. Clark, as do many of our best science students, understood the importance of music, literature, and religion.

The Clark Honors College library, which hosts 2,300 senior theses by undergraduates representing all the disciplines on campus, stands as the best proof that University of Oregon undergraduates command the ability to advance knowledge and understanding in the arts, social sciences, and the sciences. Our best students remain committed to interdisciplinary methods, trespassing often into disciplines outside their majors. A recent Clark Honors College student, who finished an outstanding thesis in the sciences, dedicated her research to Ernest Hemingway. Why would a science student choose Hemingway for a tribute? The student took a course on Hemingway, taught by Suzanne Clark, the daughter of Robert D. Clark, which inspired her to enter Hemingway’s world of literature. Of course, Hemingway was a scientist as well, having served Academy of Natural Sciences as a research scientist on a project involving marlin in Cuban waters.

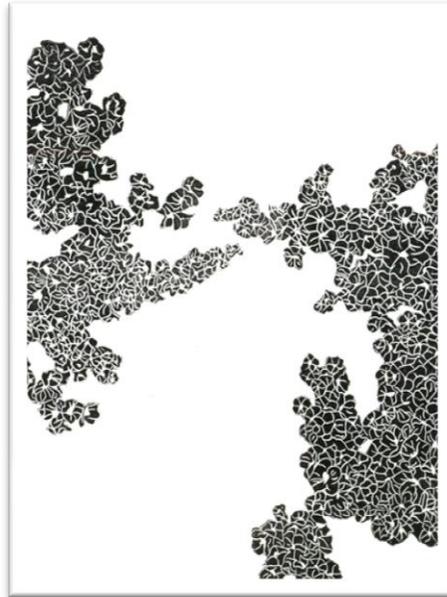
I am thrilled with the publication of the third issue of OUR Journal. Kelsey Ward, the executive editor, should be proud of this peer-reviewed journal, placing truly exceptional scholarship by our undergraduate students in the spotlight.

*David Frank is the Dean of the Robert D. Clark Honors College and Professor of Rhetoric. Author of six books and twenty-three articles in the leading journals in his field, Frank’s most recent publication, *Frames of Evil: The Holocaust as Horror in American Film*, co-authored with Caroline J.S. Picart, critiques the use of visual rhetoric and narrative devices in *Schindler’s List* and other popular films to explain evil in the world. In February 2008, during the height of the Democratic election primaries, Frank, who is also the director of the UO forensics program, was interviewed on National Public Radio about Barack Obama’s rhetoric of consilience.



Artist Statement: “Untitled number 3”

Christina Lin*, School of Architecture and Allied Arts



Texture is something we deal with every day. It deals with every one of our human senses; touch, smell, hearing, sight and taste. New experiences within these senses happen every moment and I think we unconsciously try and capture them because they carry a meaning. This idea has been a reoccurring theme in my work for my entire artistic practice. In my artwork, I delve into different mediums including print work, sculpture and music. I invite the audience to interact with each of the pieces and, depending on the first sense used, to explore how it would come out in other senses. Could you imagine feeling the texture of something two-dimensional or hearing something and seeing it come to life in 3-D?

In "Untitled number 3", a linoleum block was cut, inked and run through a press. The negative space was carefully considered in balancing with the intricate pattern. The inspiration behind the design was about organic materials. I looked to fractal patterns found in everything in nature as a jumping off point in my design of the pattern. I really wanted the patterns to flow into one another and become part of a greater whole while still appreciating the finite details as well.

*Christina Lin is currently a Master of Architecture student in the School of Architecture and Allied Arts. Her work includes prints from lino, wood block, etching and aquatinting. She has shown in the Jacob Lawrence Art Gallery in the School of art at the University of Washington and has been influenced by many artists through her work at the Gallery IMA in Seattle. She graduated from the University of Washington in 2008 with her degree in Visual Arts focusing on printmaking and received a minor in architecture. She currently resides in Eugene, Oregon. To contact her, email clin9@uoregon.edu.



Restoration Monitoring on the McKenzie River, Oregon

Michelle Rau*, Jenna Stewart, Zachariah Kezer, Rebecca Martin, Ben Miller, Sean Silverstein, Olga Slivka, Dane Swanson, Valarie Truelove, Thomas Van Hevelingen, Tyler Woods, Krystal Young**, Environmental Studies Program.

ABSTRACT

In the spring of 2012, we, the Stream Stewardship Team from the University of Oregon's Environmental Leadership Program (ELP), conducted post-monitoring surveys at a side channel of the Middle McKenzie River (side channel 4) to compare with baseline monitoring data collected by the 2011 ELP Restoration Stewardship Team. The goal of this restoration project was to enhance juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*) rearing habitat within the channel. In 2011 the U.S. Forest Service placed large woody debris (LWD) in five sections of the channel after baseline monitoring to increase the complexity of the streambed within the channel and to create a distribution of sediment optimal for salmon spawning habitat. We conducted pebble counts, cross-channel surveys, and a longitudinal profile of the stream to observe changes since the addition of LWD. Median pebble size decreased downstream of the LWD placements at gravel count 1 and increased upstream at gravel count 2. The percent of embedded sediment decreased at both gravel count sites. We also detected noticeable changes in the stream morphology at four of the five cross-sectional surveys as well as along the longitudinal profile. Sediment size distribution and the formation of pools at the downstream end of the channel showed an initial change in stream morphology since 2011, but further monitoring is warranted in order to fully assess the effects of LWD on streambed complexity and salmon spawning habitat.

1. INTRODUCTION

The McKenzie River Watershed consists of 857,364 acres of diverse ecosystems that are culturally and ecologically indispensable to the Willamette Valley (Runyon 2000). Additionally, the McKenzie River provides drinking water to over 200,000 Eugene area residents (McKenzie Watershed Council 2002). The McKenzie River also supplies abundant habitat for numerous

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**Authors are members of the University of Oregon Environmental Leadership Program, a collaborative, interdisciplinary service-learning program housed in the University of Oregon Environmental Studies Program. For more information, visit http://envs.uoregon.edu/elp_program/. To access the ELP Stream Stewardship Team website, visit <https://sites.google.com/site/elp2012streamstewardship/home>.

plant and animal species, including threatened species such as spring Chinook salmon and Oregon chub (*Oregonichtys crameri*) (McKenzie River Trust 2012). The river and its banks also offer recreational opportunities such as fishing, camping, rafting and kayaking. Its numerous recreational uses and ecological attributes make the McKenzie River an integral part of the Willamette Valley.

Many changes in the McKenzie River have occurred over the past 50 years due to human development, such as altered water flows as a result of damming and reduced stream channel stability caused by bank erosion. Increasing development along the lower reaches of the river has confined the river's natural meandering state. A naturally meandering river is characterized by braided sided channels, which create gravel bars and ponds, as well as slow peak flows during high water events. Side channels are a valuable component of salmon-bearing rivers such as the McKenzie. They provide gentle flows, which are an essential element of spawning habitat for many salmonid species in this region. Slower water velocity enables the proper sized sediment and debris for salmon spawning ground to remain in the streambed, rather than peak flow events sweeping them away (United States Forest Service 2010). Even though channelization, damming, and other anthropogenic activities have degraded much of the original McKenzie River habitat, the McKenzie Watershed Council has concluded that the McKenzie River still contains some of the most pristine water on earth (McKenzie Watershed Council 2002).

The health of McKenzie River side channels is critical for water quality and habitat complexity; however, development along the channels has reduced the input of essential ecosystem components such as large woody debris (LWD). The removal of large trees for development on the riparian banks reduces shade and litter fall and prevents logs from naturally entering the river. These logs, which are essential to the river's ecosystem functions, are also being removed due to an increased need for safe boating recreation (Runyon 2000). The presence of LWD in a stream channel can exert beneficial significant controls on the physical characteristics of streams. These controls influence channel type, sediment storage, and bed-form roughness (Naiman et al. 2000). LWD enhances instream habitat by creating pools, scouring pockets that provide hiding cover, and producing other shelter formations for aquatic species to spawn and rest. LWD increases habitat complexity by trapping smaller wood, branches, leaves and other organic materials that add to instream diversity (Gurnell 2002). This diversity provides algal food sources for aquatic biota, which in turn furnish a food source for aquatic invertebrates. In summary, aquatic species rely heavily on the maintenance of healthy habitats that contain LWD (Roni and Quinn 2001).

In October of 2011, the U.S. Forest Service and the McKenzie Watershed Council placed LWD at five different sites in the Middle McKenzie to enhance channel diversity and improve salmon spawning habitat. In May of 2011, the ELP's 2011 Restoration Stewardship Team (2011 ELP Team) collected baseline data by conducting stream surveys and Wolman pebble counts in McKenzie side channel 4 (Figure 1, Bonanno et al. 2011). We used the same procedures at Middle McKenzie side channel 4 along transects established in 2011. We then compared the baseline data with data collected this year to assess the effects of LWD placement on stream

morphology. These data may be used in future restoration efforts to enhance stream habitats for aquatic species.

2. STUDY AREA

The study area is located off McKenzie Highway 126 in the Willamette National Forest on the north bank of the McKenzie River at the McKenzie River Campground (approximately 60 kilometers east of Springfield, Oregon). The surrounding area is characterized as temperate rainforest, which receives high rainfall and is heavily vegetated by native herbs, shrubs and conifers. Herb species include: oxalis (*Oxalis oregana*), western coltsfoot (*Petasites frigidus*), waterleaf (*Hydrophyllum capitatum*), and cow parsnip (*Heracleum maximum*). The shrub layer is mainly comprised of vine maple (*Acer circinatum*), osoberry (*Oemleria cerasiformis*), alder (*Alnus glutinosa*), and elderberry (*Sambucus nigra*). The tree layer consists of western red cedar (*Thuja plicata*), Douglas-fir (*Pseudotsuga menziesii*), and big leaf maple (*Acer macrophyllum*) (Washington Native Plant Society 2002).

The side channel itself is approximately 400 meters long and 20 to 30 meters wide. It includes a mixture of riffles, pools, and glides. The U.S. Forest Service placed five log jams throughout the side channel (Figure 1, Bonanno et al. 2011). The side channel section remains around 6 degrees Celsius for most of the year and can increase to 16 degrees Celsius during summer months, making it optimal spawning ground and rearing habitat for spring Chinook salmon, bull trout (*Salvelinus confluentus*), and many other aquatic species and wildlife native to the watershed (Risley et al. 2010).



Figure 1. Map of Middle McKenzie side channel 4 including the locations of LWD placement, right bank cross section monuments, gravel count locations, and the longitudinal profile monument which were established in 2011 (Figure modified from Bonnano et al. 2011).

3. METHODS

The 2011 ELP Team conducted baseline monitoring at Middle McKenzie side channel 4 and established permanent rebar monuments along the right and left bank of the channel at all of their survey locations (Bonnano et al. 2011). In May of 2012, we used a Global Positioning System (GPS) to locate these monuments in order to conduct five cross-channel surveys, a longitudinal channel profile, and two Wolman pebble counts along the same transects used in 2011. To ensure data collection consistency, we used the same surveying methods used in 2011 (Bonnano et al. 2011).

3.1 PEBBLE COUNT

We conducted Wolman pebble counts at both an upstream and a downstream location. Gravel count 1 was located upstream of cross section 5 and gravel count 2 was located downstream of cross section 2 (Figure 1). We set up three transects at each gravel count and took two particle samples at every half-meter distance along the transect for a minimum sample size of 100 particles. Using blind sampling, we selected particles from the stream and measured the b-axis size class of each particle using a gravelometer (Bunte and Abt 2001). We also noted whether the particles were embedded or not embedded. We defined an embedded particle as one that is lodged or partially lodged within the streambed and has finer sediment attached to it.

3.2 CROSS SECTION SURVEYS

We conducted cross-channel surveys by lying transects between right bank and left bank monuments and taking measurements along each transect at every major observable change in elevation (Harrelson et al. 1994). We were unable to locate the left bank monument at cross section 1, so we made an estimation based on the right bank monument and length of the 2011 transect. For all cross-channel surveys, we used a surveyor's auto-level and a metric elevation rod to measure distance from the bank, water depth, and the elevation relative to the right bank monument at each point along the transect (Harrelson et al. 1994). By applying our measurements to the known elevations of the monuments, we calculated the actual elevation along the streambed.

3.3 LONGITUDINAL PROFILE

We used the same surveying methods as mentioned above when conducting the longitudinal profile. We began our transect at an upstream monument established in 2011 and placed it 241.1 meters downstream, along the edge of the channel bank. We used a surveyor's auto-level and a metric elevation rod to measure the streambed elevation and water depth systematically within the thalweg (the deepest part of the stream) (Harrelson et al. 1994). To capture the entire length of the channel, we set up multiple surveying stations and used multiple benchmarks to calculate actual elevation using the same calculations as those for the cross-channel surveys (Harrelson et al. 1994). We did not collect data near the log jams due to safety concerns.

3.4 DATA ANALYSIS

We used the same surveying methods as mentioned above when conducting the longitudinal profile. We began our transect at an upstream monument established in 2011 and placed it 241.1 meters downstream, along the edge of the channel bank. We used a surveyor's auto-level and a metric elevation rod to measure the streambed elevation and water depth systematically within the thalweg (the deepest part of the stream) (Harrelson et al. 1994). To capture the entire length of the channel, we set up multiple surveying stations and used multiple benchmarks to calculate actual elevation using the same calculations as those for the cross-channel surveys (Harrelson et al. 1994). We did not collect data near the log jams due to safety concerns.

4. RESULTS

4.1 PEBBLE COUNTS

The percent of embedded sediment in both gravel count 1 and count 2 decreased from 2011 to 2012 after the placement of LWD. The total embedded sediment decreased from 33.3% to 26.6% in gravel count 1 and from 27.6% to 23% in gravel count 2.

As compared to 2011, the 2012 sediment size distribution in Middle McKenzie side channel 4 shifted toward a smaller median pebble size with an increase in D_{84} in count 1, while median pebble size increased with no detectable change in D_{84} values for count 2 (Figure 2). For count 1, the D_{50} size class decreased from 45-64 mm to a size class of 32-45 mm and the D_{84} size class increased from 91-128 mm to 128-181 mm. For count 2, the D_{50} size class increased from 32-45 mm to 45-64 mm and the D_{84} size class was recorded at 91-128 mm both years.

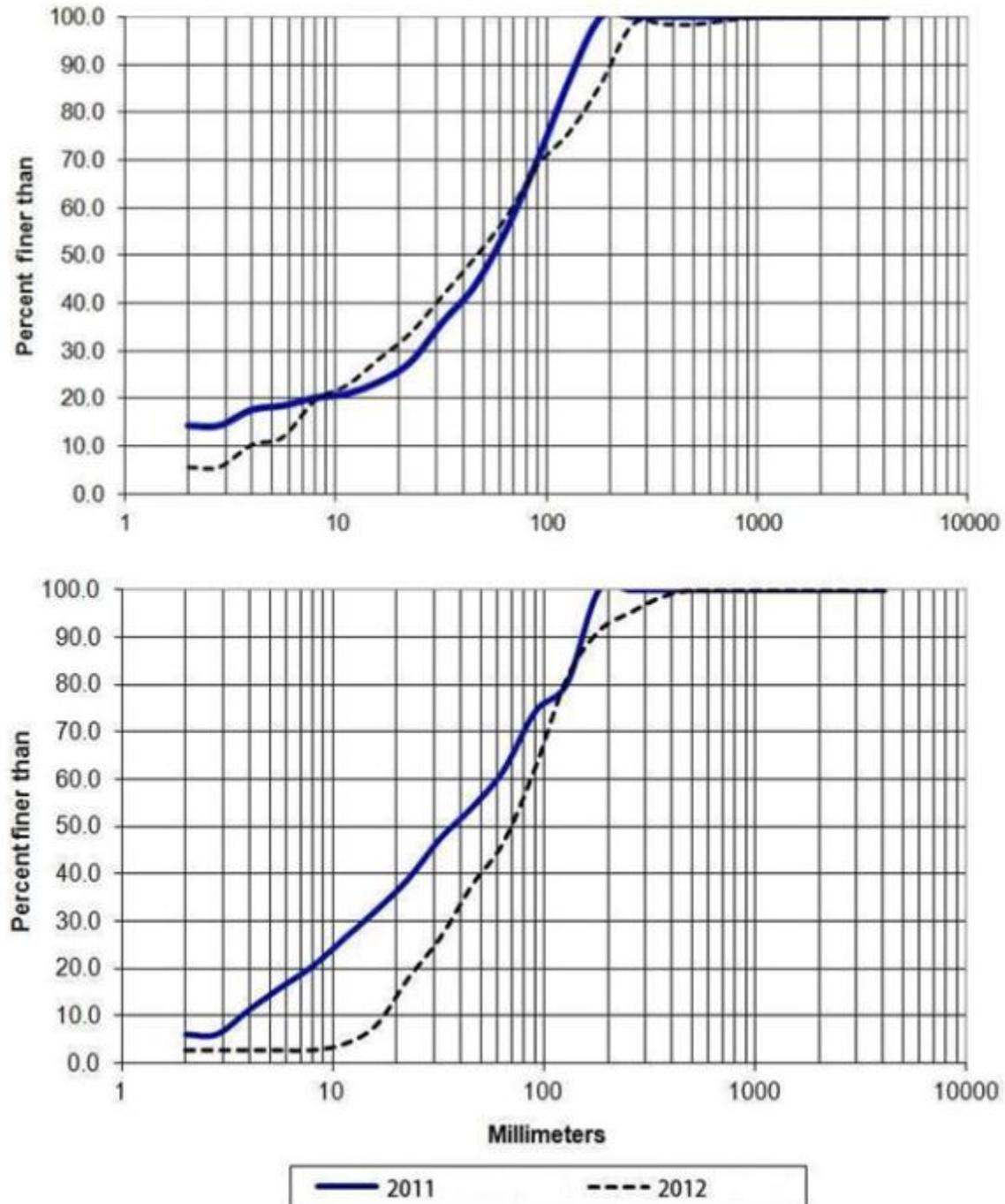


Figure 2. Comparison of cumulative sediment size distribution of gravel count 1 (top), and gravel count 2 (bottom) in McKenzie side channel 4 from 2011 to 2012.

4.2 CROSS SECTION SURVEYS

There were changes in the streambed topography at four out of the five cross sections in Middle McKenzie side channel 4 from 2011 to 2012 (Figure 3). We observed channel degradation and a decrease in streambed elevation due to scouring and erosion at both cross section 1 and cross section 2 between 2011 and 2012 (Keefer et al. 1980). The streambed lowered

in the right side of the channel at cross section 1 and in the left side of the channel at cross section 2. At cross section 3, the channel deepened by approximately one-half meter but maintained the same general morphology. We did not observe any major changes in the channel topography at cross section 4. There was minor erosion along the left bank at cross section 5 but only minor changes in streambed morphology.

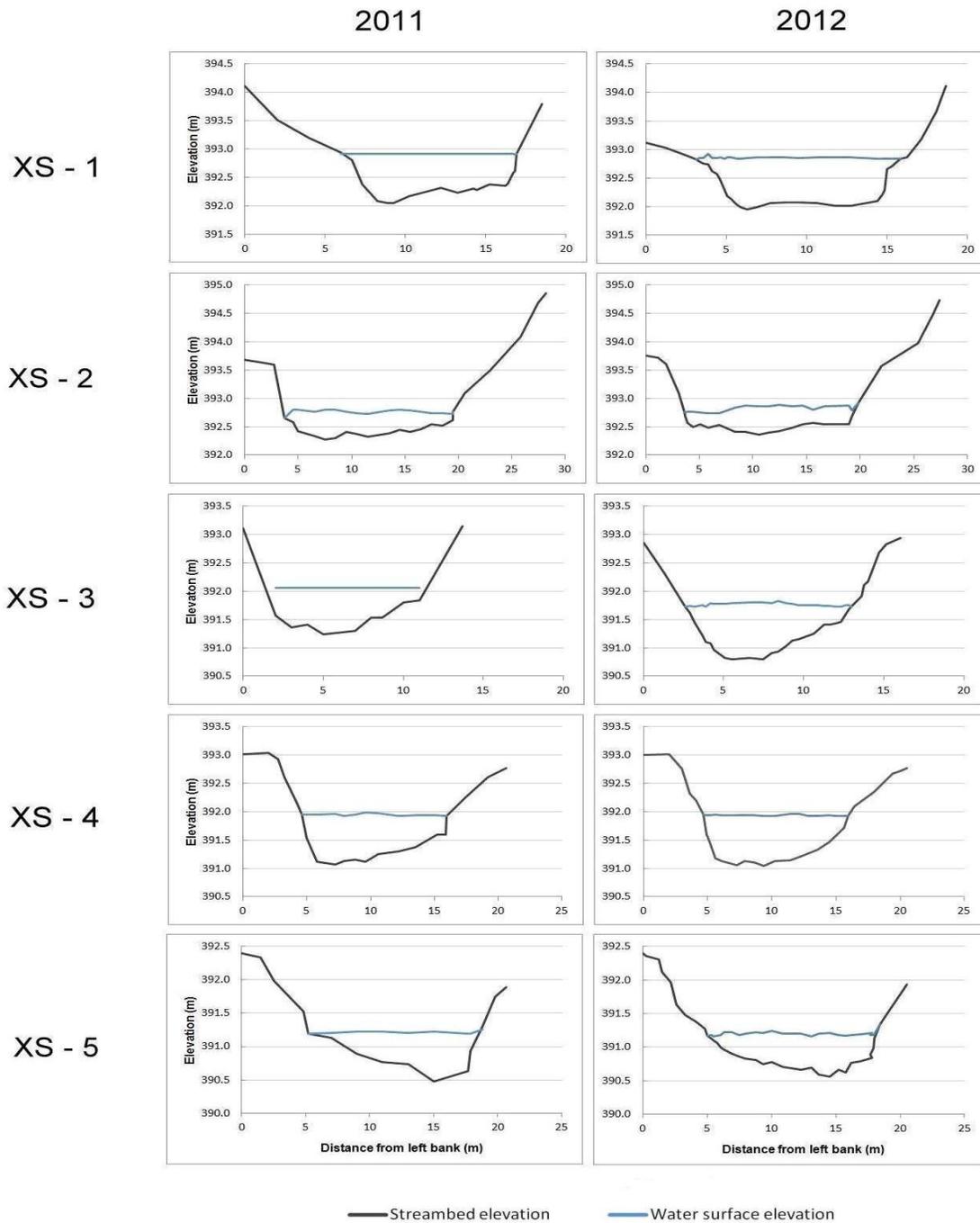


Figure 3. Comparison of channel morphology at cross section surveys (XS) 1 through 5 in Middle McKenzie side channel 4 from 2011 to 2012.

4.3 LONGITUDINAL PROFILE

We observed an increase in the complexity of the streambed elevation along the entire transect from 2011 to 2012 (Figure 4). The profile degraded due to scouring immediately downstream of the first LWD placement at 32.4 m along the transect (downstream from the permanent monument). There were aggradations of deposited sediment downstream of the last three LWD placements at 105 m, 136.6 m, and 201.5 m along the transect. The first noticeable pool formations occurred at 156.1 m near cross section 4 and before the last LWD placement at 201.5 m on the transect.

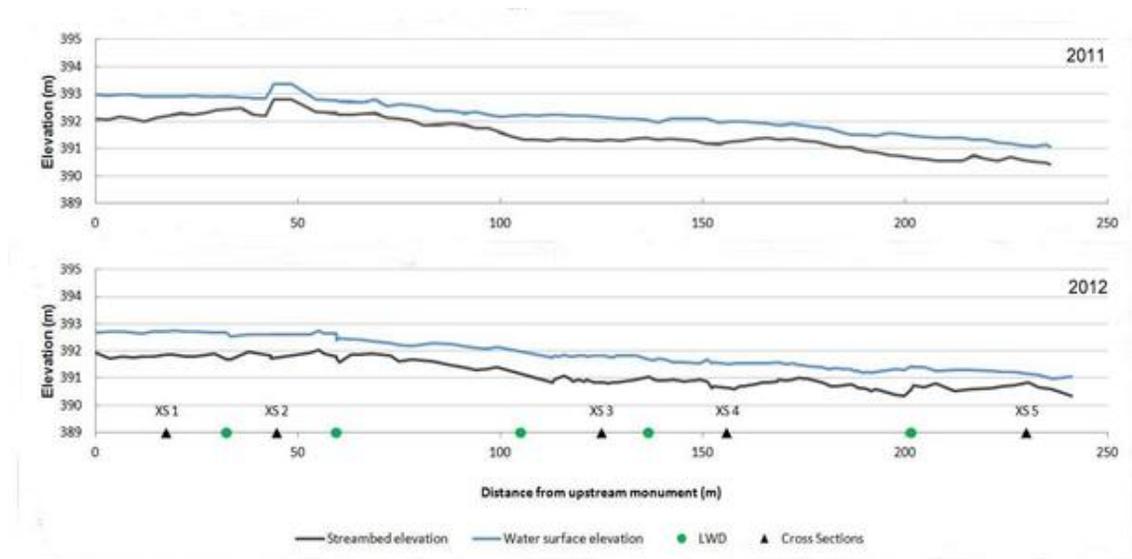


Figure 4. Comparison of streambed elevation and water surface elevation in a 241.4 m longitudinal profile of McKenzie side channel 4 from 2011 (top) to 2012 (bottom). LWD markers represent the locations of large woody debris along the length of the profile. Cross section markers represent the locations of each cross section along the length of the profile.

5. DISCUSSION

We observed changes in the sediment size distribution as well as the morphology of the streambed of the channel from 2011 to 2012 after the placement of LWD. Median pebble size decreased downstream of the LWD placements at gravel count 1 and increased upstream at gravel count 2. The percent of embedded sediment decreased at both sites. Also, we detected noticeable changes in the stream morphology at four of our five cross-sectional surveys as well as along the longitudinal profile.

Optimal median pebble size for salmon spawning habitat is within the size class of 32-45 mm (Bonanno et al. 2011). After the placement of LWD in 2011, the distribution in gravel count 1 shifted to the 32-45 mm size class while the size class in count 2 shifted to a larger size class. The percent of embedded sediment decreased from 2011 to 2012 in both gravel counts. A decrease in embedded pebbles promotes spawning habitat for salmon and other fish species that use the loose gravel to create spawning beds (Bevenger and King 1995).

The changes in sediment distribution could be due to a changing energy gradient as the result of LWD placement in which upstream scouring of the streambed transports smaller sediment downstream. This would result in less sediment being embedded and a larger median sediment size upstream. However, the changes could also be the result of annual variation in the flow regime of the channel. We observed that many of the LWD placements were bridged over the channel and did not have much contact with the water, which minimally affect changes in sediment distribution.

We also observed a trend in sediment moving downstream in our cross-channel surveys and longitudinal profile (Figures 3 and 4), which likely resulted from the LWD placement rather than annual variation in flow regime. According to our longitudinal profile, scouring was more frequent upstream while deposition occurred mostly downstream between 2011 and 2012. The proximity of these changes to the placements of LWD suggests that the LWD affects the streambed morphology.

Roni and Quinn (2001) recorded an increase in juvenile Coho salmon population density after the placement of LWD in 30 streams in the Pacific Northwest. After a single season of winter flows, we have detected the formation of pools and an improvement in sediment size for salmon spawning habitat in the downstream portion of our longitudinal profile. As the LWD sinks down over time and gains more contact with the channel, even more changes in the streambed can be expected. We did not have any control treatment for this experiment and thus can assert no definite causal conclusions regarding the effects of LWD. We did, however, find changes in the channel conducive to salmon spawning habitat since the addition of LWD in October of 2011.

Multiple sources of error and observer bias could account for discrepancies in our data. Observer bias most likely occurred in our gravel counts since we had twelve different people conducting them. During the longitudinal and cross-channel surveys, we had to shout some of the measurements across lengths of the channel and consequently may have recorded some incorrectly. Additionally, while conducting the longitudinal profile we could not always stand in the thalweg due to safety concerns and this slightly affected the accuracy of our data. Safety concerns for working adjacent to the LWD placements prevented us from taking measurements at several points along the transect of the longitudinal profile. Considering that our data were consistent with the data from the 2011 ELP team, these sources of error and bias had a minimal effect on our results. However, measures should be taken to address these sources of bias and further monitoring is warranted.

6. RECOMMENDATIONS AND MANAGEMENT IMPLICATIONS

We recommend three actions for post monitoring of LWD placement in the Middle McKenzie side channel 4. First, LWD should be monitored annually for subsequent years to observe noticeable changes in stream morphology. We recommend that future observations last for at least three years post-project (preferably longer) (Roni and Quinn, 2001). Second, elevation and orientation of the LWD should be considered in future data analysis. We noticed

that much of the LWD was barely submerged in the stream, thereby putting into question the overall effects it has on stream morphology. Lastly, previously placed LWD should be evaluated to assess which sites were most effective in positively altering streambed morphology to inform future designs for LWD placement projects.

In addition, we recommend having a single survey crew for all Wolman pebble counts conducted year to year to reduce observer bias in the field and maintain consistency. However, considering the challenge of keeping the same surveyors, strict protocols to reduce observer bias should be implemented. Stream surveyors should collect data when the water levels are low (during late spring or summer) to ensure the consistent collection of data within the thalweg. Most importantly, surveyors should collect data annually at a consistent time to ensure accurate data comparison.

ACKNOWLEDGEMENTS

The McKenzie Watershed Council and the University of Oregon Environmental Studies Program provided funding, equipment and technical support. We owe special thanks for the time and energy put forth by Jared Weybright of the McKenzie Watershed Council, Peg Boulay from the Environmental Studies Program, our technical advisor Pollyanna Lind, and Keats Conley, the Graduate Teaching Fellow who lead our team. This was a project of the University of Oregon Environmental Leadership Program.

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Stomata Density of Orchids and Cloud Forest Humidity

Sierra Predovich*, Environmental Studies Program

ABSTRACT

This experiment compares stomata density of the epiphytic *Pleurothallis aristata* and *Maxillaria sp.* orchids under experimental conditions of dry and humid environments. *Pleurothallis aristata* is in the sub-tribe Pleurothallidinae and lacks pseudobulbs, while *Maxillaria sp.* has pseudobulbs. The study seeks to determine what differences in stomata density exist between the two species, and if there is a difference in mean percent stomata open in humid and dry environments. The study takes stomata impressions from the leaves of twenty individuals of each species using clear nail polish. The results show a significant difference in stomata density between the *Pleurothallis aristata* and the *Maxillaria sp.* (Rank Sum Test: $t=55$, $n_1=10$, $n_2=10$, $p<0.05$). Additionally, both species have a higher percentage of open stomata in humid environments than in dry environments (Wilcoxon sign rank test). An explanation for these results is that *Maxillaria sp.* has a pseudobulb for water storage, has a larger leaf surface area, and therefore has higher stomata density. The study showed both species closed their stomata in drier conditions in order to reduce water loss and desiccation. The results of this experiment help demonstrate how different orchid species function in humid and dry environments, and their ability to succeed in the event of global climate change and shifting of biomes.

INTRODUCTION

Orchidaceae is one of the largest and most diverse angiosperm families in the world, including approximately 20,000 to 35,000 described species (Dressler 1981). In cloud forests and other wet forests, many epiphytic orchids live in the canopy, where species experience desiccating conditions of high winds and direct sunlight (Dressler 1993). Thick, fleshy leaves and pseudobulbs (large, bulbous formations on the stem) are both adaptations of epiphytic orchids used to store water and reduce drying (Dressler 1993). There are other morphological adaptations of epiphytic orchids such as succulence and the ability to fix carbon with Carassulacean acid metabolism, similar to members in the succulent family Crassulaceae (Dressler 1981). Orchid species that share this trait have tiny pores in their leaf surfaces (called stomata) that open at night to take advantage of the time where atmospheric humidity is highest

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(Dressler 1981). Thick, succulent leaves are characteristic of these species and they will often open stomata during the day if atmospheric humidity is high, or keep all of their stomata closed if dry conditions occur (Dressler 1981).

Stomata are found in the epidermis and cuticle layer of leaves, which provide the primary route for water vapor transfer when they are open (Hopkins 1995). Water loss and uptake are mediated by the guard cells, which can activate stomata closure if they sense water stress (Taiz and Zeiger 1991). The process of stomata closure is one of the very important protective processes to prevent severe water stress (Fitter and Hay 1987). This defense helps prevent tissue desiccation before leaves reach low water levels (Fitter and Hay 1987).

In this study, I experimented with two controlled environments: one in a wet environment and the other in a dry environment (Figure 1). The experiments were performed with individuals of two orchid species, *Pleurothallis aristata* and *Maxillaria sp.* (Figure 2), chosen because they differ greatly in overall size. *Maxillaria sp.* is much larger; *Pleurothallis aristata* is a miniature orchid. Additionally, *Maxillaria sp.* has pseudobulbs while *Pleurothallis aristata* does not. Having smaller leaves means that *Pleurothallis aristata* is more limited in the amount of water it can hold. Thus, Pleurothallids (referring to the miniature orchid sub-tribe, Pleurothallidinae) will face a more serious threat of desiccation than the *Maxillaria sp.* under identical environmental conditions. Lacking pseudobulbs means that Pleurothallids cannot depend upon their stems for water storage. Therefore, they only have their leaves for water storage and must adapt physiologically or micro-morphologically in order to prevent desiccation (K. Masters, personal communication, August 11, 2011). Thus, it is likely that Pleurothallids should evolve to have a lower stomata density and show a greater responsiveness to dry conditions by closing their stomata. Conversely, it was expected that *Maxillaria sp.* would have greater stomata density because they have larger leaves and the presence of a pseudobulb justifies the increased rate of gas exchange. Furthermore, it was predicted that in dry conditions both orchid species would have a smaller percentage of open stomata in order to prevent water loss.



Figure 1. Set up of environmental conditions. (A) Dry conditions aquaria and workspace. (B) Outdoor humid environment.

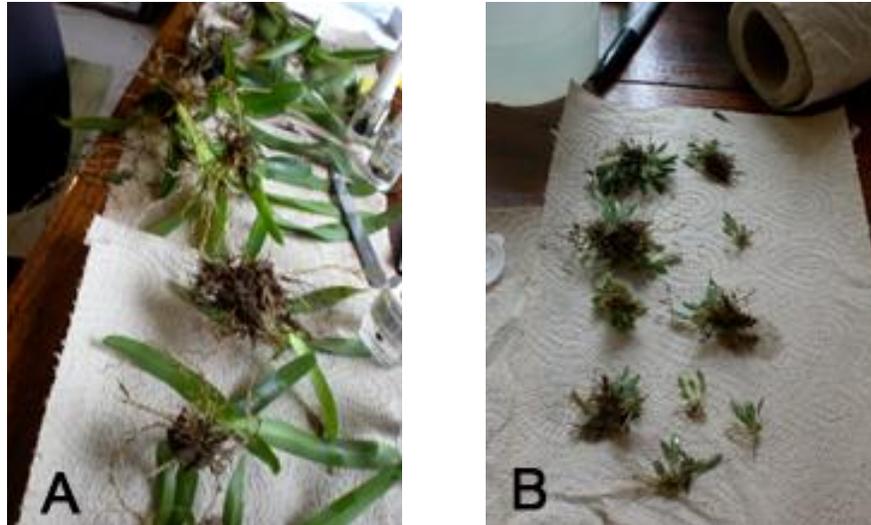


Figure 2. Orchid specimens. (A) *Maxillaria sp.* and (B) *Pleurothallis aristata*.

MATERIALS AND METHODS

Twenty *Pleurothallis aristata* individuals and twenty *Maxillaria sp.* were collected from primary or secondary growth cloud forest in Monteverde, Costa Rica. They were retrieved from locations on the property of Karen and Alan Masters as well as the biological station forest. They were removed from their epiphytic habitats by cutting out the entire root system from the host tree so as to keep the individual as intact as possible. Selection criteria for each individual required that each have at least four healthy leaves and be of medium age and size.

Once the individuals were collected, they were paired up with another individual of the same species that was most similar in size and number of leaves, and then each pair was divided into two groups: one destined for the dry, one destined for the wet experimental condition. For each specimen, the weight (g), length (cm) and surface area (cm²) of the longest leaf, width of the stem (mm), and total number of leaves were determined. The surface area was calculated using ImageJ software, which scaled scanned images of the traced leaves.

The two experimental conditions consisted of aquaria placed in either (1) an indoor, dry environment with a controlled de-humidifier, which kept relative humidity at approximately 50%, or (2) an outdoor, wet environment with a humidifier, which kept relative humidity at approximately 95%. Ten of each species were placed in a bed of moss in each environment and given three days to adjust to the humidity and temperatures. The individuals in the dry conditions were sprayed with 100-250mL of water in the morning and at night each day, and the individuals in the wet conditions had a humidifier inside the aquarium that would turn on if the humidity dropped below 92%.

After waiting three days for the orchids to adapt to the environments, the experiment was initiated on the first set of stomata peels. This consisted of painting a small stroke of clear nail polish on the largest leaf of each specimen and allowing it to dry. Once dry, the polish was

removed in a single peel with clear sticky tape, and attached onto a glass slide. When the nail polish is painted onto the leaf, it obtains an imprint of the stomata as it dries. Each slide was placed into a compound microscope and three views were taken for the peel of each leaf. In each view, the total numbers of stomata were counted, as well as the number of stomata open. From these three views, I calculated the average stomata density for each leaf, as well as the average percent of stomata open. The two sets of orchid specimens were then transferred to the opposite environment and given another three days to adjust to the new environmental condition. After the three days, the same stomata peel test was performed on each individual plant. The specimens remained in their current environments after these tests.

A final measurement was taken two days after the second set of stomata peels. Leaf cross-sections of the longest leaf of each individual plant were taken and were viewed in the cross-sections in a dissecting scope equipped with a micrometer in the eyepiece to measure the thickness of each leaf.

RESULTS

STATISTICAL ANALYSES

Since this experiment had a relatively small sample size, non-parametric statistics were used. These include the Wilcoxon sign rank test (used for comparing related samples' means), Spearman's rank correlation test of relationship (to measure the correlation of dependence between two variables), and the rank sum test of differences between means (also for comparison of two samples' means).

RELATIVE HUMIDITY AND MEAN OPEN STOMATA

In order to determine if the average percentage of stomata increased as individuals were moved from a dry environment to a humid environment a Wilcoxon sign rank test was performed four times, for each change of conditions of both *Pleurothallis aristata* and *Maxillaria sp.* From this test, results show that for *Pleurothallis aristata* and *Maxillaria sp.* the move from the dry to the wet conditions resulted in a significant increase in percent of stomata open (65.1% increase for *P. aristata*, 42.4% increase for *Maxillaria sp.*). Likewise, the results show that for both species the move from the wet to the dry conditions resulted in a significant decrease in the percent of stomata open.

The experiment was designed to determine if there was a significant correlation between leaf surface area and the mean percent of stomata open. Spearman's rank correlation test ($n = 10$, critical value = 0.648) was performed for each species in the conditions they started in and the condition they were in after the environments were switched. For both *Maxillaria sp.* and *Pleurothallis aristata* there was a significant difference in the number of stomata open from dry to wet conditions. As each species was moved from the dry to the wet environment, the mean percentage of open stomata increased significantly (Figure 3; Figure 5). Likewise, as each

species was moved from the wet to the dry environment, the mean percentage of open stomata decreased significantly (Figure 4; Figure 6).

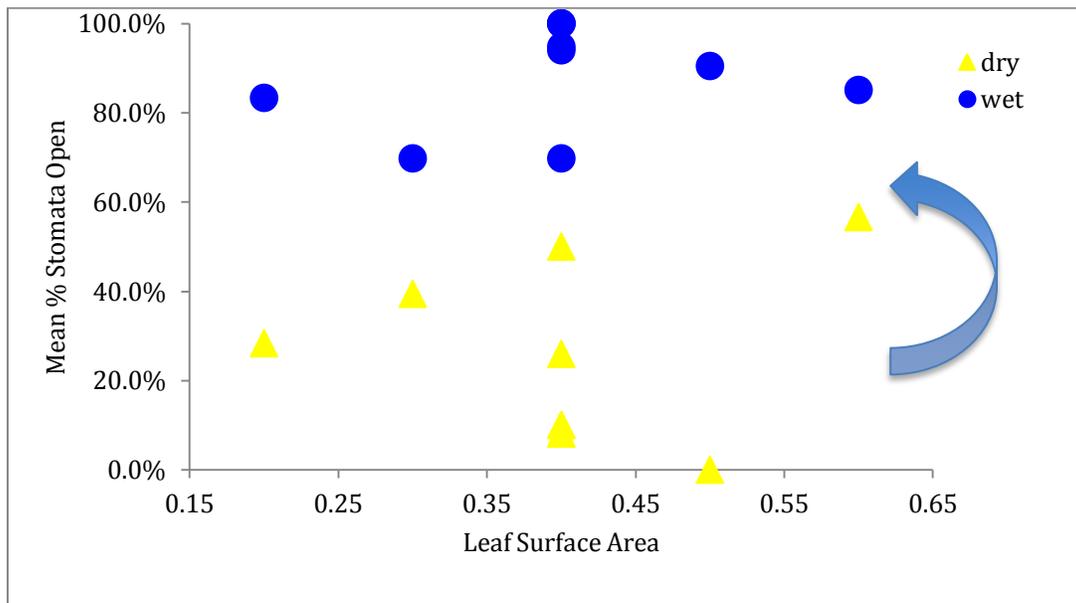


Figure 3. Leaf Surface Area and Mean Percentage of Open Stomata of *Pleurothallis aristata* from Dry to Wet Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Pleurothallis aristata* from dry to wet environments. The arrow demonstrates which environment the species was moved to. As *Pleurothallis aristata* was moved from a dry to a wet environment, the percentage of open stomata increased.

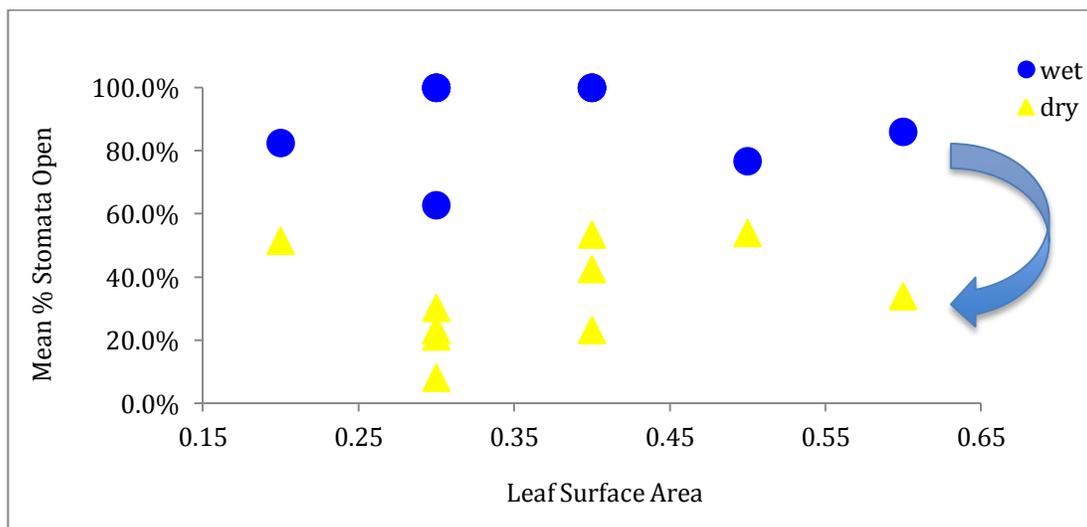


Figure 4. Leaf Surface Area and Mean Percentage of Open Stomata of *Pleurothallis aristata* from Wet to Dry Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Pleurothallis aristata* from the wet to dry environments. The arrow demonstrates the move from the wet to the dry environment. As *Pleurothallis aristata* moved from wet to dry, the percentage of open stomata decreased.

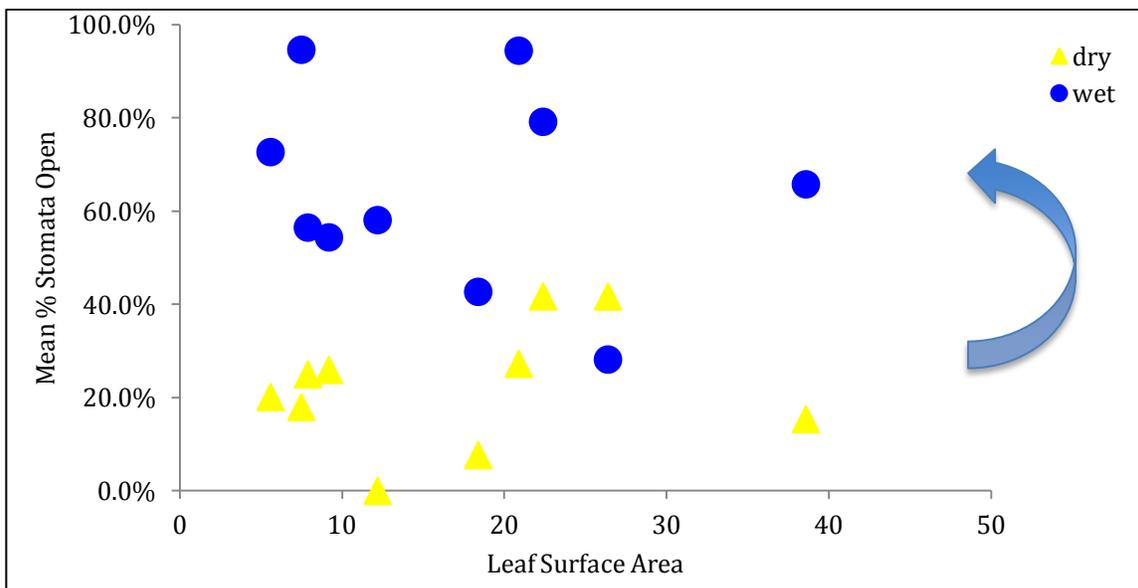


Figure 5. Leaf Surface Area and Mean Percentage of Open Stomata of *Maxillaria sp.* from Dry to Wet Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Maxillaria sp.* from the dry to wet environments. The arrow demonstrates the move from the dry to the wet environment. As *Maxillaria sp.* was moved to the wet environment, the percentage of open stomata increased.

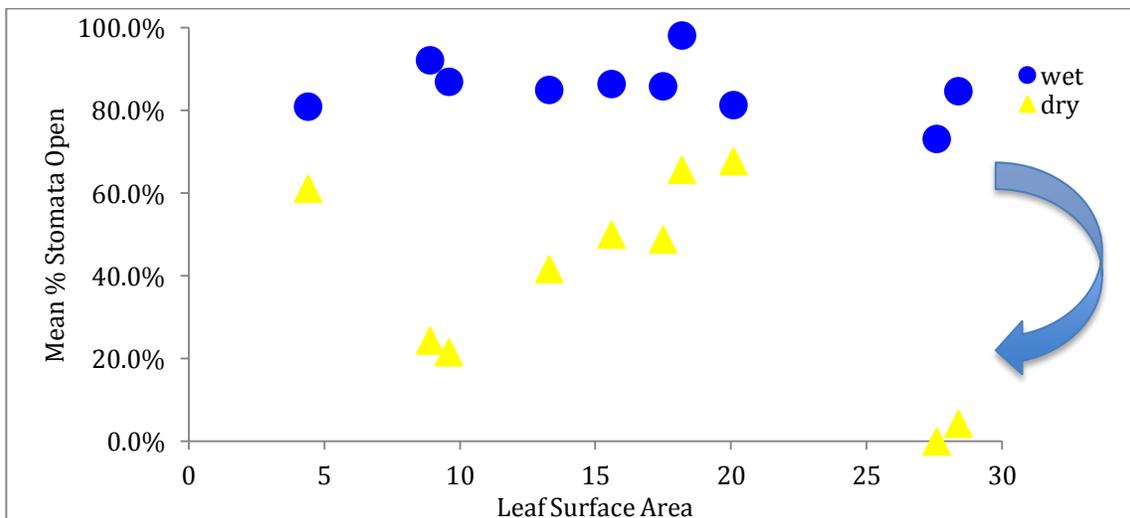


Figure 6. Leaf Surface Area and Mean Percentage of Open Stomata of *Maxillaria sp.* from Wet to Dry Environments.

This figure shows the correlation between leaf surface area and mean % stomata open in *Maxillaria sp.* from the wet to dry environments. The arrow demonstrates the move from the wet to dry environment. As *Maxillaria sp.* was moved to the dry environment, the percentage of open stomata decreased.

MEAN STOMATA DENSITY

In order to determine if there was a difference in stomata density between *Pleurothallis aristata* and *Maxillaria sp.*, a Rank Sum Test was performed of differences between means ($t =$

55, $n_1 = 10$, $n_2 = 10$, $p < 0.05$). The results show a significant difference in stomata density between the two species (Figure 7). *Maxillaria sp.* has approximately four times more stomata on average than does *Pleurothallis aristata*.

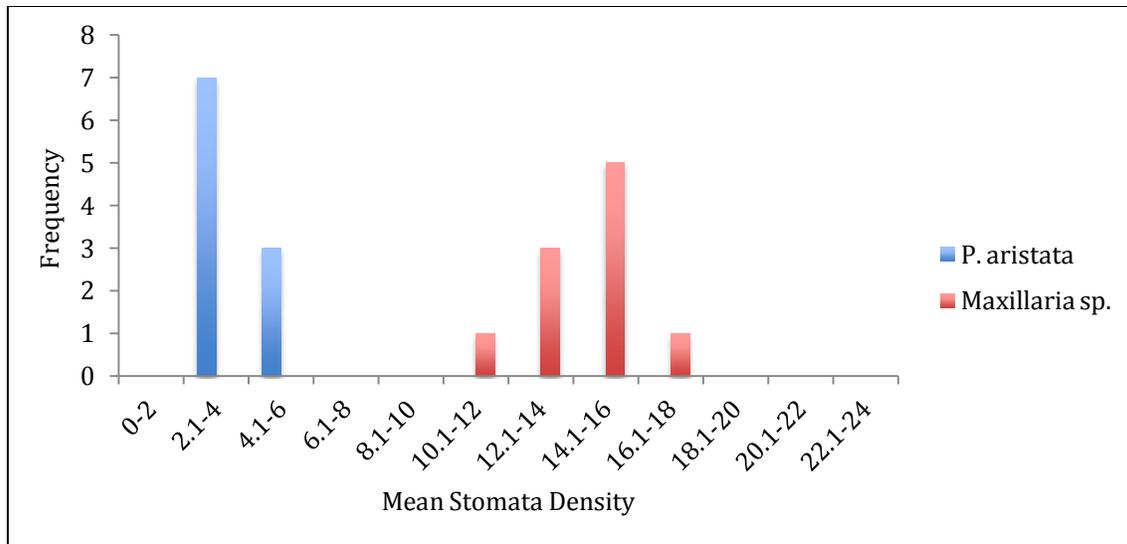


Figure 7. Mean Stomata Density Frequencies.

This figure compares the mean stomata density frequencies of *Pleurothallis aristata* ($\mu=3.8$, $\sigma=0.5$) and *Maxillaria sp.* ($\mu=14.4$, $\sigma=1.9$). There is a significant difference in stomata density between the two species (Rank sum test, $n_1=10$, $n_2=10$, $t=55$, $p < 0.05$).

LEAF SURFACE AREA

To determine if there was a significant difference in leaf surface area between the two species the same Rank Sum Test was used ($t=55$, $n_1=10$, $n_2=10$, $p < 0.05$). The results show a significant difference in leaf surface between the two orchid species (Figure 8). *Maxillaria sp.* ($x=16.4$, $sd=7.8$) has a leaf surface area that is 41 times larger than the surface area of the *Pleurothallis aristata* ($x=0.4$, $sd=0.1$) and it has four times the stomata density.

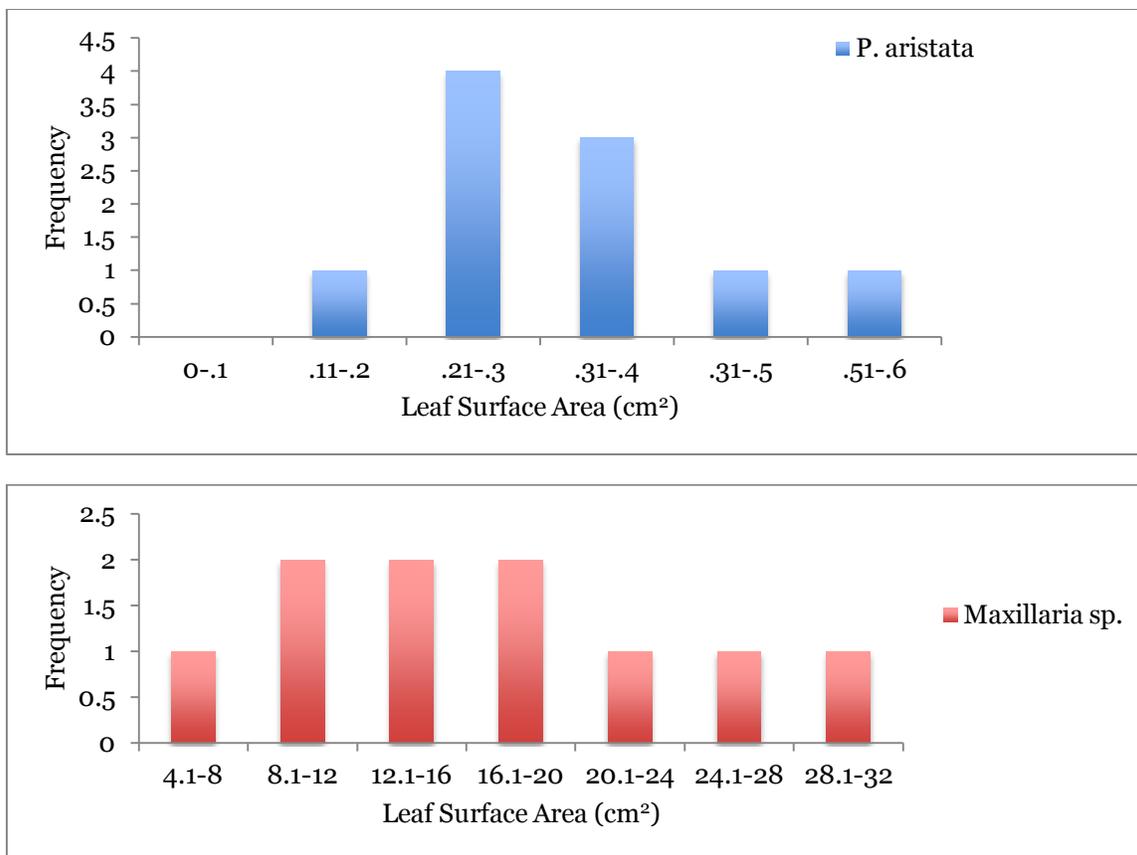


Figure 8. Leaf Surface Area Comparison.

These figures compare the leaf surface areas of *Maxillaria sp.* and *Pleurothallis aristata*. The x-axis displays intervals of leaf surface area. There is a significant difference in leaf surface area of the two species.

LEAF THICKNESS

One other factor taken into consideration was the difference in leaf thickness between the two species. A Rank Sum Test was used to derive the differences ($t=68$, critical value= 78 , $n_1=10$, $n_2=10$, $p<0.05$). There was a significant difference in leaf thickness of the two species; *Pleurothallis aristata* had leaves twice as thick as the *Maxillaria sp.* leaves (Figure 9).

DISCUSSION

The results show that *Pleurothallis aristata* and *Maxillaria sp.* differ significantly in leaf thickness, leaf surface area, and mean stomata density. These differences can be explained in a number of ways. First, Pleurothallids are orchids that lack pseudobulbs, so they must be more conservative with their water and use structures other than their stems for water storage or use other adaptations to mitigate water loss in drier environments. Having a smaller leaf surface area and mean stomata density reduces the amount of water loss through the leaves. Since *Maxillaria sp.* have pseudobulbs, it was expected that they would be less conservative in gas exchange, and therefore would have higher stomata densities. Furthermore, the Pleurothallid

species had leaves that were twice as thick as the *Maxillaria sp.*, despite their small size. It was observed that the Pleurothallid leaves were relatively waxy and succulent-like, which would justify their thicker nature if they were storing water in the leaves. The dissecting scope used to measure the leaf cross sections was used to identify presence of different tissue types. In the *Maxillaria sp.* the cross section consisted mostly of photosynthetic mesophyll. In contrast, the Pleurothallid species had a thin layer of photosynthetic cells and tissue and a large quantity of spongy and vascular tissues. This could be an adaptation of *Pleurothallis aristata* to cope with water stressed environments.

The results also show that when moving from a dry environment to a humid environment, the percentage of open stomata significantly increases in both *Pleurothallis aristata* (65.1% increase) and *Maxillaria sp.* (42.4% increase). Similarly, when moving from a wet environment to a dry environment, the percentage of open stomata significantly decreases in both species. This result is reasonable because when species of moist climates are exposed to drier environmental conditions, they reasonably respond by attempting to hold onto water to survive. Therefore, it is expected that most of their stomata would be closed in dry conditions to prevent excess water loss and desiccation.

The results of this experiment indicate that stomata density differs between the *Pleurothallis aristata*, a miniature orchid species and *Maxillaria sp.* pseudobulb-containing orchid species. The behavior of each species in wet conditions demonstrates how these two species function in the cloud forest in Monteverde, Costa Rica, where it is humid for a great portion of the year. Because most of the stomata close up in dry conditions, gas exchange is taking place at a much slower rate. This relates to issues such as climate change and the resulting biome shifts, as some species may be more likely to succeed in periods of prolonged drought or less humidity. For example, in discussing the orchids in this experiment we would expect many Pleurothallid species to be stressed in the onset of global climate change, as they have minimal water storage. Other plant species with similar water storage adaptations as Pleurothallids would also be stressed, and their populations could diminish. It is crucial to look at smaller species and their environmental adaptations when considering global climate change, as their response to change is an indicator to the success of the overall environment or biome.

ACKNOWLEDGEMENTS

I would like to thank the CIEE program for introducing me to this environment and initiating my interest in epiphytic plant species. Additionally I would like to thank Karen Masters, for her knowledge of orchids, her help with and support of my study, as well as the use of her office space to house my orchid experiment. I would like to thank my teaching assistants, Gisella Fernandez and Moncho Calderon, for helping me learn how to use new software and for supporting my project. Finally, I would like to thank my peers and fellow researchers, for being such amazing friends and keeping each other motivated.

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Assessing the Relationship Between Topography and Plant Diversity in Restored and Remnant Wet Prairies

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ABSTRACT

Wet prairies provide numerous ecosystem services and habitat for native plant species. This study examines the relationship between microtopographic variation and plant diversity in six restored and remnant wet prairies in the West Eugene Wetlands to aid future restoration projects. It was predicted that variation in elevation is influential in determining native plant community composition. Along transects within previously established macroplots, soil surface elevation and water depth were measured and percent cover of grasses, forbs, and non-forbs, and measured vegetation and litter height were determined. A linear regression was performed comparing native species richness to the topographic coefficient of variation, which yielded an R^2 value of 0.43 and a p-value of 0.16. Although these results are not statistically significant, they demonstrate a meaningful correlation between native plant richness and the coefficient of variation of topography. Further observations additionally suggest that this relationship is present. We suggest further research to determine significant results and suggest the integration of the restoration of microtopography into wetland management.

1. INTRODUCTION

Wet prairie habitats once covered 31% of the Willamette Valley ecosystem; today, 98% of these areas are severely damaged or destroyed (Wold et al. 2011). These native ecosystems are valued for their aesthetic beauty as well as for their benefits to society. Specifically, the West Eugene Wetlands provide numerous ecosystem services including carbon storage, flood control, storm water purification, and nutrient capturing and cycling. The 3,171-acre area of the West Eugene Wetlands is comprised mainly of wet prairie, which is dominated by grasses and wildflowers, and home to a variety of wildlife (City of Eugene & LCOG 2011). Wet prairie restoration in the West Eugene Wetlands is therefore an important issue for local government agencies, including the Lane Council of Governments (LCOG) and the City of Eugene.

* Authors are members of the University of Oregon Environmental Leadership Program, a collaborative, interdisciplinary service-learning program housed in the University of Oregon Environmental Studies Program. For more information, visit http://envs.uoregon.edu/elp_program/. Please direct correspondence to clairereeddustin@gmail.com.

Understanding the most feasible and effective techniques for wet prairie restoration is essential for the future of these habitats.

The role of microtopography in restored wetlands is of growing interest to wetland ecologists as restoration techniques continue to evolve and improve. Microtopographic variation is a common attribute of natural wetlands, yet is not often observed in restored or created wetlands (Bruland & Richardson 2005; Moser et al. 2007). In the West Eugene Wetlands, for example, many restored prairies occupy lands that were historically leveled for grass seed production. The presence of microtopographic variation in restored wet prairies has implications for soils, hydrology, and vegetation (Bruland & Richardson 2005). Microtopographic heterogeneity in restored wetlands can increase native species abundance and diversity (Moser et al. 2007). The promotion of native plant diversity and cover in restored wet prairies is essential to restoration projects, and is often hindered by the invasion of exotic species (Pfeifer-Meister et al. 2008).

Microtopographic heterogeneity in natural wetlands is caused by a variety of factors, including erosion, sediment accumulation, tree fall, animal tracks, and animal burrowing (Bruland & Richardson 2005). To imitate the microtopographic heterogeneity of natural wetlands, restoration scientists have used several techniques, including disking and excavation (Moser et al. 2007). Both disking and excavation use machinery to disturb soils in order to mimic the natural microtopographic variation in native wetlands.

A series of environmental conditions influence vegetative communities in wetlands, including hydrology and soils. Bruland and Richardson (2005) found that the reestablishment of microtopography by excavation in a restored wetland increased the variety of hydrologic, edaphic, and vegetative conditions. For example, soil temperature, pH, and nutrient levels varied across hummocks, hollows, and flats. Plant species diversity varied across microtopographic zones as well, with the greatest diversity in the flats and no single species occurring in all three zones. The variability in microtopography thus produced different conditions for vegetation growth (Bruland & Richardson 2005). According to Moser et al. (2007), microtopographic heterogeneity in a disked wetland promoted greater plant diversity and cover than a non-disked wetland. Disked wetlands also had greater microtopography than non-disked wetlands, and experienced greater species richness and cover. Furthermore, a study by Seabloom et al. (2003) found that plant communities were more homogeneous in a restored wetland without microtopographic reestablishment than in natural wetlands. By producing different vegetative zones, it appears that microtopographic heterogeneity increases plant diversity and cover in restored wetlands.

Wet prairie restoration values the achievement of a high percent cover of native species and high native species diversity. However, the encroachment of exotic species complicates the achievement of native plant diversity and cover in restored wetlands. Exotic plants have been hypothesized to competitively exclude native species in high-nutrient, moderate-moisture environments, thus displacing native species to low-nutrient, high-moisture environments (Pfeifer-Meister et al. 2008). Pfeifer-Meister et al. (2008) found that although two exotic grasses produced the most biomass in all combinations with two native grasses, the native

grasses were not completely excluded from high-nutrient, moderate-moisture environments. The provision of a variety of moisture conditions in restored wet prairies through microtopography may therefore have implications for reducing competition between native and exotic species.

Microtopographic heterogeneity has important implications for the restoration of wet prairies in the Willamette Valley. By promoting different vegetative conditions through soil and hydrologic conditions, microtopography can help to promote greater plant diversity and cover in restored wetlands (Bruland & Richardson 2005; Moser et al. 2007). The reestablishment of microtopographic variation may also be an important restoration tool in reducing the impact of invasive species on restored wet prairies. The goal of the Institute for Applied Ecology (IAE), LCOG, and the City of Eugene in restoring the West Eugene Wetlands is to increase the diversity and cover of native wetland flora and fauna which, in turn, will help reduce competition from and invasion by exotic species. Wetlands that have lost their microtopographic heterogeneity provide a narrow range of habitat conditions for wetland species and therefore would be expected to have less plant biodiversity. To inform local restoration efforts, we examined the relationship between microtopographic variation and native species diversity in restored and remnant wet prairies in the Willamette Valley, including the role of microtopographic variation in protecting against exotic species invasion.

2. STUDY SITES

From April 6 to May 11, 2012, we collected data from six restored and remnant wet prairie sites in the West Eugene Wetlands: Speedway, Oxbow West, Vinci, Coyote Prairie, Dragonfly Bend and Willow Creek (Figure 1). The Bureau of Land Management owns Speedway, Vinci and Oxbow West, the City of Eugene owns Coyote Prairie and Dragonfly Bend, and the Nature Conservancy owns Willow Creek. The study sites were designated as high, moderate or low quality wet prairies by IAE (Figure 1). The designation was based on qualitative assessments of percent cover of tufted hair grass (*Deschampsia cespitosa*), because this native species prevents the invasion of exotic species.

Native bunch grasses dominated all sites, except for Speedway, which was dominated by annual grasses and contained a high number of invasive species, and Willow Creek, which was dominated by annual grasses and native forbs. The endangered Willamette daisy (*Erigeron decumbens*) occurs at Speedway, Vinci, and Oxbow West. Kincaid's lupine (*Lupinus sulphureus*), also an endangered species, occurs at Dragonfly Bend. We took particular care to avoid trampling these plants during data collection.

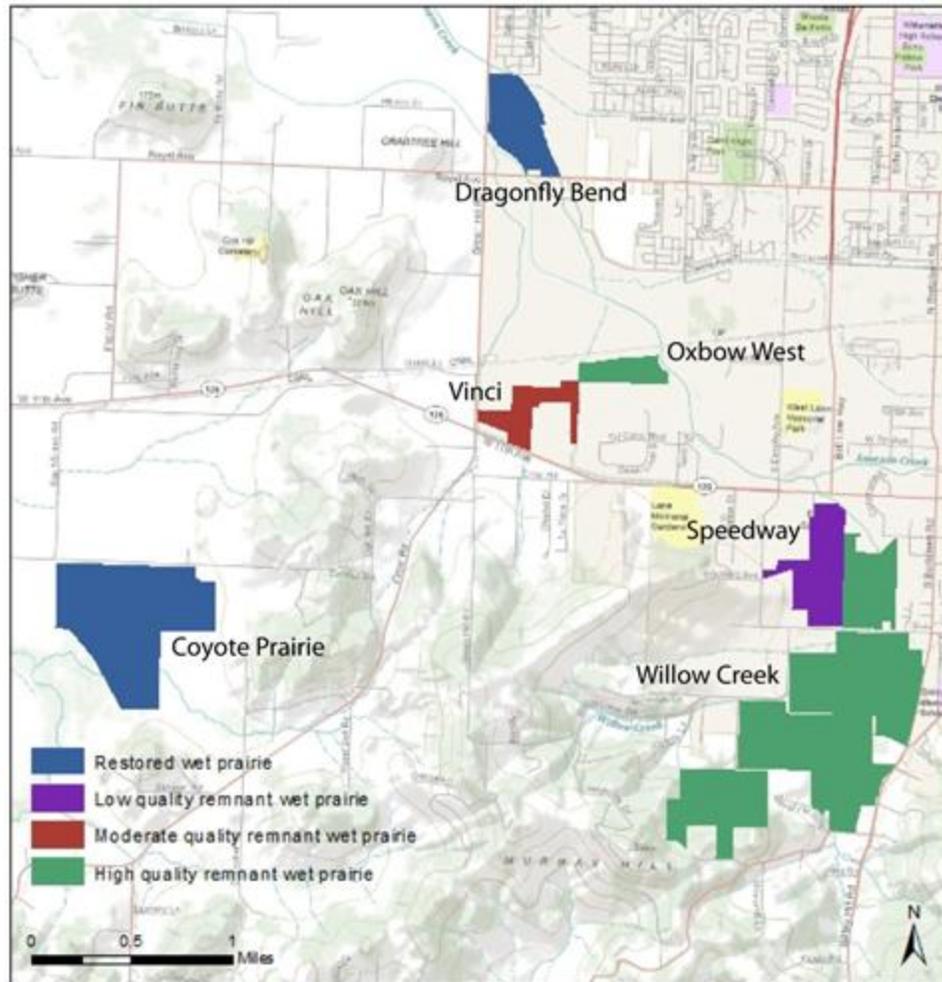


Figure 1. Location and classification of six microtopography research study sites in the West Eugene Wetlands, Eugene, Oregon.

3. METHODS

3.1. SAMPLING DESIGN

At each site, we located a point of origin within a pre-existing macroplot study area managed by IAE, LCOG, or the City of Eugene. From the point of origin, we established a baseline transect based on the site descriptions provided by IAE. Using the systematic sampling method, we set up transects perpendicular to the baseline transect, with the first transect randomly located and each subsequent transect following at an equal interval of 4-5 meters. Along each transect, the first sample point was randomly located and each following point was spaced at an equal interval of 4-5 meters for a total of 200 sample points per study site. At each sample point, we collected data for topography, water level, vegetation height, and litter (fallen dead vegetation) depth.

3.2. MICROTOPOGRAPHY & WATER DEPTH

In order to measure microtopography at each site, we used a surveyor's level (Harrelson et al. 1994). First, we set up the tripod, and secured and leveled the instrument to ensure consistency in our measurements. Next, we took a backsight reading to the point of origin, which had an arbitrary elevation of 100 meters. The height of the instrument (HI) was then calculated by adding the backsight reading to the standard elevation value of 100 meters. At each sample point along each transect, a foresight (FS) was recorded. The elevation of each sample point was calculated by subtracting the foresight from the height of the instrument ($HI - FS = ELEV$). At the approximate mid-point and end of the survey, we took another backsight to the benchmark to ensure the level had not moved or shifted significantly (Harrelson et al. 1994). In addition to measuring elevation, at each point we recorded the depth of standing water with the surveyor's rod to the nearest millimeter.

3.3. VEGETATION HEIGHT & LITTER

We used a meter stick to measure vegetation height and litter depth to the nearest millimeter. We did not manipulate plants or litter to ease the process of taking measurements. Upon resting the meter stick on top of the soil, any litter disturbed in the process was carefully replaced to its former condition. We waited for the vegetation to stop moving completely, and then recorded the height of the tallest vegetation in contact with the meter stick. In addition, we measured the depth of litter above the soil.

3.4. PLANT COMMUNITY COMPOSITION

Along each transect, we used a 1 meter x 1 meter quadrat frame to determine the percent cover of plant functional groups (grasses, rosette forbs, non-rosette forbs, and shrubs/trees). We placed a quadrat frame on the right side of the transect at a predetermined, random location along the transect. To estimate percent cover, we used the Daubenmire cover class method at intervals 0-5%, >5-25%, >25-50%, >50-75%, >75-95%, and >95-100% (NOAA 2005). For each quadrat, the total cover was equal to or greater than 100% because of the overlapping of different functional groups. To reduce observer bias, we worked in pairs while estimating percent cover. This process was repeated once or twice along each transect at randomly determined locations for a total of 20 plots per study site.

3.5. DATA ANALYSIS

We first calculated the Shannon's Diversity Index, Pielou's Evenness Index, and the topographic coefficient of variation for each site. The Shannon's Diversity Index (Equation 1) and Pielou's Evenness Index (Equation 2) were calculated using previously-collected percent cover data compiled by IAE.

$$\text{Equation 1 Shannon's Diversity Index: } H' = - \sum p_i \ln (p_i)$$

where p_i = proportion of individuals of species i

Equation 2. Pielou's Evenness Index: $J' = H'/H'_{\max}$

where $H_{\max} = \ln(S)$ and $S =$ number of species

We used the topographic coefficient of variation as a measure of site variability, which we calculated by dividing the standard deviation of the topographic elevation points by the total number of points.

The total species richness (number of species), native species richness, and introduced (non-native) species richness were calculated using data from IAE. From the data we collected in the field, we then calculated the arithmetic mean and standard deviation of the litter depth, vegetation height and water depth for each site. To assess the relationship between microtopography and species diversity, we conducted several linear regressions between native and introduced species richness, diversity and evenness versus the topographic coefficient of variation. Presence and absence data collected by IAE of obligate species (found in only uplands or lowlands) and facultative species (found in either uplands or lowlands) was compared to the coefficient of variation for each site to assess plant diversity. Finally, we assessed plant community composition qualitatively and compared plant functional groups across study sites.

4. RESULTS

4.1. SPECIES RICHNESS, EVENNESS AND DIVERSITY

Data provided by IAE showed that total plant species richness ranged from 59 species at Willow Creek to 25 species at Coyote Prairie. Willow Creek had the greatest native species richness, while Oxbow West had the lowest. Vinci had the greatest number of invasive species, while Coyote Prairie had the fewest (Figure 2).

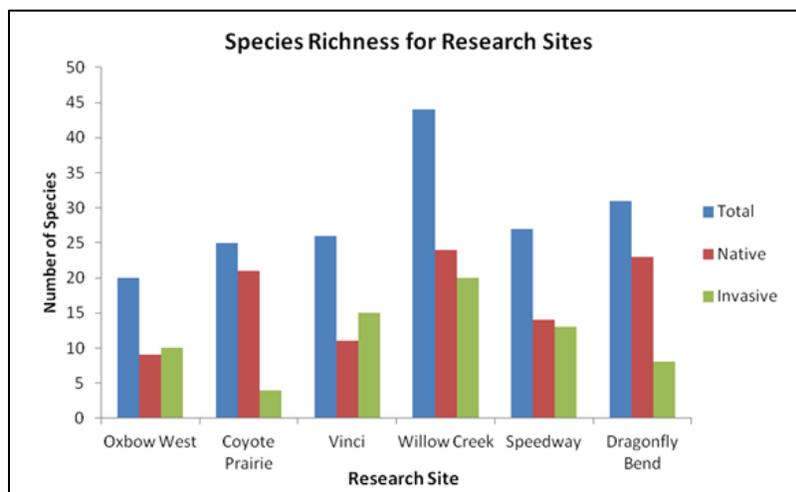


Figure 2. Total, native, and introduced plant species richness for all research sites.

Pielou's Evenness Indices ranged from a low of 0.44 for Willow Creek and a high of 0.92 for Coyote Prairie (Table 1).

Table 1. Diversity and Evenness Indices for Six Sites in the West Eugene Wetlands, Eugene, Oregon

Site	Shannon's Diversity Index	Pielou's Evenness Index
Oxbow West	1.98	0.66
Coyote Prairie	2.68	0.92
Vinci	2.13	0.65
Willow Creek	1.68	0.44
Speedway	2.20	0.67
Dragonfly Bend	3.17	0.83

Diversity indices ranged from 3.17 observed at Dragonfly Bend to 1.68 at Willow Creek (Table 1). All sites had high species diversity as measured by Shannon's Diversity Index. An index of 1.7 or higher is considered indicative of high diversity (Thorpe, personal communication, May 18, 2012). The two restored sites, Dragonfly Bend and Coyote Prairie, had the highest indices. Speedway and Vinci, the two low quality remnant prairie sites, had medium indices, whereas both high quality remnant prairie sites Oxbow West and Willow Creek had the lowest indices (Table 1).

4.2. MICROTOPOGRAPHY

The values of coefficient of variation ranged from 0.0003 to 0.0012 across sites (Table 2). Willow Creek has the highest coefficient of variation, while Oxbow West had the lowest.

Table 2. Coefficient of variation of topography for all research sites

Site	Coefficient of variation
Oxbow West	0.00030
Coyote Prairie	0.00110
Vinci	0.00056
Willow Creek	0.00120
Speedway	0.00058
Dragonfly Bend	0.00043

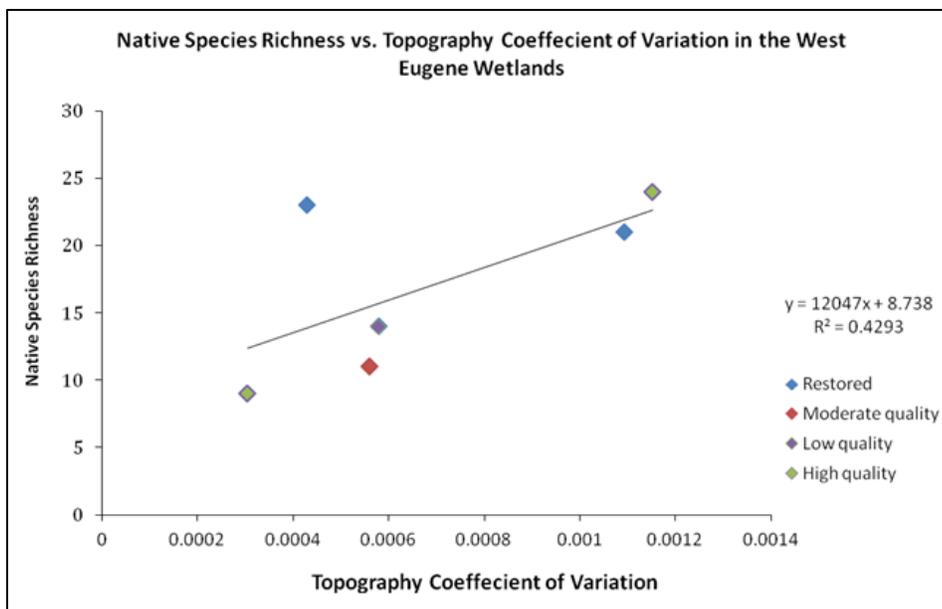


Figure 3. Topographic coefficient of variation as a function of native species richness for the six research sites.

No significant correlation was found between the coefficient of variation and Shannon’s Diversity Indices ($R^2 = 0.04$; p -value=0.7), Pielou’s Evenness Indices ($R^2 = 0.01$; p -value=0.8), total species richness ($R^2 = 0.03$; p -value=0.2) or introduced species richness ($R^2 = 0.03$; p -value=0.7).

4.3. HYDROLOGY AND VEGETATION

Mean water depth throughout the study sites ranged from 0.000 cm at Dragonfly Bend to 0.767 cm at Vinci (Table 3).

Table 3. Vegetation height, litter and water depth measured at six study sites in the West Eugene Wetlands, Eugene, Oregon

Site	Vegetation height (cm)	Litter depth (cm)	Water depth (cm)
Oxbow West	18.37 ± 10.29	0.618 ± 1.25	0.282 ± 0.686
Coyote Prairie	11.87 ± 12.24	0.509 ± 0.670	0.00830 ± 0.292
Vinci	13.30 ± 11.54	0.617 ± 1.34	0.767 ± 1.32
Willow Creek	13.13 ± 7.58	1.25 ± 1.74	13.13 ± 7.58
Speedway	16.66 ± 10.96	0.515 ± 1.00	0.207 ± 0.744
Dragonfly Bend	24.07 ± 15.35	0.186 ± 0.625	0.00 ± 0.00

The two restored wet prairies, Coyote Prairie (0.083 cm) and Dragonfly Bend (0.00 cm), had the lowest mean water depth. There was no significant relationship between mean water depth and mean vegetation height ($R^2 < 0.20$) or between mean water depth and mean litter depth ($R^2 < 0.01$).

The mean vegetation height ranged from 13.13 cm (Willow Creek) to 24.07 cm (Dragonfly Bend) with high standard deviations (Table 3). A linear regression comparing vegetation height to litter depth indicated that as vegetation height increased litter height decreased ($R^2 = 0.38$).

The plant community composition data revealed that bunchgrasses dominate the majority of the sites, with Oxbow West and Dragonfly Bend exhibiting especially high percent cover of bunchgrass (Table 4).

Table 4. Plant Community Composition Data

Site	Grasses			Rosette Forbs			Non-Rosette Forbs		
	Rhiz. & stolon.	Bunchgrasses	Annual/single blade	<15 cm	15-30 cm	>30 cm	<15 cm	15-30 cm	>30 cm
Oxbow West	1	3	1	1	1	1	1	1	1
Coyote Prairie	1	2	1	1	1	1	1	1	1
Vinci	1	2	1	2	1	1	1	1	1
Willow Creek	1	2	1	1	1	1	1	1	1
Speedway	2	1	3	2	1	1	2	1	1
Dragonfly Bend	1	3	2	1	1	1	1	1	1

1=0-5%; 2= >5-25%; 3= >25-50%; 4= >50-75%; 5= >75-95%; 6= >95-100%

Speedway was dominated by annual/single blade grasses and also had the highest percent cover of rhizomatous grasses, rosette and non-rosette forbs, shrubs and trees and litter cover. The percent cover of rhizomatous grasses and forbs greater than 15 cm was generally low throughout the six study sites.

4.4. FACULTATIVE AND OBLIGATE PLANT SPECIES

Willow Creek had the greatest richness of facultative, facultative upland, obligate wetland and obligate upland species. Oxbow West and Dragonfly Bend also had a high species richness of facultative, facultative wetland and facultative upland categories. Speedway and Coyote Prairie have low species richness of facultative, facultative wetland, obligate wetland and obligate upland categories (Table 5).

Table 5. Facultative and obligate presence/absence data across all sites West Eugene Wetlands

	Oxbow West	Vinci	Speedway	Dragonfly Bend	Coyote Prairie	Willow Creek
Total FAC	7	7	5	6	4	12
Total FACU	14	13	13	5	5	17
Total FACW	7	9	6	10	7	9
Total OBL	2	1	1	3	1	3
Total UPL	2	4	2	4	2	4
Total NL	2	3	0	3	4	11
Total	34	37	27	31	23	56

FAC=Facultative; FACU=Facultative upland; FACW=Facultative wetland; OBL=Obligate wetland; UPL=Obligate upland; NL=Not listed.

5. DISCUSSION

5.1. SPECIES DIVERSITY

We observed that Willow Creek, one of the high-quality remnant sites, had the highest species richness, as well as highest introduced and native species richness. These observations can be explained by the management practices of prescribed burning in return intervals of two to five years, as well as mowing and hand-pulling of exotic species. This produces an environment in which introduced species are not competitively dominant and do not outcompete the native species observed at the sites, allowing natives to dominate. Generally, we observed high native species richness in high-quality and moderate-quality sites. Invasive species richness varied across all sites and was likely dependent on the management practices of each site.

Willow Creek and Oxbow West's low evenness index can be explained by the observation that these sites are dominated by a few native plant species. Both sites are high-quality remnant prairies actively managed using techniques such as prescribed fire. In this context, low evenness indicates high percent cover of native species. The other four sites had high evenness indices indicating that no single species dominated. However, these sites varied in quality, so evenness could not be used as an indicator of native species cover.

Shannon's Diversity Index incorporates both species richness and evenness, and therefore provides a more complete indicator of diversity. Willow Creek's lower diversity index supports the observation that a few native plant species dominate this site. Diversity indices of over 1.7 for each site indicate that all sites have a high number of species and are not dominated by a few common species. The high SDI indicates that there is a high number of native species, and no species, either native or introduced, is competitively dominant at each site. The high SDI for the two restored sites, Coyote Prairie and Dragonfly Bend, demonstrates that these sites have a high nativeness due to their high diversity index and their active management for the removal of introduced species.

5.2. MICROTOPOGRAPHY

The relationship between native species richness and the coefficient of variation was not statistically significant ($R^2=0.43$; $p\text{-value}=0.16$) and therefore did not support our hypothesis that native plants diversity in wetland prairies increases with topographic variation. Although not statistically significant, there was a positive relationship that explained 42.9% of the observed variation. We believe our results, in combination with patterns in the data, indicate that there is a relationship between these variables. When we computed the regression without data for restored sites the results were statistically significant. This is important to recognize for management and future research because the history and management of restored sites is so different from remnant sites.

We recommend further research to assess the hypothesis that microtopography is positively correlated with site diversity and evenness indices. For example, an extended study that includes a larger sample size for microtopography and percent cover data may produce more significant data than observed during a short pilot study. Within the scope of this study, due to high variability among sites, detecting relationships was difficult. In future studies we would recommend a study design that reduces unnecessary variability between study sites by comparing sites with similar history and management.

5.3. HYDROLOGY

Seasonality explains the lack of precision of the water depth data, as indicated by the large range and standard deviations of the water depth data. Precipitation and site hydrology varied greatly throughout our study as we collected data over a six-week period. This effect was likely produced by high variation due to seasonal changes in precipitation and site hydrology, confounding a relationship between water depth and species diversity. In order to collect more comparable data, water depth data should be collected during a shorter period of time when all sites are subject to the same weather conditions and precipitation.

5.4. VEGETATION

The observed relationship between plant height and litter depth did not correlate with topographic variation. Our plant community composition data indicated that bunchgrasses

dominated most sites. High-quality sites were dominated by bunchgrasses and forbs. Low-quality sites had a high percent cover of introduced plants such as rhizomatous grasses and rosette forbs. This observation is not surprising considering the habitat quality designations are partly based on the level of introduced species.

5.5. FACULTATIVE AND OBLIGATE SPECIES

The observed relationship between plant height and litter depth did not correlate with topographic variation. Our plant community composition data indicated that bunchgrasses dominated most sites. High-quality sites were dominated by bunchgrasses and forbs. Low-quality sites had a high percent cover of introduced plants such as rhizomatous grasses and rosette forbs. This observation is not surprising considering the habitat quality designations are partly based on the level of introduced species.

6. CONCLUSION

Although not statistically significant, we believe that our results suggest that the presence of microtopography in remnant and restored wetlands plays an important role in the presence of native plant species. Research sites with high variation in microtopography appear to host more native and specialized plant species, suggesting a wider range of niches available for species. The variation in microtopography provides hummocks and hollows that define particular niches that support native species colonization and prevent colonization by invasive species (Bruland & Richardson 2005).

Wet prairie restoration continues to be a priority for restoration ecologists in the Willamette Valley because of the rarity of these habitats. The methods used in restoration projects must therefore be the most effective in restoring native species cover and diversity. While our pilot study suggests that there is a relationship between variation in microtopography and increased native plant richness, we recommend further research to assess relationships between microtopography and plant community composition in sites with similar histories and management practices. If this relationship is confirmed, we recommend that wet prairie management protocol include the restoration of microtopography to increase the cover and diversity of native plant species. The presence of introduced species at low quality sites could possibly be reduced through practices that include the restoration of microtopography. These methods will increase the colonization of native plants by decreasing competition with introduced species that prefer a more homogeneous flat terrain.

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The Potential Role of Dietary Intake in Explaining Postoperative Muscle Loss in Total Knee Arthroplasty (TKA)

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ABSTRACT

Post-operative muscle loss has been identified as the greatest contributor to osteoarthritis patients' long-term strength deficits, explaining 77% of muscle weakness one to three years after total knee arthroplasty (TKA) for the treatment of osteoarthritis in the knee. Essential amino acid supplementation may help reduce atrophy, but other factors could have an equally influential role. Patients' food intake could likewise affect atrophy, and thus dietary intake must be measured in order to determine whether essential amino acids reduce muscle loss following TKA. Subjects with a minimal dietary intake could exacerbate muscle loss because fasting causes the body to break down skeletal muscle protein to liberate amino acids for use as gluconeogenic precursors in the liver. As a result, individuals with different diets may experience differing degrees of muscle atrophy. This investigation seeks to test the hypothesis that higher rates of muscle atrophy in subject groups could be explained by comparatively poorer dietary intake. For this study, subjects completed a total of three 72-hour food logs before surgery, then two, and six weeks post-TKA. During these periods, bilateral quadriceps muscle volume was determined using magnetic resonance imaging (MRI). Baseline and six-week whole-body dual-energy X-ray (DEXA) scans were also performed to compare changes in lean tissue and fat mass. Data collected indicated there was no significant difference in dietary intake between the control group and treatment group, suggesting that any muscle saved in the treatment group was a result of essential amino acid supplementation.

As the average age of our population increases, we are faced with new problems and pathologies to combat in our search for a higher quality of life. The current average lifespan (including both men and women) is almost 78 years of age; however, evidence suggests that the average lifespan is increasing by almost half of a year annually (Arias, 2007). While such a feat should be celebrated as a marvel of modern health advancements, this increased life expectancy, in combination with the aging "baby boomers" generation, will cause significant growth in the elderly population. Current predictions suggest that by the year 2050, the population of individuals over the age of 65 will more than double from 40 million to 88 million (Johnson,

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2010). Healthcare facilities must be prepared to support this surge and develop techniques to efficiently manage pathologies common to the elderly population.

Osteoarthritis (OA) of the knee involves gradual wear and tear of the articular cartilage in the joint of the knee, which allows for bone on bone rubbing. Osteoarthritis is currently the most prevalent chronic disease in the United States, affecting 60% of Americans over the age of 65, especially women (Parsley et al., 2010). Total knee arthroplasty (TKA) is the most common surgical treatment for osteoarthritis, and due to the prevalence of the disease, it is estimated that by the year 2030, 3.48 million TKAs will be conducted annually in the United States (Kurtz, 2007), with two-thirds of TKAs predicted to be performed on elderly women. Although the surgery has shown success in alleviating chronic knee pain, TKA has not been successful in completely restoring full physical function in women (MacDonald, 2008; Parsley et al., 2010). This inability to recover physical function has been labeled as the “never catch up syndrome” (Lavernia, 2009), the result of quadriceps weakness which is much more pronounced among women; women produce around 40-50% less force than men when tested two years following surgery (Silva, 2003).

Muscle atrophy is the greatest contributor to long-term quadriceps weakness, which can explain 77% of strength deficits one to three years following TKA (Meier, 2009). An interesting aspect of TKA is that muscle atrophy presents in both the operative and the non-operative quadriceps muscle groups. This factor should be considered when developing therapies to prevent muscle loss, because weakness in the non-operative leg is associated with the measured decrease in physical function one to two years following surgery (Zeni, 2010). Furthermore, for the older women that make up the majority of patients, significant muscle loss is especially detrimental because these patients are limited in their ability to increase muscle mass (Bamman, 2003). As a result, it is imperative that therapies are designed to prevent bilateral muscle atrophy following TKA in order to improve long-term functional health.

There has been a growing interest in essential amino acids (EAA's) in relation to muscle protein turnover. Of the 20 total amino acids, ten have been labeled as essential while the remaining 10 are considered non-essential amino acids. An essential amino acid is simply one that the body cannot create on its own and therefore must be obtained by dietary means.

Previous studies have shown support for the hypothesis that EAA supplementation can result in increased muscle protein synthesis, stimulating muscle growth. One such study demonstrated that EAA supplementation after a single bout of exercise led to an increase in muscle protein synthesis when compared to synthesis from the exercise alone (Dreyer, 2008). Previous studies have also shown that as we age, we begin to obtain higher levels of anabolic resistance (resistance to normal muscle growth) to amino acids at low dosages—thus decreasing the stimulatory effect of dietary intake on muscle protein synthesis (Johnson, 2010). Because of this increase in anabolic resistance, many elderly people can remain in a catabolic state even while ingesting the daily-recommended amount of calories and protein. But if the daily-recommended consumption is inadequate, perhaps the solution would be to simply increase patients' essential amino acid intake above previously recommended standards. This dietary

change could then negate the anabolic resistance and eventually lead to a net anabolic state of muscle growth.

These results may have implications that could slow the rate of muscle atrophy in older populations, but EAA supplementation should not be the only focus in this area of research. Instead, the patient's entire diet should be taken into consideration. The amount of fat, carbohydrates, and protein consumed could all have potential effects on the rate of muscle atrophy, with or without EAA supplementation. The following review discusses insufficient dietary intake's potential to accelerate muscle loss due to decreases in blood glucose levels.

BIOLOGICAL REVIEW

GLUCOSE

Glucose is a six-carbon simple sugar that is the end product of carbohydrate digestion (Hall, 2011). Carbohydrate digestion begins as soon as food enters the mouth, when enzymes present in saliva begin to break down complex carbohydrate sugars known as polysaccharides. The food then proceeds through the stomach and on to the small intestine, where any remaining polysaccharides are completely broken down into the monosaccharide glucose (Freeman, 2008). Once carbohydrates are reduced to this form, glucose is absorbed through the small intestine into the bloodstream, where it is circulated throughout the body to be utilized as a substrate in the creation of the body's ubiquitous form of energy: adenosine triphosphate (ATP).

GLUCOSE UTILIZATION IN AEROBIC RESPIRATION

Whether it is the stomach, skeletal muscles, or any other organ in the body, cells require a consistent inflow of glucose to function. The manner in which cells utilize glucose follows a four-stage mechanism that includes glycolysis, pyruvate decarboxylation, the TCA cycle, and oxidative phosphorylation (Freeman, 2008). These processes take place sequentially, and while all four of these processes are important in the overall creation of ATP and glucose utilization, one of the foci of this paper is the TCA cycle and how it may be affected by differing levels of food intake.

GLUCOSE REGULATION

The mechanism of particular interest to this investigation is glucose control and regulation. As stated, the elderly population often experiences difficulty in maintaining a healthy level of food intake. If food intake is low, blood glucose levels will begin to decrease as well. An individual is generally said to be euglycemic when his or her resting blood glucose concentration is within 90 to 108 milligrams glucose per deciliter of blood (mg/dL). If this level rises above 126mg/dL, a person is said to be hyperglycemic; conversely, if this level drops below 70mg/dL, a person is said to be hypoglycemic and may develop symptoms accordingly (Houston, 2006). While these values represent only the resting blood glucose level, the concentration can be augmented or attenuated depending on the state of the individual. Some of the factors that can

influence the blood glucose level include continual fasting, the ingestion of a meal, or exercise. This study is primarily focused on the effects of low dietary intake on future muscle atrophy; therefore, the most relevant factor is the health effects of continual fasting, in which a subject does not ingest adequate amounts of food for extended periods of time.

If an individual is continually unable to maintain an adequate dietary intake, the body will recognize the subsequent low blood glucose level and will begin to defend the normal level by activating several mechanisms. This can include breaking down stores of excess glucose in a process called glycogenolysis, burning excess fat in the form of triglycerides in a process known as lipolysis, or finally, proteolysis, by which muscle protein is broken down into amino acids to be used to generate necessary glucose (Brooks, 2005). While the focus of muscle synthesis is often placed on protein intake, it is apparent that if caloric intake is not maintained, muscle can still deteriorate via amino acid metabolism in order to defend blood glucose levels, even with adequate amounts of protein.

AMINO ACID METABOLISM

Amino acids are the foundation and building blocks of proteins in the body; a single synthesized protein may contain hundreds of amino acids in its structure, and the largest storehouse of proteins in the body is found in the skeletal musculature. Because skeletal muscle is comprised of so many of the body's amino acids, it follows that amino acid metabolism would lead to atrophy of the skeletal muscles.

Like stored glycogen and triglycerides, amino acids can also be used to generate glucose during times of need, such as fasting. Indeed, metabolism of amino acids occurs every morning because the body is in a natural catabolic state after not receiving any form of sustenance since dinner the night before (Brooks, 2005). This catabolic state will continue until the individual ingests his or her first meal of the day—thus providing the body with sufficient glucose to cease amino acid metabolism and muscle atrophy.

Amino acids can be extracted from muscle tissue and carried in the blood to the liver as a precursor to gluconeogenesis (Ruderman, 1975). This process, known as the glucose-alanine cycle, is capable of providing the 130 grams of glucose per day needed to fulfill the minimum glucose requirements of the brain, nerves, and kidneys (Brooks, 2005). This feat is triggered during fasting, when various amino acids in skeletal muscle are subjected to a reaction with α -ketoglutarate—the keto acid of glutamate—in a process catalyzed by an amino acid transaminase (Brooks, 2005). When the reaction is complete, the original amino acid loses its amine group, thereby reducing it to its keto acid form, while α -ketoglutarate accepts the amine group to form glutamate. The newly produced glutamate then experiences another round of transamination with the high levels of pyruvate located in the skeletal muscle. Glutamate loses its amine group to become α -ketoglutarate, while pyruvate accepts the amine group to form the amino acid alanine (Brooks, 2005). This mechanism allows many different amino acids to be transformed into alanine, one of the few amino acids capable of being mobilized from skeletal muscle and released into the bloodstream.

Once alanine reaches the mitochondrial matrix of the liver, it undergoes one of three processes: another round of transamination, oxidative deamination, or gluconeogenesis. Transamination will occur to reform pyruvate—the keto acid of alanine, but perhaps more importantly, pyruvate is a gluconeogenic precursor. If, however, alanine experiences oxidative deamination, the amine group will be removed, and the leftover carbon skeletal of alanine can be utilized in aerobic respiration.

In order to utilize amino acids in the formation of glucose, muscle protein must be broken down, resulting in increased muscle atrophy. But if daily caloric and protein requirements are met, it would seem that there would be enough glucose already present to avoid breaking down large stores of muscle protein. This will be of particular interest to this research, which tests whether muscle atrophy can be reduced after surgery via adequate dietary intake and amino acid supplementation. Non-fasting individuals will show increased levels of insulin, which has the ability to decrease rates of muscle protein catabolism; therefore, a patient with satisfactory dietary intake should be expected to experience decreased rates of muscle atrophy following surgery (Saltiel, 2001).

RELATION TO TCA CYCLE

Although alanine is the primary amino acid extracted from skeletal muscle, it does not represent the overall composition of the amino acids present in the skeletal muscle. This means that there is some form of local metabolism occurring within the muscle that is responsible for the interconversion of amino acids to alanine as described by the glucose-alanine cycle discussed in the previous section (Owen, 2002). Upon completion of transamination, there are remaining carbon skeletons that can produce one of five products—oxaloacetate, α -ketoglutarate, fumarate, succinyl CoA, and pyruvate—depending on the original amino acid being metabolized. Pyruvate can readily be shuttled to the liver as a precursor to gluconeogenesis; however, the first four products of transamination are TCA cycle intermediates necessary for the cycle to function.

The TCA cycle is the third step in the creation of ATP following pyruvate decarboxylation and before oxidative phosphorylation. This process is composed of eight steps, each using a distinct enzyme or intermediate to carry out reactions necessary to propagate the cycle (Hall, 2011). The steps most relevant to this research are the induction of acetyl CoA—the end product of pyruvate decarboxylation—to the cycle and the use of the intermediates that are provided during amino acid metabolism. During times of fasting, TCA intermediates are removed from the TCA cycle and used in the liver to create glucose via gluconeogenesis. As the various intermediates are used and taken out of the cycle, the reactions ongoing in the TCA cycle cannot be completed; this leads to a buildup of acetyl-CoA that is unable to enter the cycle. Such buildup stresses the system and can lead to further muscle loss.

AMINO ACID SUPPLEMENTATION

It will be of interest to observe the effects of the amino acid supplementation on the rate of muscle atrophy. Essential amino acids have been shown to increase muscle synthesis, and therefore, should be able to slow down muscle atrophy by offsetting muscle loss due to fasting. Conversely, a nonessential amino acid supplement could also decrease muscle atrophy because, instead of skeletal muscle breaking down in order to supply alanine to the liver, the supplement would take its place. An increase in nonessential amino acids should therefore decrease demand for the breakdown of muscle protein. With this in mind, both patients receiving nonessential amino acid supplementation and patients receiving essential amino acid supplementation should be expected to show decreased muscle atrophy following surgery as compared to an untreated patient. It is only the degree to which each supplement attenuates muscle atrophy that is left to be determined.

INTRODUCTION

As discussed, TKA surgery is becoming more and more prevalent, and by the year 2030, it is estimated that 3.48 million surgeries will be conducted every year (Kurtz, 2007). Thus it is imperative to find methods to decrease the associated muscle atrophy following surgery. Recent studies have shown that the quadriceps muscle group may atrophy more than 10% following surgery (Meier, 2008). Under normal muscle atrophy rates, an elderly individual will lose 10% muscle mass in approximately two decades; therefore, the equivalent of two decades of muscle mass may be lost following a single surgical procedure. Because the majority of patients receiving total knee surgery are both overweight and elderly, it is often the case that this muscle will never be regained. Consequently, the focus must be on preventing atrophy from occurring at all following surgery, since it cannot be corrected easily.

As noted, essential amino acid supplementation is currently being studied as a possible preventative measure for muscle atrophy following TKA surgery. Essential amino acids, leucine in particular, are anabolic and stimulate muscle protein synthesis. Conversely, non-essential amino acids are not anabolic, thus providing the rationale for supplemental groups. However, dietary intake by each subject within the study is a key component that may influence the eventual total muscle loss regardless of whether the subject receives essential or nonessential amino acids. Essential amino acid supplementation has been shown to activate anabolic pathways for muscle synthesis, but it might not have a large impact if the subject is not ingesting an adequate amount of calories, because muscle protein will be broken down via proteolysis to supply a substrate for the synthesis of glucose. Therefore, to optimally reduce the rate of muscle atrophy following surgery, the subject should ingest adequate amounts of both calories and protein.

One problem that arises in this study is the investigators' inability to dictate the subjects' diets during testing, which could impact the magnitude of muscle atrophy. All subjects were allowed to eat as they chose during the testing period as long as they recorded it in the food logs during the selected recording times. As a result, it is reasonably plausible that there will be no

significant differences in dietary intake found between two groups of subjects. This study, however, is not intended to be taken as exhaustive by any means. Instead, if there are no significant differences found in dietary intake, any differences in muscle atrophy rates between groups following surgery could be attributed to whether or not the subject was ingesting essential amino acids or nonessential amino acids. Such a conclusion would then have future implications for the implementation of essential amino acids in the surgical protocol for TKA.

The general hypotheses for this study is that there will be a significant difference in dietary intake that will partially explain differences in muscle atrophy following surgery between a placebo group with a nonessential amino acid supplementation (non-anabolic) and a treatment group with an essential amino acid supplementation (anabolic). Furthermore, a secondary hypothesis is that either form of supplementation will allow for the majority of subjects to reach the recommended protein intake of at least 0.8g/kg/day with a caloric intake of at least 2000 calories per day.

Subjects were tested according to the following specific hypothesis: Lean muscle mass in both the TKA leg and the untreated leg for subjects who meet the nutritional standards above will remain closer to preoperative muscle mass after surgery. This specific hypothesis will be tested with the implementation of the following measure: The total mass, lean muscle mass, and fat mass of both the TKA leg and the untreated leg will be measured before and after surgery and at specifically selected intervals.

METHODS

For this study, 18 subjects (12 women and six men) were selected in the range of 60-80 years of age, who were all relatively healthy older men and women, and who were scheduled to have elective total knee replacement surgery with orthopedic surgeons at the Slocum Center for Orthopedics and Sports Medicine in Eugene, Oregon. Subjects were excluded from the study if they matched any of the following exclusion criteria:

1. Dementia or related mental issues that could potentially put subject at risk as determined by the surgeon.
2. Previous TKA and/or THA surgery.
3. Untreated endocrine disease.
4. Significant heart, liver, kidney, blood, or respiratory disease.
5. Peripheral vascular disease.
6. Active cancer.
7. Recent (within six months) treatment with anabolic steroids or corticosteroids.
8. Alcohol or drug abuse.
9. Inability to have MRI.

Once selected, the subjects were placed in either an experimental group or a control group using a double blind methodology. The experimental group received 20g of mixed essential amino acids twice daily for a period of seven days before surgery and 14 days after surgery. The

control group received 20g non-essential amino acids in the form of alanine twice daily given at the same time intervals. The first 20g for both groups of subjects was taken at 1000 a.m., and the second was taken at 2:00 p.m.

Subjects were also required to fill out three 72-hour food logs - one seven days before surgery, one 14 days after surgery, and once 42 days after surgery. These logs accounted for all food eaten within the 24 hour period, and specific notes were taken describing what was consumed with the supplement. The amount of fat, carbohydrate, and protein consumed by each individual was calculated from these logs.

All subjects in the study received an MRI preoperatively and again at two and six weeks following total knee replacement surgery. Images for the MRI were taken from the Anterior Superior Iliac Spine (ASIS) to the tibial plateau. Total muscle volume of the quadriceps muscle group as well as total fat, subcutaneous fat, and intermuscular fat were determined from these images.

Additionally, subjects were required to receive two DEXA scans—one seven days before surgery and one six weeks following surgery. These scans were used to find the body composition (fat, lean, and total tissue volume) at each point and to calculate percent change between the two.

Separate analysis of covariance (ANCOVA) were conducted to test for the effects of the treatment for four primary outcomes: six-week TKA leg lean mass, six-week control leg lean mass, six-week TKA leg mid-section five-slice value, and six-week control leg mid-section five-slice value. This test compares measured variables to determine whether they change significantly during the course of the study. Subject characteristics that were found in univariate correlations to be significantly related to outcomes were included as covariates in all models: gender, age, body mass index, tourniquet time; and baseline values of dietary fat intake, carbohydrate intake, and protein intake. Baseline values for each outcome were also included as a covariate for analysis. The effect sizes (Cohen's d) were found using covariate-adjusted values from the ANCOVAs at each time point ($d = \text{absolute value} [(mean_1 - mean_2) / \text{pooled standard deviation}]$). Models were conducted using data from all of the subjects (N=18), and then again using data from only females (N=12) in order to generate statistics for future research on female-only elderly populations. The procedure for analyzing the secondary outcomes was the same as the one used for the primary outcomes.

RESULTS

Table 1. Baseline values

	Placebo Group Mean±SE (N=9)	Treatment Group Mean±SE (N=9)	Sig ¹
Subject Characteristics			
# Females	6 (66.7%)	6 (66.7%)	1
Age	70.4±1.6	69.3±1.6	0.63
Body Mass Index	28.8±0.8	33.8±1.8	0.02
Tourniquet Time (min)	43.6±4.9	44.0±1.5	0.39
Fat Intake	48.7±5.3	60.2±4.6	0.12
Carbohydrate Intake	169.7±13.5	180.0±18.8	0.66
Protein Intake	63.2±10.6	73.2±3.4	0.38
Calorie Intake	1,358.4±113.3	1,573.0±102.3	0.18
Baseline Values			
TKA leg tissue (g)	13,328±863.7	15,803.3±1,053.8	0.1
TKA leg lean mass (g)	7,938.3±909.0	8,614±658.1	0.55
TKA leg fat (g)	5,390.0±547.8	7,187.9±853.1	0.11
Control leg tissue (g)	13,315±978.9	15,653.8±1,184.2	0.16
Control leg lean mass (g)	7,954.3±990.6	8,485.1±600.2	0.65

Significant values from chi-squared or t test, as appropriate. Observed (unadjusted) values are presented.

Preliminary data were collected for each subject within the placebo group and the EAA group. Baseline values were recorded and the significance value between groups was found. The only significant difference between the groups was Body Mass Index (BMI) but both groups had a BMI high enough to indicate the subjects were overweight. None of the other baseline values were significantly different, suggesting that all subjects as a whole were similar, and any deviations in baseline numbers could not be used to suggest they had influence on significant differences in muscle atrophy following surgery.

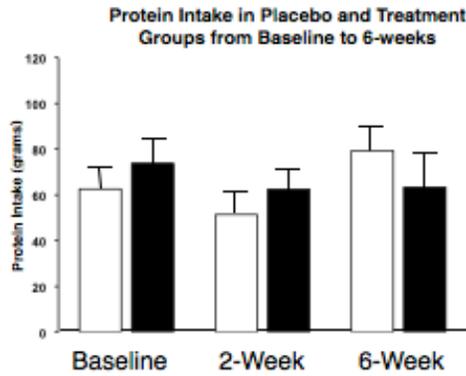
Table 2. Dietary intake

	Control Group Mean±SE	Treatment Group Mean±SE	Sig1
Fat Intake			
Baseline (g)	47.5±5.3	61.4±5.3	0.12
2 Weeks (g)	33.5±5.8	51.6±5.8	0.08
6 Weeks (g)	59.0±7.0	52.0±7.0	0.55
Carbohydrate Intake			
Baseline (g)	187.9±17.7	161.8±17.7	0.37
2 Weeks (g)	146.7±22.7	165.3±25.8	0.62
6 Weeks (g)	202.3±25.8	166.7±25.8	0.41
Protein Intake			
Baseline	62.6±9.7	73.8±9.7	0.48
2 Weeks	51.4±6.0	62.5±6.0	0.26
6 Weeks	79.4±6.8	63.4±6.7	0.16
Protein Intake g/kg body mass			
Baseline	.743±0.109	.910±0.109	0.35
2 Weeks	.628±0.070	.742±0.070	0.33
6 Weeks	.993±0.080	.785±0.080	0.13
Calorie Intake			
Baseline	1,436.2±129.2	1,495.2±129.2	0.78
2 Weeks	1,086.1±127.3	1,376.1±127.3	0.18
6 Weeks	1,625.6±180.0	1,527.7±180.0	0.74

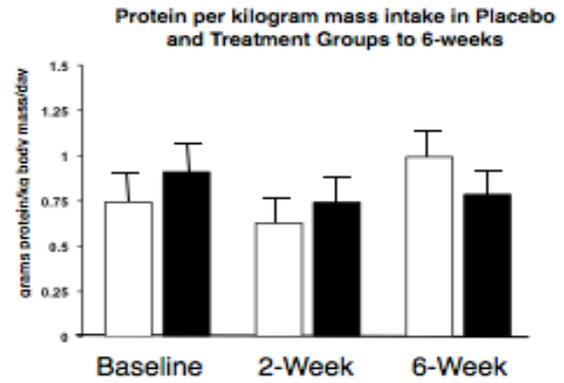
Separate ANCOVA models specified for each time point. Covariates in all models were age, sex, BMI, and tourniquet time. Also covaried in two-week and six-week models were baselines scores of the outcomes. Covariate-adjusted values are presented in the table.

Each subject completed food logs for all of his or her dietary intake, and the values for fat, carbohydrates, protein, protein per kilogram mass, and calorie intake at each interval were calculated. When each value between the placebo and EAA group were compared, all values at each time interval were found to be non-significant.

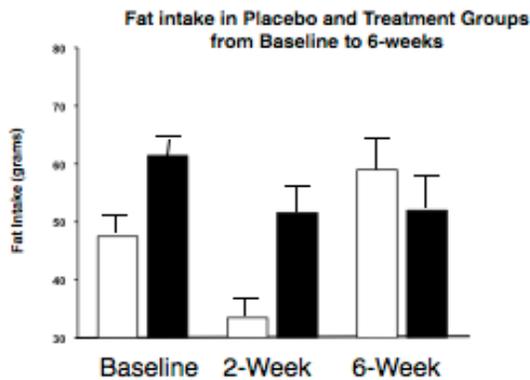
□ Placebo ■ Treatment



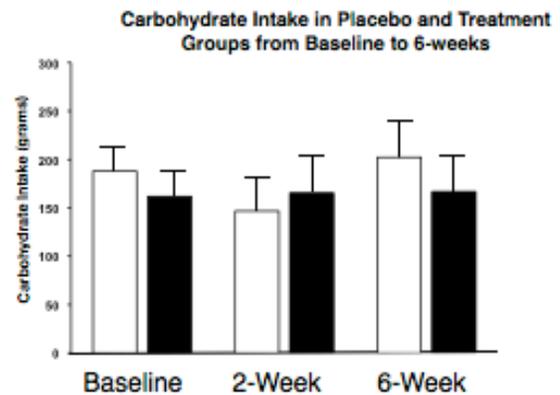
Graph 1: Shows the fluctuations of protein intake throughout the trial for both groups. Differences between the two groups at each time interval were non-significant.



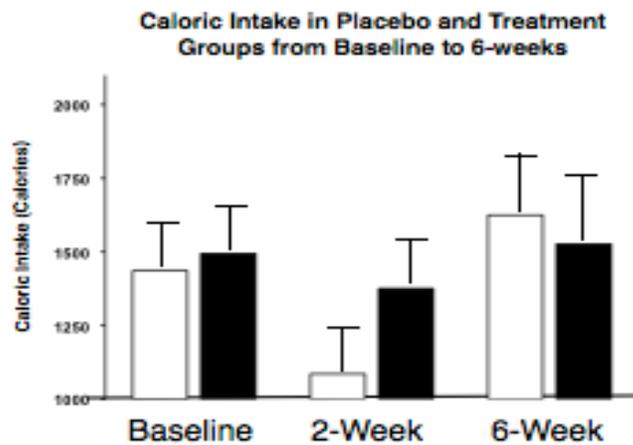
Graph 2: Shows the fluctuations of protein per kilogram mass intake throughout the trial for both groups. Differences between the two groups at each time interval were non-significant.



Graph 3: Shows the fluctuations of fat intake throughout the trial for both groups. Differences between the two groups at each time interval were non-significant. Despite a large difference in averages at two weeks post-surgery, large standard error led to differences that were non-significant.



Graph 4: Shows the fluctuation of carbohydrate intake throughout the trial for both groups. Differences between the two groups at each time interval were non-significant.



Graph 5: Shows the fluctuations of calorie intake throughout the trial for both groups. Differences between the two groups at each time interval were non-significant.

Table 3. Mean percent change between placebo and treatment group between baseline two weeks.

	Placebo Group (%)	Treatment Group (%)
Fat intake	-26.8*	-7.06
Carbohydrate Intake	-18.2	7.62
Protein Intake	-29.0**	-5.22
Protein/kg mass/day	-27.1**	-4.97
Calorie Intake	-27.4**	-4.32

Shows mean percent change within groups between baseline and two week values.

* indicates a trending value for the placebo group in fat intake.

** indicates significant differences in placebo group for protein intake, protein/kg mass/day, and caloric intake.

The mean percent change was used to find the average difference between the two-week values and the baseline values for each nutritional value that was measured. A negative value demonstrates that value decreased two weeks after surgery within the group. The majority of the percent change values show declines in each nutritional value, except for carbohydrate intake within the EAA group. Significant decreases were found within the placebo group from baseline to two-week values in protein intake, protein per kilogram mass intake, and calorie intake. There also was a trending value found in the fat intake for the placebo group from baseline to the two-week value.

Table 4. Quadriceps data before and after surgery

Outcome Variable	Baseline Mean±SE	2w followup Mean±SE	6w followup mean±SE	Effect Size of 2w	Effect Size of 6w
TKA leg lean mass (g) (n=15)					
Control Group	8,358.7±617.6	NA	6,811.4±192.1	NA	1.74
Treatment group	8,246.3±558.1	NA	7,681.0±173.6		
Control leg lean mass (g) (n=15)					
Control Group	8,915.2±633.8	NA	7,072.5±99.8	NA	3
Treatment Group	7,644.3±572.8	NA	7,848.3±89.7		
TKA leg mid-section 5-slice (g) (n=17)					
Control Group	127.3±9.1	108.6±7.3	105.8±4.7	0.46	0.69
Treatment Group	127.7±9.9	118.7±7.9	115.6±5.1		
Control leg mid-section 5-slice (g) (n=17)					
Control Group	150.6±10.0	133.2±5.2	133.6±3.9	0.31	0.51
Treatment Group	142.2±10.9	138±5.7	139.6±4.2		

Column 2 and column 3 values were adjusted for gender, tourniquet time, age, body mass index, fat intake, carbohydrate intake, protein intake, and baseline values of the outcome variable. Column 1 values were adjusted for gender, age, body mass index, tourniquet time, fat intake, carbohydrate intake, and protein intake. Effect sizes (Cohen's d) were calculated on adjusted values at each time point: $d = \text{absolute value} [(\text{mean}_1 - \text{mean}_2) / (\text{pooled standard deviation})]$. Analysis of covariance resulted in a statistically significant treatment effect on covariate-adjusted TKA leg lean mass at 6 weeks: $F(1)=7.45$; $p=0.041$. Analysis of covariance resulted in a statistically significant treatment effect on covariate-adjusted control leg lean mass at 6 weeks: $F(1)=21.03$; $p=0.006$.

Whole leg lean mass was taken for both the control and TKA leg for all subjects as well as muscle mass within a small, five-slice section of the quadriceps muscle. Whole leg mass was taken with a DEXA scanner and only recorded at baseline and six weeks after surgery, while the five-slice section was taken with an MRI at baseline, two weeks, and six weeks. The effect size was found between groups for each variable, and an effect size of $d=0.2$ suggests minor relevance while a value above one suggests strong relevance. Data from both DEXA and MRI scans suggest greater muscle loss in the control group following surgery.

DISCUSSION

Essential amino acids have shown potential to attenuate the rates of muscle atrophy following total knee arthroplasty, but because there are many factors that could potentially influence levels of atrophy, each variable must be accounted for between subjects. Factors including the subjects' activity level, the total time the surgery lasts, and dietary intake before and after surgery could all affect the rate of muscle atrophy. The goal of this study was to

determine whether dietary intake played a part in determining the amount of muscle lost or muscle saved in the quadriceps following total knee arthroplasty. This seemed like a prudent question because skeletal muscle houses the majority of protein within the body, and without adequate caloric intake, the body will begin to break down stores of protein in order to maintain the blood glucose level in circulation. The data, however, failed to support the hypothesis that there would be significant differences in dietary intake between subjects in the placebo group and subjects in the treatment group that could partially explain the differences in muscle mass following surgery.

Through analysis of the food logs, it became apparent that there was no significant difference between the control and treatment group for any of the recorded dietary measurements: fat intake, carbohydrate intake, protein intake, protein per kilogram intake, or caloric intake at any of the selected intervals before and after surgery (all p values >0.05). Despite this lack of significant differences in dietary intake, there are still significant differences between the placebo group and EAA group in regards to the lean mass in the quadriceps and a mid-section five-slice piece in the middle of the quadriceps of the affected leg (effect size of 1.74 & 0.69 respectfully). The five-slice section in the middle of the quadriceps is of particular interest because muscle lost here is less likely to come as a direct loss from the physical surgery. The area proximal to this section is likely to be affected from the tourniquet that prevents blood flow to the quadriceps muscle, while the area distal to this section will accrue ongoing damage from the surgical process.

Although no support was found for the initial hypothesis, these results suggest the administered essential amino acids given to the EAA group were able to spare muscle by limiting the rate of muscle atrophy when compared to the placebo group receiving nonessential amino acids. From previous studies, it appears that essential amino acid supplementation leads to various changes in gene regulation in which muscle protein synthesis is upregulated. Following surgery, the upregulation of muscle protein synthesis would then buffer the rate of muscle atrophy. In order to definitively prove the efficacy of essential amino acid supplementation in reducing muscle loss following TKA, continued research must be conducted to determine whether other variables could possibly account for differences in quadriceps muscle saved or lost following surgery. If there continued to be no significant differences between the two groups in terms of these variables, it would be even more suggestive that essential amino acids were capable of reducing the rate of muscle atrophy following TKA.

For the purpose of this study, dietary intake was not shown to account for any of the differences between groups in terms of muscle lost; however, proper nutrition could still be beneficial in this respect. Dietary intake for both groups had a decreasing trend between baseline and two-week values. For each individual, this could exacerbate muscle loss, but because both groups were subjected to the same decrease at this level, there was no significant difference between the two groups. One particular measurement of interest was the grams of protein per kilogram body mass per day measurement. Even with the added protein supplement for both groups, subjects rarely met the new recommended requirement of one gram of protein per kilogram body mass per day. Although the protein supplement is administered in order to

help reduce muscle loss, subjects should still be practicing healthy nutrition in order to bolster the effects of the supplement.

One interesting finding resulting from this study was that only one of the subjects managed to regularly ingest the recommended 2000 calories a day as demonstrated in the collected food logs, while all other subjects fell short of that requirement. This peculiarity arose from the fact that the majority of the subjects for the study were overweight (average BMI: placebo group: 28.8; TKA group: 33.8); however, this could be attributed to a lack of energy output due to the limited mobility of the subjects both before and after surgery. If energy output is low, then fewer calories would suffice for daily activities while still leading to increases in BMI. Moreover and perhaps more importantly, the lack of energy output before surgery can most likely be credited to pain arising from the damaged knee, thus limiting mobility, while the continued lack of energy output following surgery is a direct result from the surgery itself. This, if nothing else, demonstrates why finding a less deleterious method of TKA surgery is an important step in healthcare. An alternative explanation for a low dietary intake could be the result of inaccuracies in the self-reported food logs. Measures are currently being taken to have healthcare staff record food intake of the subjects following surgery in order to achieve more accurate measures.

It is evident that current protocol for TKA is lacking in its ability to fully restore patients to optimal health because of substantial muscle atrophy. Although the surgery has been proven to be a successful method for eliminating the original pain stemming from osteoarthritis in the knee, there is significant muscle loss in the quadriceps following surgery that leads to ongoing muscle weakness and decreased physical function. Essential amino acid supplementation could be an additional method in the surgical procedure to help reduce this high rate of muscle atrophy. Although essential amino acid supplementation seems to have a positive effect in preventing high levels of muscle loss, it is apparent that other factors could be responsible for part of the differences in muscle loss between a control group receiving nonessential amino acids and a treatment group receiving essential amino acids. Research should continue in this field, but evidence from this preliminary study suggests that dietary intake is not responsible for eliciting any of the differences in muscle saved following surgery.

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