PMUSER

Proceedings of Manitoba's Undergraduate Science and Engineering Research

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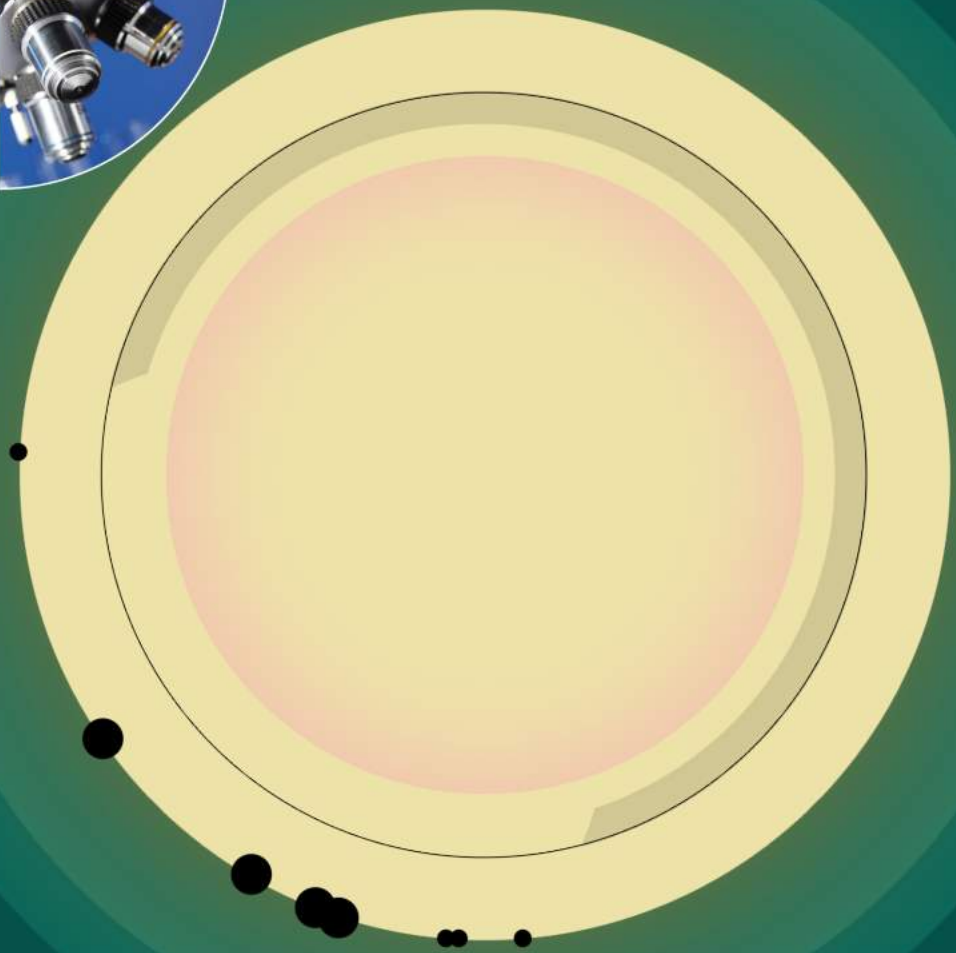

Frontiers of Undergraduate Research

Invited Submission

2018

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I4

Tradition is the knowledge and practices that have been passed to the next generation, accomplished by written language, oral testimony, natural observations, or scientific peer-review. Evolution is the change within a community where new, effective knowledge or practices are utilized. Evolution is the result of basing what we envision on a traditional knowledge and research is a bridge between tradition and evolution. Research builds on existing knowledge and introduces new practices through experimentation. Knowledge evolution through research is only possible when we are privileged to a wealth of tradition. For example, research of botanical species and their medicinal properties evolve the traditional knowledge of pain management, pharmacotherapy, and human-environmental relations. Traditional practices continue to evolve through research and development. We identify what is most effective and efficient, and we accept the discontinuation of out-dated practices and knowledge. Knowledge transference, within certain cultural practices, may feature social protocols that ensure the accuracy and prevent the loss of traditional knowledge. Time has evolved. Traditional knowledge and practices have also evolved. Anything and almost everything is accessible online, including PMUSER's journal. If this is available online, why continue to print physical copies? This is simple. Human behaviour analysis indicates people approach physical publications with more authority and attention than they do digital publications. The way people recognize physical and digital publications affects what they experience. One is viewed as traditional and is revered by a community, while the other is viewed as evolutional and is utilized to create efficiencies and effective protocols. A physical publication may be considered outdated in this era. While partially true, the publication of PMUSER in print is to influence those publishing and those reading to consider contributing. Some view physical publication of their research as a milestone in their lives. Others view the digital publication as the only way forward. Until a culture and a tradition of a solely digital publication of knowledge transference is viewed and accepted by readers and contributors as tradition, PMUSER meets tradition and evolution on common ground. Acknowledging the tradition of knowledge transference and the evolution of research and scientific truths, PMUSER enables both sides to continuously evolve without forgetting where our roots are.

Best Paper Award

Developing a Genotyping Scheme for Mycobacterium abscessus *Complex Using Whole Genome Sequencing Data*

37

Winner

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Chloé W. Schmidt

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About PMUSER

Proceedings of Manitoba's Undergraduate Science and Engineering Research (*PMUSER*) is an open-access refereed journal that is published annually and hosted by the University of Manitoba. The journal accepts research or review manuscripts written by undergraduate students from any science or engineering related faculty or manuscripts on any topic concerning or related to science or engineering. Upon submission, each manuscript undergoes a double blind peer-review process by two undergraduate/graduate students from a pool associated with the respective research area. Our mission is to provide opportunities for student communities to explore the frontiers of undergraduate research and add additional value and learning opportunities to students' degree programs through rigorous exploration, analysis, and presentation.

Aims & Scope

The focus of *PMUSER* is three fold: author, reviewer, editor. Through preparing and revising a manuscript, students are recognized for their research, have an opportunity for out-of-classroom learning, and further develop communication skills. Through learning the role of a peer-reviewer and reviewing manuscripts, students learn to critically evaluate the scientific literature and to critique ideas in the broader world. Through the editorial board, an interdisciplinary team arises to provide mentorship to undergraduate students and valuable leadership and teamwork skills. The three prongs of our focus unite in preparing students for careers in science or beyond, and the stepping stones to get there. Available online: http://ojs.lib.umanitoba.ca/pmuser/about/editorialPolicies#focusAndScope.

Image Credits

Evan Tremblay, *cover*
Maria Nickel, *page 1 inset*
ANASA/ESA/Hubble Heritage, *page 1 star background*
Chloé Warret Rodrigues, *pages 2 & 3, Cover Art runner-up*

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Open Access Policy

This journal provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge. Available online: http://ojs.lib.umanitoba.ca/pmuser/about/editorialPolicies#openAccessPolicy.

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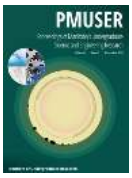
Instructions for Authors

Undergraduate authors are encouraged to submit research or review articles in or related to any science or engineering discipline. Detailed instructions are available online: http://ojs.lib.umanitoba.ca/pmuser/about/submissions.

Peer-Reviewers

PMUSER's peer-reviewers apply each year and are primarily graduate and undergraduate students, with expertise supplemented by faculty. Training is provided through a University of Manitoba Co-Curricular Record certified workshop. Those interested in reviewing for the journal should email the editor-in-chief for application forms. Peer-reviewers for Volume 4 were: John P. Aguilar, Sarah Arnold, Jun Bae, Kevin R. Bairos-Novak, Tom Booth, Jennifer Doering, Keri Everitt, Kristen Fleet, Andrew Hogan, Kat Kratzer, Janelle Boram Lee, Duc Minh Nguyen, Franklin Ogidi, Michaela Palmer, Davinder Partola, and Rani Ramachandran.

About the Cover



The cover features the the 2018 Cover Art Contest winner, *Anabasis* by Evan Tremblay.

"The image, Anabasis, *is an interpretation of the journey of discovery, represented as the piercing of the circle of the world.*
The physical and biological forces explored by the papers in this volume of PMUSER are shown abstracted, blurring the line between orbits and cell walls, on a field of either the sky or the deep sea.
The pierced wall of the circle speaks to the tearing-through of mystical borders to knowledge through scientific inquiry, giving motion and direction to what would otherwise be a static, theoretical existence. The form invites contemplation: it inspires questioning."
The back cover continues Tremblay's exploration of this theme.

Letter from the Editor-in-Chief

Volume 4: A Publishing Year of Firsts

With the publication of Volume 4, *Proceedings of Manitoba's Undergraduate Science and Engineering Research* (PMUSER) marks a number of firsts. With an expanded editorial board encompassing the breadth of the scope of PMUSER , we're delighted to share with you the largest volume in our seven-year history.

This volume includes two issues, not only the regularly published issue 1 of student research and review papers but also an Agriculture Special Issue devoted to highlighting student successes and research accomplishments in the Faculty of Agricultural and Food Sciences. In future publishing years, we hope to have a special issue for each of the faculties and areas of study that PMUSER 's scope encompasses.

The year 2018 also sees the inaugural cover-art contest. The winning image, *Anabasis* by Evan Tremblay, is featured on the cover of this issue (Volume 4, Issue 1), and the runner-up, submitted by Chloé Warret Rodrigues, is featured on the Table of Contents of this issue. We also proudly announce that Michelle Wuzinski is the winner of the Volume 4 Best Paper Award!

With the publication of Volume 4, PMUSER has embraced the "Manitoba" in our name. For the first time we have received and are publishing submissions from outside the University of Manitoba. Two articles have come from Brandon University and an invited submission from students in the Interlake School Division. We look forward to continuing these relationships and to building awareness of the opportunities that PMUSER offers students through all of Manitoba's degree-granting institutions.

Also this year, PMUSER :

- is publishing poster abstracts and titles in Issue 1,
- is publishing online-only supplementary information for an article, and
- developed a University of Manitoba Co-Curricular Record (CCR) approved peer-review workshop.

The workshop provides students with, very often, a first exposure to peer-review, what is expected of the reviewers in the publishing process, and is a fantastic opportunity for all students to gain insight into scientific publishing that they may not otherwise have.

Workshop participants were enthusiastic about the workshop and material presented. Their feedback will surely make it even more valuable in 2019!

Inside this issue, the research published covers many facets of science and engineering. Research that I am particularly excited to publish in this issue includes the effect of repeat space-exposure on tomato seeds, perceptions of underrepresentation of women in STEM fields, revised submissions from class review papers of "The Chordates" class in the Department of Biological Sciences, and the entirety of undergraduate summer and honours research contained in this large volume.

After reading the issue, I urge you to attempt the puzzle included on the final page. Following a traditional logic grid puzzle style, it is entirely informed by the particular articles published in PMUSER 's Volume 4 Issue 1. It is sure to be no small feat of mental maneuverings; we look forward to congratulating successful solvers!

Good luck!

Thanks for reading,

Matthew J. D. Doering
PMUSER Editor-in-Chief

Student Profile

Undergraduate Student Fights Against Epidemic of Heart Disease in Canada

Fourth-year biochemistry student Matthew Stecy completed his Honours project and gave his final presentation April 7, 2018. His research project, entitled *Regulation of Scleraxis by microRNA* investigated whether the production of the protein scleraxis, which promotes the development of cardiac fibrosis, can be inhibited in live mouse cells.

"Learning hands-on how the whole [research] process works has been really valuable for my own personal development. Pursuing a career in medicine, I think it's really important to understand the fundamentals. There's so much new research coming out and I think it's really important for physicians to keep up to date," Stecy said.

Cardiac fibrosis is a heart condition whereby fibroblast cells in the heart overproduce extracellular matrix (ECM) proteins. ECM proteins normally help give structure to tissues of the heart, but when overproduced, as in cardiac fibrosis, the tissues of the heart thicken beyond normal and make it difficult to pump blood. This overaccumulation of ECM, over time, not only leaves the tissues rigid but it also leaves the heart exhausted from being overworked.

Stecy started working in the laboratory of Dr. Michael Czubryt, molecular pathophysiology professor at the U of M Department of Physiology and Pathophysiology. Dr. Czubryt's lab, at the St. Boniface Hospital Albrechtsen Research Centre, studies how genes influence heart diseases. With the aid of sophisticated technologies, they study the genes that may be involved in normal and abnormal heart conditions. Dr. Czubryt and his team were the first to identify the scleraxis protein as an activator of the production of the ECM protein collagen whose overproduction contributes to cardiac fibrosis.

"I love that [Dr. Czubryt] is doing research that has practical applications that relate to the human body," said Stecy on why he chose his research topic. "I relate much better to humans and diseases, and that's what interested me."

Taking advantage of the current knowledge of how microRNAs regulate messenger RNAs, Stecy's research tested the interaction between miRNA-7087 and scleraxis. He also wanted to see whether the production of scleraxis messenger RNAs and scleraxis protein can be inhibited in live mouse cells. While previous studies have investigated the role of microRNA and messenger RNAs in cardiac fibrosis, the role of miRNA-7087 in regulating scleraxis had yet to be, until now. *In silico* mathematical models were used to decide which microRNA to choose, from a pre-existing database of microRNAs of possible regulators of scleraxis.

"We used three different programs, and they all identified miRNA-7087 as the front runner, and so it was a pretty obvious choice to investigate how it works," Stecy explained.

For undergraduate students, the prospect of starting and successfully completing a research project is often daunting. But students must realize that they will be working in the context of a much larger project, and under the guidance of mentors who provide tremendous support all the way through.

"Reach out to people [whose research] you're interested in," is Stecy's advice to students who might be interested in research. "Principal investigators and researchers on campus are typically really open to having students work for them. They love people who are interested in what they do." He said students should not only go into labs for the experience, but also to find research that they are genuinely interested in.

Undergraduate students receive invaluable mentorship through working under senior researchers and principal investigators, but graduate students often also play a role in this mentorship experience. Matthew pointed out that PhD candidate Raghu Sundaresan, although not his principal investigator, was "instrumental" in the success of his project.

"I was really lucky to have awesome graduate students to teach me things," stated Matthew as he recounted the benefits he gained from the research experience at Dr. Czubryt's lab, starting last summer. "I would watch and learn, slowly becoming independent and helping with the graduate students' experiments."

The biggest challenge, for Stecy, was working with the NIH-3T3 mouse cell line because they were easily infected with foreign materials and had to be kept sterile at all times, using meticulous aseptic techniques.

"One little slip-up can lead to a week or two's work down the drain," he added. He acknowledged that his undergraduate microbiology courses gave him the foundation for that good technique. "The techniques I learned in those [microbiology, biochemistry, and organic chemistry] labs were really helpful."

"Regardless of what I end up doing next year, I hope to continue in research." Speaking generally about the advantages he gained through his research experience, and his future plans, Stecy said, "Having this experience — learning how to read and analyze papers, learning how statistics work, and whether you can trust the methods — are all important for where I want to go with my career."

— David Zirangey

Originally published at pmuserjournal.wordpress.com

Student Profile

Tackling Multidrug-Resistant Bacteria Through Novel Approaches

While some students quickly learn that research might not quite be the path for them, many others get a solid conviction that they are on the right track.

After completing his honours research project, fourth-year U of M Chemistry student Liam Berry has decided research is the career path for him.

Berry's work with Prof. Frank Schweizer, faculty member in the Departments of Chemistry and Medical Microbiology & Infectious Diseases, focused on the development and evaluation of novel antimicrobials against multidrug-resistant bacteria. The Schweizer group specializes in organic and medicinal chemistry.

"Last year in America, a woman died from a bacterial infection that was resistant to every class of antibiotic," said Berry, referring to the widely-covered 2017 case of a Nevada woman who died from a multidrug-resistant bacterial infection after treatment with 26 different antibiotics.

The first case of antibiotic resistance was reported in 1940 when an *E. coli* strain was observed to deactivate penicillin by producing an enzyme called penicillinase. This happened a little over a decade after penicillin was discovered in 1928. The discovery of penicillin revolutionized medicine and benefited humanity in immeasurable ways, and diverse antimicrobials have been discovered since. However, microbes have been shown to continuously adapt resistance mechanisms to hinder the action of these antimicrobials.

The development of new antimicrobials is currently unable to keep pace in the evolutionary arms race between humans and disease-causing microbes.

Antimicrobial resistance (AMR) is a growing public health concern across the globe. AMR-related deaths worldwide were estimated at 700,000 per year as of 2014, and will reach an estimated 10 million by the year 2050 if necessary actions are not taken. The increased frequency of AMR infection cases suggests we are approaching a "post-antibiotic era" where common microbial infections that were once easily treated may now cause mortality. "Superbugs" may become more than just a buzzword.

Different antibiotics have different methods of penetrating the protective membranes of bacteria. Berry's project focused on two modes of cell entry. One type of antibiotic — pore invaders — combats gram negative bacteria by inhibiting DNA synthesis, which requires it to enter the bacterial cell. It enters the cell through tiny pores that can be found in the protective membrane of the bacteria. Fluoroquinolones are a family of antibiotics that use this mode of action. Cationic antimicrobial peptides — membrane destabilizers — are another family of antibiotics, which combat bacteria by destabilizing the chemical structure of the protective outer membrane. Once the structure is destabilized, these antibiotics are then able to penetrate the membrane.

Levofloxacin is a clinically used pore invader, and Colistin is a type of membrane destabilizer. Liam's research goal was to determine whether these two modes of cell entry could be combined in a single antibiotic, while retaining the original modes of action.

The Schweizer lab has successfully made twelve different derivatives of the pore invading antibiotic and tweaked each by adding various parts of the membrane destabilizing molecule. They expected that the resulting hybrid antibiotic may have "dual action" because it will be able to enter the bacteria through pores, as well as by destabilizing the bacterial protective membrane, increasing the amount of antibiotic able to enter the cell.

They tested these twelve hybrids against five different bacterial species, but none of them were effective antibiotics on their own.

One of the primary ways that bacteria develop resistance is by expelling antibiotics as they enter the cell. Colistin is not susceptible to expulsion (efflux), so further research at the Schweizer lab will explore whether the hybrid antibiotics can avoid a similar fate or prevent other currently used antibiotics from being expelled. The results of this next phase will determine whether the antibiotic hybrids they developed can overcome bacterial resistance, and whether they can be used in combination with other antibiotics.

"This whole process taught me how to be productive," said Berry who took a full course load with his research.

"It probably would scare some people, but I think people should do things that scare them sometimes. It is a good learning experience," he added, acknowledging the personal transformation that he has experienced through the discipline and dedication that his research project demanded.

Berry gave his final project presentation on April 7, 2018. He was runner-up in the biochemistry oral presentations of the Western Canadian Undergraduate Chemistry Conference (WCUCC) held at the University of Winnipeg from April 30 to May 3, 2018. He also won third place at the inaugural Manitoba Chemistry Symposium in May 2018 at the University of Winnipeg, where he represented the Schweizer group in the undergraduate oral presentation. Berry has decided to put off medical school to pursue a master's program once he completes his undergraduate degree.

— David Zirangey

Originally published at pmuserjournal.wordpress.com

Student Profile

Is the Migration of Manitoba's Purple Martin Population Mistimed with Prey Emergence?

Final year ecology and environmental biology honours student Ellyne Geurts has loved birds since she was a child. After learning about avian diversity as an eight-year-old she started to see and recognize bird species everywhere. It wasn't long before she began bird watching and keeping a bird observation list as a hobby.

Having always wanted to work with bird conservation, she was thrilled when the opportunity arose to study purple martins (*Progne subis*) and investigate a proposed mechanism for their population decline — "ecological mismatch".

"When my supervisor, Dr. Kevin Fraser, suggested the idea of ecological mismatch with his study species, purple martins and their prey odonates (dragonflies and damselflies), I was excited to work on this project."

Purple martins are a migrant bird species that travel thousands of miles every year to their overwintering sites in South America and back to North America for the summer. The martins are one of the fast-declining aerial insectivores in North America, but, like other aerial insectivores, the decline is poorly understood.

"I wanted to know if the timing of purple martin breeding in relation to the timing of peak odonate abundance affected martin breeding success such as average nestling mass or number of fledged young," Geurts said.

"Ecological mismatch has been proposed because more northern regions of the globe generally experience more seasonality and temperatures are increasing more rapidly in the north with climate change," she explained.

Insectivores may be at risk of mismatch given that they are generally slower than their prey in responding to changes such as earlier springs. And if these species breed in more northern latitudes like the Manitoban purple martin populations do, they may be at even greater risk of population decline given that they have relatively short summers to arrive, adjust to the environment, feed, breed, and reach maturity.

As timing of ideal weather conditions is changing yearly, time of odonate emergence and time of arrival of martins from the south may be mismatched, possibly leading to misalignment between the purple martins' peak breeding time, coinciding with high energetic needs, and prey abundance.

"We stayed up all night after having done fieldwork during the day, catching and banding birds until sunrise," Geurts described as a memorable moment — alongside designing and making of her own dragonfly traps.

Over the summer she would go out to the field to monitor nests and band adult martins for migration studies.

To test the ecological mismatch hypothesis she measured whether there was asynchrony between purple martins' peak breeding time and their prey odonate abundance. But she also needed to measure whether the level of asynchrony, if any was observed, had any relationship to the purple martin reproductive success.

Once the adult martins were caught, their morphometric measurements were taken and then they were either tagged with GPS or with archival light-level geolocators. The GPS and geolocators were used to monitor the bird migration as part of the "Hemisphere to Hemisphere (H2H)" and "migratory connectivity" projects the ABC lab runs.

Martin nestlings and their parents were counted and summed up to estimate the population size. A total of 56 nests were observed every 2 to 3 days to determine number of fledglings per brood and the average brood mass. These measures helped determine the reproductive success of the martins in the Winnipeg, Manitoba region for the year 2017.

Geurts's results showed that there was some asynchrony between the timing of peak abundance of odonates and the timing of peak energetic demand of the purple martins. The timing of peak abundance of odonates was on July 7, 2017 — not too far off from the timing of peak energetic demand of the purple martins, on July 3 and July 15.

"Number of fledglings and average nestling mass [reproductive success] were not related to the degree of synchrony between timing of peak odonate abundance and martin breeding," she concluded.

These results spelled good news because it meant that an ecological mismatch did not occur in the Manitoban population she sampled in 2017. But this also leaves the puzzle of the reason behind martin population decline largely unsolved.

"Several things changed in my undergrad life as I became involved in research. Lab assignments began to feel easier as research experience granted me skills in literature searching and study design. I felt more confident in my abilities."

In addition to developing her writing skills, she cultivated an interest in statistics as she tried to understand her research results using various statistical measures. This newfound interest drove her to do a minor in statistics.

Besides her sheer grit and her ability to be organized with her time, Geurts attributes her ability to successfully complete her thesis to the comradery she shared with fellow honours students.

— David Zirangey

Originally published at pmuserjournal.wordpress.com

University of Manitoba Faculty Profiles

Dr. Jim Roth
Associate Professor, Biological Sciences
What opportunities do undergraduates have in your lab?

Much of the work in my lab involves understanding how feeding relationships among organisms drive changes in population sizes; i.e., how predator-prey interactions affect population dynamics.

We reconstruct diets of wildlife using stable isotope analysis on a variety of tissues of different species, and this method that requires a lot of sample preparation (freeze drying, lipid extraction, homogenizing, and weighing).

We also have thousands of photographs each year from trail cameras at Arctic fox dens that need to be viewed to document fox activity, including patterns of occupancy, litter sizes, and use of dens by other wildlife species. In addition to our field work, these activities in the lab provide many opportunities for students to gain research experience.

Why is research experience valuable for undergraduates?
Research experience gives students an understanding of how science is done, and how we figure out the way the world works. By participating in research, students learn if it's something they'd like to pursue later on, and become more competitive for other opportunities in science.

What value do undergraduates get from publishing?
Science builds on what has been learned in previous research, and publications are how we communicate what we've learned. Peer-reviewed publications are the litmus test by which research validity is evaluated, and publications are the standard measure of research productivity required to secure funding for more research and training.

Experiencing the process helps students understand how we communicate science and the standards we must meet.

What does it take to be successful in biology?
Hard work and persistence! Grades are important, but getting some experience is key. You need to develop skills, as well as gain knowledge. Writing ability and quantitative skills are particularly valuable.

What advice would you give students in biology?
Commit to learning as much as you can in class, and gain as many different types of experiences outside of class as possible. Biology is a diverse discipline, addressing levels of organization from molecules to ecosystems, so figure what you enjoy doing. If you like it, you'll work hard at it. If you work hard at it, you'll become good at it. If you become good at it, you could get a job doing it!

Dr. Denice Bay
Assistant Professor,
Medical Microbiology & Infectious Diseases

Why is research experience valuable for undergraduates?
That's a great question! If you are pursuing an undergraduate degree, let's say in microbiology, you are essentially studying to be a researcher and getting research exposure; "getting your hands wet" so to speak, will be the most valuable experience that you will have.

In the lab, you have the opportunity to combine the theoretical aspects, learned in class, with practical skills. It is also a great opportunity to gather valuable information on the strengths and weaknesses of the field. In addition, you are in an environment that fosters scientific thinking, where you are free to explore and learn, which does not only solidify what you've been learning in class but is also an excellent way to determine whether research is the appropriate career path for you.

By participating in research, students learn if it's something they'd like to pursue later on, and become more competitive for other opportunities in science.
— *Dr. Jim Roth*

What opportunities are available for undergraduate students within the Department of Medical Microbiology & Infectious Diseases?
We have a number of labs on the med-micro floor, which includes eight core members, and this number is expected grow in the next five years. The majority of this core conducts HIV research, antibiotic resistance, emerging and re-emerging pathogens in regard to viral infections.

In addition to our core members, we have a vast network of cross-appointed faculty members from a variety of departments, from Immunology to Microbiology at the main campus. We also have members within the Public Health Agency of Canada, Cadham Provincial Labs, and the National Microbiology Lab.

Finding a lab that interests you in our department is definitely possible. The limiting step is the timing for finding available positions whether as a summer, co-op, or graduate student.

This is why we are holding an open house January 9, 2019 from 4 to 6 p.m. in Room 540 BMSB to bring awareness to our department. You will get to know what the Department of Medical Microbiology & Infectious Diseases is doing and which professors are accepting students and at what level. We are also starting, in the new year, a summer undergraduate research award for two undergraduate students valued at $7,000 each, for students to work in a lab in the Department of Medical Microbiology for the summer of 2019. These are just some of the ways, if you are interested in our department, to get a taste of the environment and the types of projects that you could be involved with.

Getting research exposure, "getting your hands wet" so to speak, will be the most valuable experience that you will have.
— Dr. Denice Bay

What value do undergraduate students get from publishing their summer or co-op projects?
The value in publishing as an undergraduate student is that it sets you apart from other students that don't have publications when applying for graduate school or other professional programs. For all of the students that are working in my lab, I don't want them working on anything that is not going to be published whether as a paper on its own or contributing to another publication as this is an important training aspect.

The value in publishing as an undergraduate student is that it sets you apart from other students that don't have publications when applying for graduate school or other professional programs.
— Dr. Denice Bay

However, lab research is a two-way street. The student needs to put in enough work that will then lead to a publication, and this will also depend on the lab you are working in. If publishing is something you want to get out of your summer research experience, talk to the supervisor whose lab you are interested in and express this interest.

Keep in mind that not all supervisors take on summer students to contribute to a publication; you may be reorganizing a freezer. So, if publishing is something you are adamant to get out of your summer work, then make that clear and look for a professor that will provide those opportunities.

What do feel it takes to be successful in research?
Everybody is different. If you are excited, engaged, have some level of organization and you're okay with the fact that you will not succeed all time then you will do well in this field.

In addition, having perseverance, the willingness to "dust yourself off", and, most importantly, the ability to recognize an opportunity are essential skills. So, don't give up! Having that willingness and resiliance to want to learn will get you to where you want to be.

Proceedings of Manitoba's Undergraduate Science and Engineering Research
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Editorial

A Vision Quest Experience of a History of Knowledge

River S. Guillas[1]

[1]Faculty of Nursing, University of Manitoba, Winnipeg, MB, R3T 2N2
Corresponding Author: R. S. Guillas (guillasr@myumanitoba.ca)

Tansi, tawaw // Hello, and welcome; there is room, come in. I've said my salutations and used words in Nēhiyawewin // language of peoples from Nēhiyaw descent. I lay on tarp-covered moss inside a tent, observing constellations with only gravity between us. It is relieving during Sâkipakâwipîsim // Leaf-Budding full moon on May 29th, 2018. The second night of a four-day Vision Quest, where the heat has dissipated, and my mind has ascended to connect stars like dots on paper. This moment is a fragment of memory-recollection, constantly on my mind. Manito Api // Bannock Point is a sacred site where ceremony takes place. The Indigenous practice of fasting dates back over millennia, and the remaining infrastructure of Manito Api is approximately 1,600 years in age. Manito Api is a site of Anishinaabe // Ojibwe traditional territory.

Through traditional knowledge passed down generation to generation, the objective research and Ceremonial Protocols of our ancestors gave us the power to survive a genocide

Kânata has been, and still is, home to over 700 defined, distinct, and diverse Nations who speak over 60 languages. Derived from Kânata // a settlement, Indigenous languages influence what is now known as Canada. We, Indigenous peoples, connect with the spirit of all things Land and Skies. We observe there is no pause or static period in the continuum of tradition and evolution amongst the First Nations, Inuit, and Metis Nations. Our languages have fluid states that transcend the honesty of human perspectives. Language is a pillar in any culture, which is why colonial media perpetuates reality based not in truth, but in honesty.

Honesty is a form of subjectivity, where our human bodies elicit a response. Honesty, to simply explain, is human emotion. Most effective of these emotions are paranoia, rage, selfishness, and identity. The epitome of these humanistic emotions would be Capitalism. Non-Indigenous peoples hoarding millions or billions of dollars in profits while less than 200 km away, Indigenous peoples starve from lack of food/water resources. This is applicable across the planet. In Canada 150 First Nation reserves have poisoned water and boil advisories due to non-Indigenous corporations extracting natural resources next door. Honesty is observed as genocide, where a truth is hidden by the monuments to our sins.

The Truth? Some feel it is boring, factual, and objective, never elevating the heart rate or giving people a rush of epinephrine. Truth is a language spoken by the Moon and Sun. From observing honesty through a microscope, one must then observe truth through a telescope. Animikii // Thunderbird, spiritual beings of water in Anishinaabe legend, is not considerate of human honesty. Truth is no matter how deeply you believe bacteria cannot kill you, they still do. Leaving only scientific findings and proven Protocol, truth is "the bigger picture" to all living beings and their complex relations.

With bloodlines that run deeper than any oil well, Indigenous Peoples feel and sense the spirit of the living on Kânata. Through traditional knowledge passed down generation to generation, the objective research and Ceremonial Protocols of our ancestors gave us the power to survive a genocide.

From observing honesty through a microscope, one must then observe truth through a telescope

While 127 million Indigenous peoples occupied Turtle Island in the 1400s, we now have 70 million. This population decline of 57 million Indigenous peoples across Turtle Island // North America is due to genocide. Statistics Canada published a population of 1,673,780 Indigenous peoples for fiscal year 2016, which is approximately the same as 600 years ago. We, Indigenous peoples, are recovering from extensive genocide with the first rise in our population in a long period. We have much farther to go.

Centuries of Indigenous-led fur trades with non-Indigenous people founded the economy of today's society. High and unrelenting demand for Indigenous knowledge transference and sacred traditions led to cultural changes within Indigenous nations. From the 1400s to late 1800s, peoples of both Indigenous and non-Indigenous descent, now referred to as Metis, began to emerge. Indigenous and Metis Nations across Canada faced criminal apprehension of their land and material properties by the Hudson's Bay Company, and eventually the Royal Canadian Mounted Police. Often through swindling, in the form of Scrips, the Hudson's Bay Company sold the stolen land to the Government of Canada.

Legislative recognition over Manito api // Manitoba by this colonial state was a trojan horse. The efforts to recognize Manitoba as a province were led by the honourable politician Louis Riel. Upon learning Indigenous peoples were utilizing language and white privilege in asserting legislative recognition, the colonial state executed Louis Riel and introduced the numbered Treaty Acts in his absence. In knowing Indigenous Nations could not utilize the English language like Louis Riel and other Metis Nations, the colonial state legislated numbered Treaty Acts to perpetuate their ongoing genocide. Riel symbolizes a last resort of one kind, as Traditions, and a new hope of another, as Evolutions.

The colonial state disregards the numbered Treaty Acts, instead legislating the Indian Act. Through the Indian Act of 1876, people were given social authority and jurisdiction to continue in the abduction and murders Indigenous peoples. This federal law gives jurisdiction to the provincial governments of Canada to control Indigenous peoples through institutional oppression. Instead of murdering like the previous four hundred years, in 1876 they began to obfuscate genocide through 139 Indian Residential Schools and 29 Segregated Indian Hospitals, through imprisonment and foster care, and through court systems that acquit murderers like Gerald Stanley, Raymond Cormier, and Peter Khill.

These institutions were given legislative powers from the Colonial state to abduct Indigenous peoples and forcefully colonize their minds. Each Indigenous child was subsidized at $0.35 cents a day, paid by the colonial state, $0.15 cents extra granted to Indian Residential Schools housing children with Tuberculosis. When they became ill and/or injured from mental, sexual, spiritual, and/or physical abuse, Indigenous peoples were then sent to the Segregated Indian Hospitals. All 29 of these federal institutions performed medical, surgical, experimental, and unethical practices on Indigenous peoples.

Where 150,000,000 Indigenous Peoples once facilitated a wealth of knowledge and wisdom transference, we now have 570,000,000 (non-Indigenous) Peoples facilitating fascistic ideologies and capitalist governments.

Almost as if intentional, media outlets constantly run narratives through different perspectives, by different companies (owned by the same corporations). Media went from revering scientific truth to upholding honesty in the form of reality. No matter how truthful the facts, dishonesty will always bear more influence over an audience. People are more attracted to honesty guised as reality over the truth. Honesty is a form of subjectivity, where our human emotions elicit a response of a certain type. Truth leaves only scientific findings and proven protocols to be digested, an audience may sigh "Never enough".

We have, in the College of Nursing, a population of 7% Indigenous Peoples. When addressing these concerns to my fellow r epresentatives, they were unintentionally stereotypical of Indigenous students (our GPAs are too low, we don't apply, we lack qualities of strong students, etc.). These stigmatizations of us echo on, so easily absorbed by those with the best intentions, and are endlessly repeated. I attended the Manitoba Undergraduate Healthcare Symposium where Dr. Barry Lavallee spoke of Indigenous Health and wellbeing. We had a student derogatorily demean the research of our health and wellbeing. We experienced continuous taunting throughout, this non-Indigenous student felt so propelled to assert his version of Indigenous health and wellbeing. Anything to suppress and invalidate the knowledge transference of Indigenous research. Our own student body does this, even in the presence of those who directly contributed to or are related in the research performed.

Anything to suppress and invalidate the knowledge transference of Indigenous research

It is the right and responsibility of Indigenous Nations to ensure the knowledge transference of our Sacred Traditions and Traditional Knowledges

There are still those within Canadian society who cannot (or will not) see the facts as presented. It is time we shave the blinding wool that misleads our societies. It is time rather than blind ourselves to scientific realities, we deafen ourselves to those speaking scientific fallacies and falsehoods. It is the right and responsibility of Indigenous Nations to ensure the knowledge transference of our Sacred Traditions and Traditional Knowledges. No form of colonial media, organization, or state shall interfere with this process. If they try to do so again, they will discover future generations will never allow it. They will find architects of tomorrow's labyrinths are catching up to them. They will find we do not become weaker from starvation of education, food/water sources, shelter, employment, community relations, and pursuit of Life's Purpose. We become stronger warriors. A warrior is not one of anger and malice, they are one of awareness and acceptance.

I speak from experience when I claim we do not become weaker from starvation, through education, food/water sources, shelter, employment, community relations, and pursuit of Life's Purpose. As I lie under the stars, night three of no food or water, I realize how little we need in this life beyond the connection with other human beings. Like gravity, material greed restrains our human experiences as spiritual beings.

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Perspectives

SSEP: Dare to Dream Big Things

Carter Ives[1*], Graeme Perrie[1*], Adam Stamler[1*], Mrs. Maria Nickel[1]

[1]Interlake School Division #21, Stonewall, MB, R0C 2Z0
*Equal contributors listed alphabetically
Corresponding Author: Maria Nickel (mnickel@isd21.mb.ca)

SSEP (Student Spaceflight Experiments Program) is a great program. It inspires students to give it their all and strive for greatness at an academic level. Students are able to have an experience that impacts their lives forever. We are very grateful to have this opportunity to participate in the SSEP program. We also believe that Tomatosphere™ is another great organization that encourages students to strive for academic excellence by planning, predicting, and observing.

Tomatosphere™ lets students compare germination of tomato seeds sent to space (or treated in simulated conditions) and untreated seeds. The SSEP is a Flight Experiment Design Competition for microgravity research proposals of projects to be done in a mini-laboratory. After going through a two-step review process and a flight safety review, experiments are revised and are incorporated into the payload of the ferry vehicle to the International Space Station (ISS).

This project started by us coming together for the first time and just coming up with possible ideas for our project. At first we had wanted to send up animal DNA to see how it was affected by long-duration space flight, but then we realized that we couldn't. We then started to talk about what we could do that related to agriculture since Carter Ives is an agricultural enthusiast. We then started to talk about a project that we had previously done, with Tomatosphere™ seeds, where we grew seeds that had been to space once. We then decided to add to that project and send them up for a second time and we went from there.

The biggest challenge was the time limit. The three of us had so many ideas and things that we wanted to add to the project. Checking grammar, spelling, and forming proper sentences was very time consuming. The proposal had to be the best. Overcoming the time limit wasn't easy but we managed it. We spent many hours in class and at home writing and rewriting to get our proposal done in time. The load was shared between the three of us evenly to limit the stress level.

Just through participating in the SSEP project there are many take-aways, but by winning the SSEP there are even more. We believe that SSEP will take us very far and will give us a lot of benefits in the future. We believe that this will help us get jobs in the future and maybe even university scholarships! SSEP has enabled us three to become excellent team workers in and out of school. It has taught us valuable life skills such as taking others' advice, collaboration, and team work. We will take all of these lessons throughout the rest of our lives.

The students' experiment flew from the Kennedy Space Centre in Florida on the SpaceX Dragon on Monday August 14, 2017 and the experiment and mission patch were captured to the ISS on Wednesday August 16, 2017. Students from the school division also participated in SSEP Mission 3. Winning experiments were able to fly with a mission patch as part of the payload. Mission patches have been part of the NASA space program since Project Mercury first put astronauts in space in the 1960s.

Students in grades 5 – 6 were involved in patch development and 176 patch designs and write-ups were created. After being narrowed down to 12 finalists, the top-voted design per class, the entire school — students, teachers, administrators, custodians, and support staff — voted to select the mission patch winner.

For SSEP Mission 11, 1,959 proposals, developed by 9,870 students from grades 5 and up, were submitted from 21 participating communities across North America. The proposals were all reviewed and 913 were sent to Community Review Boards to select three finalists from each community. The National SSEP Review Board selected the winning proposal from each community which would fly to the ISS.

Figure 1: *Left: SSEP Mission 11 Mission Patch, By Wallace Glaspey In my mission patch, I put a rocket to show that we can use science to learn more about our solar system. I put the earth to represent where we live and who we are. I put the Stonewall quarries to represent our awesome town. I put the rings encompassing the earth, with our school name (whose space club planned a winning experiment for SSEP Mission 11), to represent that we can do anything when we put our minds to it. I also put stars in the background to represent that we only understand approximately 4% of the cosmos.*
Right: Tomatoes grown from Tomatosphere™ seeds.

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Research Article: Invited Submission

Can Tomatosphere™ Tomato Seeds Germinate After Two Exposures to Space, in Mars-Like Conditions?

Carter Ives[1*], Graeme Perrie[1*], Adam Stamler[1*], Mrs. Maria Nickel[1]

[1]Interlake School Division #21, Stonewall, MB, R0C 2Z0
*Equal contributors listed alphabetically

Corresponding Author: Maria Nickel (mnickel@isd21.mb.ca)

Abstract

Tomatoes are commonly used throughout the world as a nutritious food source. Tomatoes are one of the only fruits to have had their seeds exposed to the harsh conditions of space travel. If humans are ever to colonize other planets then there will be a need for seeds to travel through space to those extraplanetary destinations. The objective of this study was to compare germination and growth of twice-space-exposed (TSE) tomato seeds with control-ground-truth (CGT) seeds. We found that the twice-space-exposed seeds had similar germination rates to the ground truth seeds but lower mortality. The TSE seeds were also taller from 18 to 56 days after planting than the CGT seeds. These results show that the frequency of space exposure is not a limiting factor for seed germination and growth.

Keywords: International Space Station, tomatoes, Tomatosphere, space research, germination

1 Introduction

Tomatoes grew in the wild before being cultivated from 700 A.D.[1] They were not cultivated earlier because they were thought to be poisonous.[1] They originated in South America in the Andes mountain range of Peru, Bolivia, Chile, and Ecuador.[1]

Tomatoes are rated the seventh most important crop in the world — the first six are maize, rice, wheat, potatoes, soybeans, and casava. Tomatoes are the most economically important vegetable globally (sold fresh and processed into many different forms and products).[2] While sold as a vegetable[3], tomatoes are botanically classified as a fruit. Tomatoes are used in many recipes and are highly nutritious with high amounts of vitamin A, vitamin C, carotenes, lycopene, and other vitamins and minerals.[2] While there are about 7,500 varieties of tomatoes grown globally [4], only one type of Canadian tomato seed has been to space. That is Tomatosphere™ seeds from 2016 (Heinz 9478 F1 Hybrid Tomato Seed) (these seeds were in space on the International Space Station (ISS) for five weeks April — May 2015). We chose the Tomatosphere™™™ seeds because we had previous experience (spring 2016) growing them. We also chose this experiment to expand our knowledge of deep space exploration and habitation on Mars or other planets.

Our proposal is to see if the Tomatosphere™ seeds, which have previously been exposed to microgravity and cosmic radiation environment along with entering and exiting Earth's atmosphere, will grow on Earth after being re-exposed to similar conditions on board the International Space Station (ISS). We will see if the growth rate and size of the tomato plant changes due to these environmental changes and compare control-ground-truth (CGT) tomato seeds, from Heinz tomato company not exposed to cosmic radiation, microgravity, and exiting and entering the Earth's atmosphere, with the Tomatosphere™ seeds that have been exposed to microgravity, cosmic radiation exiting, and entering the earth's atmosphere two times (twice-space-exposed; TSE seeds). We want to learn if a space flight can carry seeds without special handling and whether it can be used to start a food source after multiple exposures to cosmic radiation, microgravity, and the exiting and entering planets' atmospheres. This is important if humans are ever to perform interstellar travel and colonize other planets.

2 Methods

2.1 Experimental Materials

Tomatosphere™ seeds (tomatosphere.org; tomatosphere.letstalkscience.ca) were chosen was because we did the Tomatosphere™ project, during the spring of 2016, that was created by former Canadian Space Agency Astronaut Dr. Robert Thirsk, University of Guelph, Stokes Seeds, Let's Talk Science, and the Heinz tomato company. We also chose the Tomatosphere™ because we want to expand our knowledge base on the Tomatosphere™ seeds that we grew

in the spring. Our group is also very interested in deep space travel and space agriculture.

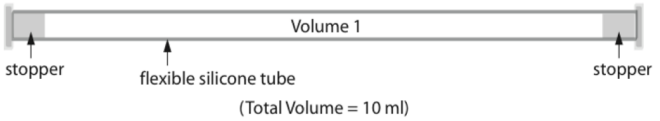

Figure 1: *Schematic of the type 1 fluids mixing enclosure (FME) mini-lab used to send the experiment to pack the seeds for space travel.*

2.2 Pre-Flight

We followed all safety procedures to ensure no contamination to the fluids mixing enclosure (FME). We cleaned and sterilized the FME according to Student Spaceflight Experiments Program (SSEP) directions, including the wearing of gloves, safety goggles, hospital face mask, and lab apron when handling the space FME and the ground truth FME.

Prior to flight, the space FME was filled with three cotton balls (Compliments Jumbo cotton puffs, Sobeys Ltd., Serial # 5574233979) and 15 Tomatosphere™ seeds from package D (Heinz 9478 F1 Hybrid Tomato Seed) were placed inside. The FME was then filled with the other two cotton balls to keep the seeds in place and prevent physical damage to them. The same procedure was followed with the ground truth FME with an additional 15 Tomatosphere™ seeds from package D.

The space FME was then be packaged in a container and shipped to Nano Racks, Houston, Texas, USA for flight. The ground truth FME was placed in the same type of container and stored in the dark at room temperature.

2.3 On-Board the International Space Station

There were no crew interactions with the space FME and no interactions with the ground truth FME, it remained in the science storage lab throughout.

2.4 Post-Flight Analysis

As soon as the seeds returned, we grew them in the Compact Three Tier Sunlight Grow garden (Veseys, www.veseys.com) to ensure all plants get an equal amount of replicated sunlight to ensure the same treatment for all plants. All seeds were planted individually into peat pellets with sterile soil at 12:05 p.m. on March 22, 2018. Each peat pot was watered with 25 mL water on days 7, 10, and 20. Each received 40 mL water on days 24 and 42.

Observations were made after 2, 10, 18, 20, 24, 32, 34, and 56 days for the 15 TSE seeds and the 15 CGT seeds not exposed to space. Results were graphed using MS Excel.

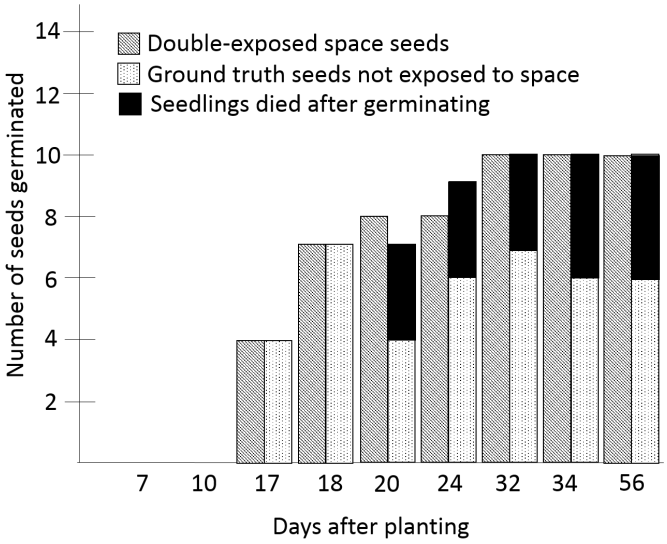

Figure 2: *Germination success of twice-space-exposed (TSE) and control-ground-truth (CGT) seeds at each observation.*

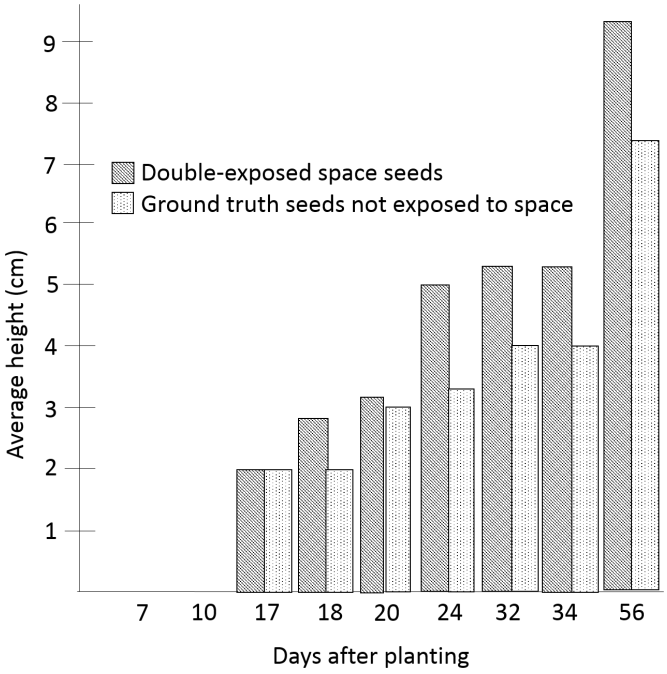

Figure 3: *Average height of germinated twice-space-exposed (TSE) and control-ground-truth (CGT) plants at each observation.*

3 Results & Discussion

The goal of our experiment was to compare growth between CGT that have not and TSE tomato seeds that have been to space two times and been exposed to all the accompanying environmental changes.

The first seeds germinated by day 17 (Fig. 2) and all germinated plants had two leaves that were light green in colour.

On day 18 all germinated plants had two light green leaves. By day 20, all plants had leaves that were noticeably larger and three of the CGT seedlings had died. Multiple leaves started growing on each plant by day 24. Leaves were larger and branches were noticed by day 32 on the TSE seeds. By day 56, thicker stalks and taller plants were seen for the TSE germinated seeds than those not exposed to space. Germination success at each observation day (Fig. 2) and plant height (Fig. 3) are presented.

Our results show that short-term, repeated space travel may be beneficial to the growth of the germinated plants. This in contrast to seeds in space for six years that showed a decrease in growth compared to the control [5]. However, our results are consistent with those of Martinez et al.[6] who found that early stages of tomato growth are accelerated following exposure to magnetic fields like those experience by our space FME during its exit and entry into Earth's atmosphere. From this literature and our results, duration of space flight appears to be more important than number of trips into space in reducing tomato plant growth. However, we have answered our question as to what effect the repeated entering and exiting of atmospheres has on tomato seeds and have found an improvement in growth.

4 Conclusions

We conducted this experiment is to see if astronauts can grow seeds in microgravity while on long-duration space flights as well to see if they can continue to regrow tomatoes as a food source on other planets such as Mars. This will help when we colonize multiple planets and travel back and forth from and to these planets.

We found that twice-space-exposed tomato seeds germinated faster and grew taller than the tomato seeds not exposed to space. Future work is needed to expand the number of seeds tested with space travel, the number of generations of seeds exposed to space, and whether there are any changes in nutritional content in the fruit from plants grown from those experiments.

5 Acknowledgements

Let's Talk Science Canada and Tomatosphere™ provided the seeds, SSEP granted the opportunity, and our teacher Mrs. Nickel made the experience happen!

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Research Article

Perceptions of Underrepresentation Among Students in STEM Fields: An Empirical Analysis

Danielle Saj[1], Annemieke Farenhorst[1], Tracey Peter[1]

[1]Dept. of Sociology, University of Manitoba, Winnipeg, MB, R3T 2N2

Corresponding Author: D. Saj (sajd@myumanitoba.ca)

Abstract

Gender bias in science has been thoroughly researched and it is well known that women are underrepresented within post-secondary Science, Technology, Engineering, and Mathematics (herein STEM) programs. Limiting women's participation in science carries heavy consequences for both the economy and scientific community. Therefore, gender inequality must be addressed with urgency. This research is focused on the following research questions: 1) are there gender differences in how students perceive the underrepresentation of women in STEM; 2) are there gender differences in student support for initiatives that could enhance gender equity in STEM? Not surprisingly, the results suggest that women consider proportionate gender representation to be more important than men (61.9% vs. 39.6%; χ^2 [2, 158]=7.05, p=0.029, Cramer's V=0.211). Further, when considering their own experiences, 20% of female respondents reported feeling underrepresented at university. These perceptions were more common among women studying STEM subjects than other subjects (33% vs. 14%; χ^2 (1, 339)=16.9, p<0.001, Cramer's V=0.22). Women expressed a greater level of support than men for many programs that would address this issue and a greater level of interest in participating in them. This interest was heightened among women who felt underrepresented. This suggests that women desire opportunities to connect with like peers through outreach and mentorship programs. These solutions require increased levels of resources, as well as the cooperation of those who do not identify as underrepresented individuals. Post-secondary institutions should consider this as they develop new ways of addressing this issue.

Keywords: Underrepresentation, Gender Bias, Students, STEM, Women

1 Introduction

Recent data has shown that although women earn a greater number of post-secondary degrees, they remain underrepresented among STEM degree earners[1]. Within Canada's prairie region, female students are less likely to major in Agriculture, Chemistry and Physics, Computer Science, Engineering, General Science and Mathematics than male students[2]. Within this region, the only STEM subject area where a greater number of female than male undergraduate students declare majors is Biology[2]. Similar findings have been documented within this subject area, across degree levels[3]. This suggests that there is generally a more equal gender distribution within biology, but also that the women earning STEM degrees remain highly concentrated within certain science subjects such as biology and are scarcely found within other disciplines[1]. For example, within the prairie region less than 15% of Engineering majors are female[2]. This is the lowest rate of female representation in any STEM discipline across all of Canada[2]. These statistics suggest that additional efforts are required to address such gender disparities across STEM subject fields.

Failure to address the gender gaps within these fields has many negative consequences for the economy and the scientific community, and therefore the underrepresentation of women in STEM is an issue which should be addressed with urgency. By ignoring the availability of female labour we inhibit the development of scientific knowledge and economic growth within the STEM industry as a whole[4]. This is particularly troubling as new STEM jobs are emerging at an increasing rate and additional labour will be required to fill these positions (Dasgupta and Stout, 2014). Although post-secondary STEM programs can play a critical role in responding to this trend by training the future workforce[2], it remains clear that these institutions have been relatively unsuccessful at closing these gaps. Women's labour is in no short supply as women make up a significant portion of both the student population within post-secondary institutions and the labour market[5]. Therefore, women should play an important role in the STEM workforce[4, 5].

Unfortunately, when women exit STEM programs the knowledge and strengths they bring to the discipline are lost[6]. Women have the ability to make valuable scientific contributions[6] and as underrepresented individuals, their

experiences allow them to make unique contributions[7]. Incorporating a greater number of perspectives provides new possibilities for scientific research[7]. Therefore, efforts to increase gender representation should be viewed as a strategy for strengthening diversity within STEM and a way of developing scientific inquiry[6].

Women navigating paths towards STEM careers must complete relevant training within post-secondary institutions. Unfortunately, the climate women enter within many university STEM programs has been identified as a factor which prevents their retention and achievement[8]. Many women report that they have faced gender bias, as well as discrimination and harassment throughout their education[6]. For example, women may be prevented from accessing the same experiences within science and technology throughout their developmental years and continue to see a lack of equal opportunities for women in science throughout their academic careers, which contributes to limited experiences[5]. From an early age boys are socialized in a way that encourages them develop an interest in activities that relate to STEM, whereas girls are socialized to develop an interest in other areas[5]. Therefore, girls may not receive the same exposure to these activities that boys do[5]. For example, this may include learning about technology through video games[5].

Due to the limited number of women in STEM programs, women lack social support which may allow them to deal with adverse situations. The development of interpersonal relationships is considered to contribute to a stronger sense of belonging among STEM students[9]. Further, Rainey *et al.*[9] observed a correlation between a student's sense of belonging within their major and the number of students of that gender studying that major. For instance, male students were more likely than female students to indicate that they felt like they belonged in their STEM major[9]. Furthermore, the underrepresentation of women in STEM creates multiple barriers to establishing successful role model and mentorship relationships[6]. Many female STEM students feel they do not have female role models to look up to throughout their education[4, 6]. This would suggest that women in STEM require additional ways of networking with like peers and role models[4].

Therefore, post-secondary institutions should be interested in taking steps to address unsatisfactory program climates and feelings of underrepresentation among students as this could enhance the quality of the learning environments within the institution. In response to the issue of women's underrepresentation in STEM, many post-secondary institutions have begun to seek out and initiate strategies which encourage and support women's participation in STEM. The present study has two main objectives:

1. to identify factors that influence perceptions of underrepresentation among post-secondary students, which would include gender and program of study;
2. to assess students' interest in initiatives which create supportive networks for female STEM students.

2 Methods

Research Questions

Building on the literature, our work is guided by the following research questions: 1) Are there gender differences in how students perceive the underrepresentation of women in STEM? and 2) Are there gender differences in student support for initiatives that could enhance gender equity in STEM?

2.1 Data Collection

The method employed for this study was the was that of the University of Manitoba Student Equity Survey (UMSES)[10]. The survey addressed a great variety of themes as the overall purpose was to better understand the potential differences in university climate for students in STEM and non-STEM fields. The areas of discussion included: 1) choice to pursue current academic program; 2) anticipated career path; 3) opinion on equity programs in terms of the importance of such programs and predicted participation in such initiatives; 4) perceptions of underrepresentation at university in general and within their program; 5) incidents of discrimination and harassment experienced or witnessed; and 6) General demographic information.

This study specifically addresses opinions on equity initiatives (UMSES discussion point 3), perceptions of underrepresentation (UMSES discussion point 4), and general demographic information (UMSES discussion point 6).

2.2 Description of Variables

The measures included in the survey were designed specifically by the Principal Investigator of the umses, Jenna Rapai as part of her M.Sc. research. Her decisions were informed by a general review of the literature, public information, and personal experiences[10]

The first factor (Factor 1) pursued in analysis was gender. A crude definition of gender which allowed students to differentiate between "Female", "Male", and "Other" gender identities was pursued for statistical purposes. Due to an inadequate sample size of respondents who reported marginalized gender identities, this study cannot account for the experiences of these students. In social research, researchers are

Table 1: *Questions used to measure students' perception of their own underrepresentation in STEM (Perception 1).*

#	Perception Descriptor
1a	Do you feel underrepresented in any of the courses you take?
1b	Do you feel underrepresented in your program?

Table 2: *Common explanations of why women are underrepresented in STEM used to measure students' perceptions (Perception 2).*

#	Perception Descriptor
2a	Male students are disruptive towards female students in learning
2b	Women are more interested in arts than science
2c	Women in STEM are unfairly evaluated at a higher standard
2d	Examples in education biased to males
2e	There is a lack of female professors to act as role models
2f	Males are hostile toward women in STEM
2g	There are not enough female professors who can act as role models for female students in STEM
2h	Hiring committees consist mainly of men
2i	Policy makers consist mostly of men

Table 3: *Community building (CB) initiatives.*

#	CB Initiative Descriptor
CB 1	Outreach within elementary schools
CB 2	Outreach within high schools
CB 3	Read brochure or literature
CB 4	Voluntary workshops for faculty
CB 5	Guest speakers who are members of underrepresented groups speaking about their experiences
CB 6	Voluntary workshops for students
CB 7	Mandatory workshops for students
CB 8	Mandatory workshops for faculty
CB 9	Blogs or Twitter feeds
CB 10	Apply to a specific program to be eligible for scholarships and bursaries

Table 4: *Structural change (SC) initiatives.*

#	SC Initiative Descriptor
SC 1	More opportunities for part-time students
SC 2	Longer library hours
SC 3	Electronic library resources
SC 4	Formalized mentorship programs
SC 5	Centre providing support
SC 6	Conferences

bound by ethics protocols which require a minimum of five similar cases in order for results to be shared. Therefore, this study centers around differences between female and male experiences within the university setting.

The second factor (Factor 2) pursued in analysis was program of study. Students had the opportunity to indicate which academic faculty they were enrolled in. During data analysis the researchers differentiated the responses by "STEM program" and "non-STEM program". The "STEM program" category included the Faculties of Science, Engineering, and Agriculture.

In order to measure students' perceptions of their own underrepresentation (Perception 1, Table 1), questions were asked with options for responses of "Yes", "No", and "Sometimes". "Yes" and "Sometimes" considered indicators of perceptions of underrepresentation. In our analysis, students who answered yes to at least one of these questions are described as "feeling underrepresented". Within this study the focus is limited to examining perceptions of underrepresentation on the basis of gender identity.

In order to measure students' perceptions of the underrepresentation of women in STEM (Perception 2, Table 2), the survey questions addressed common explanations of why women are underrepresented that have been discussed across the literature on the topic. Students then expressed their level of agreement with the statement based on their perceptions of why women may be underrepresented on a likert scale as possible responses ranged from "Strongly agree" to "Strongly disagree".

The survey questions also focused on the importance of various proposed initiatives and students' predicted participation in them. These initiatives were proposed by the original authors of the survey and were based on student initiatives that could be implemented by universities at no or low cost, initiatives that could appeal to all students, and initiatives that are clearly focused on advancing women students in STEM, including initiatives that might require substantial funding.

A first set of initiatives were based on community building (CB) programs (Table 3). Students expressed how important each initiative was with their options ranging from "Very important" to "Very unimportant" and rated their predicted level of participation from "Very likely" to "Very unlikely".

The questions regarding a second set of initiatives also addressed potential changes to the organizational structure (structural changes: SC) of the institution (Table 4). Students expressed how important each initiative was with their options ranging from "Very important" to "Very unimportant" and rated their predicted level of participation from

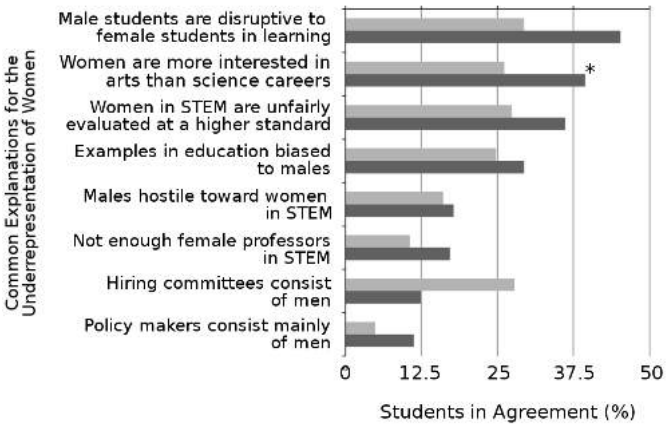

Figure 1: *A comparison of how male (grey bars) and female (black bars) respondents rated their agreement with common explanations of why women are underrepresented in STEM (Perception 2). Each of the common explanations variables were proposed by the original authors of the survey. Significant differences between men and women have been indicated with asterisks (*).*

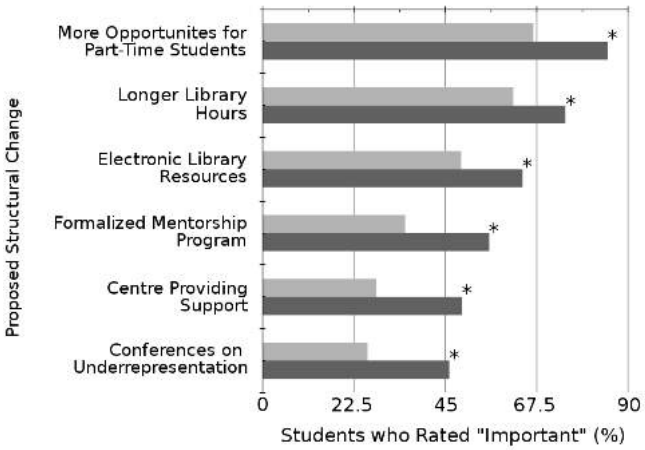

Figure 3: *A comparison of how male (grey bars) and female (black bars) respondents rated the importance of proposed structural changes aiming to address the underrepresentation of women in STEM. Each of the structural initiative variables were proposed by the original authors of the survey. Significant differences between men and women have been indicated with asterisks (*).*

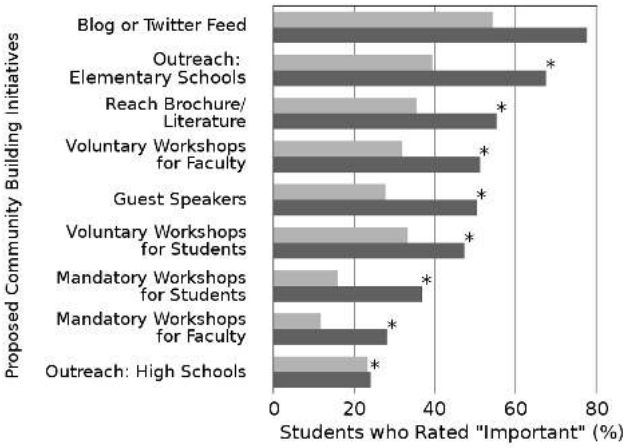

Figure 2: *A comparison of how male (grey bars) and female (black bars) respondents rated the importance of the proposed initiatives aiming to address the underrepresentation of women in STEM. Each of the initiative variables were proposed by the original authors of the survey. Significant differences between men and women have been indicated with asterisks (*).*

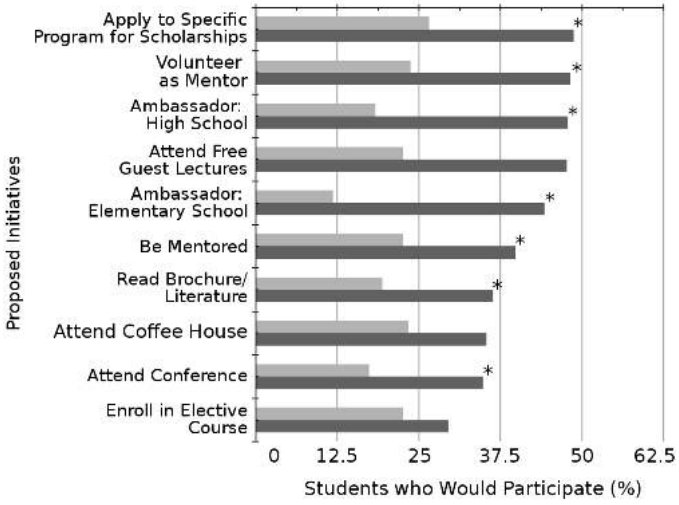

Figure 4: *A comparison of how male (grey bars) and female (black bars) respondents rated their predicted participation in the proposed initiatives aiming to address the underrepresentation of women in STEM. Each of the initiative variables were proposed by the original authors of the survey. Significant differences between men and women have been indicated with asterisks (*).*

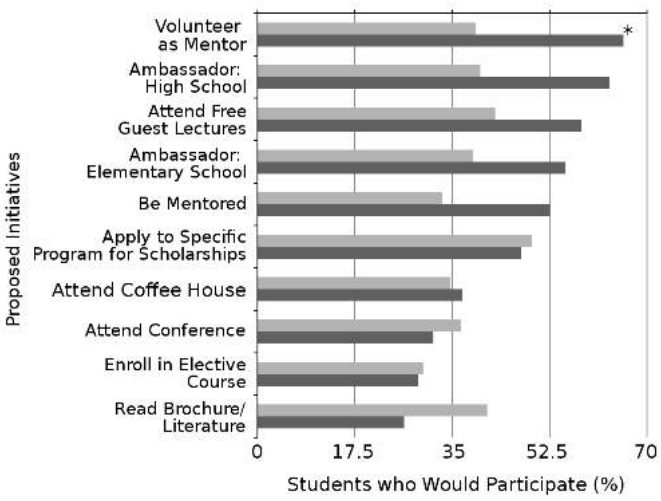

Figure 5: *A comparison of how female STEM respondents who perceive and do not perceive that they are underrepresented rated their predicted participation in the proposed initiatives aiming to address the underrepresentation of women in STEM. Significant differences ($p<0.5$) between men and women have been indicated with asterisks (*); grey bars: did not feel represented, black bars: felt represented.*

"Very likely" to "Very unlikely".

The sample was composed of male and female undergraduate and graduate students at the University of Manitoba, with a targeted oversampling framework of students within STEM faculties. The gender distribution also included an overrepresentation of female students, with a total of 369 female and 163 male respondents. With regards to recruitment, the email addresses of students currently enrolled in undergraduate programs were obtained by the research team and all respondents were recruited through an email invitation. All of the surveys were filled out online using an online survey program. Once responses were collected, the data was analyzed by the research team using SPSS. Bivariate statistics were generated using chi-square.

3 Results

3.1 Underrepresentation

3.1.1 General Perceptions of Gender Underrepresentation

Students' responses illustrate clear differences in perceptions of underrepresentation within university in general and within their program of study, based on respondent's gender (Factor 1) and status as a STEM vs. non-STEM student (Factor 2). In relation to Perception 1, students' perceptions of how their own identities are represented varied. Overall, 20% of female respondents reported feeling underrepresented at university, regardless of their program of study. However, female students enrolled in a STEM program (33%), were significantly more likely than those in non-STEM programs (14%; χ^2 [1, 339]=16.9, p<0.001, Cramer's V=0.22) to indicate that they feel underrepresented. In relation to Perception 2, students expressed differences in how important they regarded the representation of marginalized identities. STEM students from marginalized groups were more likely to think it is important that their respective identities are proportionately represented. For example, 61.9% of female STEM students thought proportionate gender representation was important, compared to 39.6% of male STEM students (χ^2 [2, 158]=7.05, p=0.029, Cramer's V=0.211).

3.1.2 Opinions on Possible Causes of Gender Underrepresentation

In relation to Factor 1, generally among the STEM population, males and females expressed similar levels of agreement with the proposed explanations of why women are underrepresented (Fig. 1). For example, in response to statements such as "Male coworkers in STEM fields sometimes behave in a hostile way towards women in their fields" (Perception 2f), 26.3% of females and 21.3% of males were in agreement. This difference was not statistically significant.

In response to the proposed explanations of why women are underrepresented, the only significant difference observed between male and female STEM students was Perception 2b, "Women are more interested in arts than science careers". In response to this statement male students (25.0%) were almost two and a half times more likely than female students (10.5%) to agree (χ^2 [2, 157]=12.69, p=0.001, Cramer's V=0.284).

Female respondents who reported feeling underrepresented due to their gender were significantly more likely, than female respondents who did not feel underrepresented on the basis of their gender, to agree with "there are not enough female professors that can act as role models for female students in STEM", (Perception 2g) (42.1% vs. 22.7%; χ^2 [2, 113]=6.6, p<0.05, Cramer's V=0.24).

Among the overall population of students, female students were significantly more likely than male respondents to agree with Perception 2i, that policy makers consist mostly of men (45.2% vs. 29.4%; χ^2 [2, 525]=17.6, p<0.001, Cramer's V=0.18), Perception 2h, hiring committees consist mainly of men (39.4% vs. 26.4%; χ^2 [2, 524]=21.3, p<0.001, Cramer's V=0.20), and Perception 2g, that there are not enough female professors teaching in STEM disciplines (36.1% vs. 27.3%; χ^2 [2, 527]=6.5, p<0.05, Cramer's V=0.11).

3.2 Support for Initiatives

3.2.1 Gender Differences

In relation to Factor 1, support for a number of initiatives aiming to increase gender representation varied greatly between STEM men and women (Fig. 2), with many statistically significant differences. STEM women were significantly more likely than men to indicate that almost all of the initiatives were important. The only exception was reading a university blog or Twitter feed (CB Initiative 9). Roughly a quarter of both men (23.4%) and women (24.1%) who responded indicated that this would be important. Interestingly, among both female and male STEM students the initiatives or programs which were believed to be of the greatest importance were based around outreach, with significantly greater rates of support from female students. Over three-quarters of STEM females (79.0%) and more than half of males (60.4%) thought that CB Initiative 2, outreach programs to high schools, was important in order to promote the participation of a given gender in a field where they are underrepresented (χ^2 [2, 158]=7.01, p=0.030, Cramer's V=0.211), while nearly two-thirds of female students (63.8%) and less than half of males (43.4%) reported that CB Initiative 1, outreach within elementary schools would be important, (χ^2 [2, 158]=7.19, p=0.027, Cramer's V=0.213).

3.2.2 Structural Changes

In relation to Factor 1, it is clear that female STEM students were significantly more likely than male STEM students to support policy amendments related to the structural initiatives (Fig. 3). These suggestions are considered to be "structural" in nature as they would require a higher level of involvement of university administration and would potentially change the organizational structure of the academic institution. When questions center on issues of childcare, for instance, female STEM students were more supportive than male STEM students of SC Initiative 1, providing more opportunities for part-time studies, as it may help students with children to complete their degree in a timelier manner (85.0% vs. 66.7%; χ^2 [1, 206]=9.6, p<0.01, Cramer's V=0.22).

A significantly higher number of female than male respondents also supported SC Initiative 2, extending library hours, in order to assist students with young children 74.6%; χ^2 [1, 208]=4.0, p<0.05, Cramer's V=0.14). There were significant differences observed between female (55.8%) and male respondents (35.1%) in regard to SC Initiative 4, the implementation of formalized mentorship opportunities in programs with gender underrepresentation. Significantly more women than men were in support of such an initiative (χ^2 [1, 207]=8.8, p<0.01, Cramer's V=0.21). SC Initiative 5, the creation of a centre that would provide support for programs with gender underrepresentation, was also thought to be significantly more important among women than men (49.1% vs. 28%; χ^2 [1, 207])=9.6, p<0.01, Cramer's V=0.22)(Fig. 3).

3.3 Participation in Initiatives

3.3.1 Gender Differences

In relation to Factor 1, results regarding respondents' predicted participation in such initiatives also varied greatly between male and female STEM students (Fig. 4). The greatest variances were in response to CB Initiative 2, high school (41.9% vs. 21.2%; χ^2 [2, 157]=9.03, p=0.011, Cramer's V=0.240) level and CB Initiative 1, becoming an ambassador in an outreach program at the elementary school level (37.1% vs. 21.6%; χ^2 [2, 156]=11.32, p=0.003, Cramer's V=0.269). Among female STEM students, half of the proposed initiatives received over 40% of respondents' interest (Fig. 4). Notably, programs that involved "role modeling" were among those that received such levels of support. For example, among the community building initiatives this would include CB Initiative 2, becoming an ambassador in an outreach program to high schools (47.8%), and Initiative 1, becoming an ambassador in an outreach program to elementary schools (44.2%); and among the structural initiatives this would include SC Initiative iv) participating in a formalized mentorship program as a volunteer peer-mentor at the university (48.2%).

Although not shown, female STEM students who thought the proposed initiatives and programs were important were significantly more likely to express an interest in participating in them.

3.3.2 Differences Based on Perceptions of Underrepresentation

In relation to Factor 1, differences in respondents' interest in participating in the initiatives were also analyzed among STEM females who felt underrepresented and those who did not (Fig. 5). Most of these differences were insignificant. However, female STEM students who felt underrepresented were significantly more likely than those who did not feel underrepresented to express interest in SC Initiative 4, by both participating in the formalized mentorship program as a volunteer peer-mentor (65.8% vs. 39.2%; χ^2 [2, 112]=7.6, p<0.05, Cramer's V=0.26) and receiving mentorship from another peer (52.6%), CB Initiative 2, becoming an ambassador in outreach programs both at the high school (63.2%) level, CB Initiative 1, becoming an ambassador at the elementary school (55.3%) level, as well as CB Initiative 5, attend-

ing free guest lectures given by members of underrepresented groups (58.3%).

4 Conclusions

We observed that women report feeling underrepresented at the university at a greater rate than men and further, women who study within STEM programs are more likely to feel underrepresented than those who study within other fields.

In response, these women particularly desired the implementation of programs which help to strengthen their social ties within STEM. The results revealed that STEM women overwhelmingly indicated that they would likely participate in mentorships and outreach programs, while many also recognized that there could be structural changes made within the University which would ensure women are supported in STEM programs and are better connected with their like peers. Furthermore, support and predicted participation in the proposed initiatives was also greater among women who indicated that they felt underrepresented themselves, which suggests that these women are eager to find new opportunities to connect with like peers in their programs. Post-secondary institutions should prioritize their role in improving the learning and working climate for their members and should develop a response to women's underrepresentation which considers these findings. Based on these findings we would propose two recommendations to post-secondary institutions.

Firstly, increased support should be provided to underrepresented gender groups. Many of these students are eager to enact change by participating in community building initiatives, but would require resources to establish such programs. There is a large portion of literature which supports the benefits of community building initiatives for underrepresented students in STEM. Recent research by Robnett[11] argues that in order to reduce the negative effects of gender bias, supportive networks can be created through various initiatives targeting women studying in STEM fields. Her recommendations include developing interventions to meet the specific needs of the women in specific STEM programs and partnering with outreach programs. Therefore, discretion over the structure of such programs will be held by each institution. However, in order to insure that the structure reflects the needs of the students who are engaged in the program, post-secondary institutions should seek input from students as they develop these programs. In addition to students' prior notions about STEM fields or the ways students believe they are perceived by their peers, the sense of community within their program is an important factor that influences retention and achievement[12]. There could be many benefits to implementing community building programs which would be received by the student's and post-secondary institutions, including a greater level of inclusivity within University STEM programs, higher rates of enrolment and retention and improved academic performances among women.

In order to ensure that the learning climate within STEM programs becomes a more socially inclusive place for students of all gender identities, men and women who do not feel underrepresented would need to be engaged in this process. Encouraging a broader audience to take part in learning and discussing these issues would require creative efforts. Many STEM students and professors may vary in their understanding of equity, equality and diversity issues; however, in order to effectively carry out solutions, sharing of this knowledge will be critical. For example, one proposed solution could be discussing these issues within the courses that students are taking. This could help to familiarize everyone with the experiences of underrepresented students and ensure information about strengthening gender equity will be communicated in a consistent manner to all.

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Research Article

Analysis and Prediction of Patterns in Futures Trading Datasets Using LSTM

Beom-Jin Park[1], Christopher Chmelyk[1], Daniel Heslop[1], Gu Zhengyu[1]

[1]Dept. of Computer Science, University of Manitoba, Winnipeg, MB, R3T 2N2

Corresponding Author: B. Park (parkb346@myumanitoba.ca)

Abstract

One of the most promising tools in recent years for the analysis and prediction of time series data, which includes financial market data has been the use of neural networks. While the complexities inherent in market prediction have confounded machine learning techniques, newer deep learning techniques such as long short-term memory (LSTM) show promise in their ability to predict using time series datasets. This paper will explore the feasibility of using a recurrent neural network (RNN) with LSTM as a predictive tool for use with futures trading data, and determine the time ahead with which this particular tool can maintain its predictive accuracy. Using a dataset comprised of all futures trading occurring on the Bourse de Montréal (TMX) during a nine-month period from January to September 2015, we assessed the predictive effectiveness of an RNN in predicting the price of front-end contracts for the futures symbol BAX. We found that while an RNN provided a degree of short-term predictive capability, this capability did not extend beyond a couple of days. Although it failed as a trading instrument to predict futures prices, the RNN could detect, identify, and reflect underlying trends in the data, indicating the tool may hold promise in the detection of trading patterns.

Keywords: Machine Learning, Deep Learning, Time Series Dataset, Prediction, Futures Trading, Market Prediction

1 Introduction

For the obvious reason of profit, mankind's interest in market prediction has existed for as long as markets themselves. While many predictive tools and methods have been employed over the years, relatively recent advances in computing power have enabled new strategies to be attempted. Prediction is one of the most extensively applied tasks in time series data mining[1]. This results from the fact that prediction is a logical extension of data mining processes. There have been many methods used for the purposes of prediction, such as reinforcement learning with Q-learning, Bayesian networks, and recurrent neural networks.

Since their inception, neural networks have been considered one of the best solutions for prediction in financial markets[2]. Because of the complex and chaotic nature of financial market data, traditional statistical methods of prediction and analysis can be of limited utility. This is where machine learning and neural networks may prove useful[3]. By training recurrent neural networks to map input sequences to output sequences for applications in sequence recognition or time series prediction, we can often get an accurate predictive output. This requires a system to store and analyze information computed from past inputs in order to produce the desired useful resulting output. Since recurrent neural networks (RNNs) have an internal state that well-represents time series, they are uniquely suited for the task of prediction relating to time series datasets. The construction of a recurrent neural network is such that it allows retaining of past inputs for analysis while weighting it according to its timestep or 'distance' from the current index. A recurrent neural network can be used to transform an input sequence into an output sequence, whereas static artificial neural networks (ANN), which do not have that context memory, cannot store information for an indefinite period.

Analysis of market data is difficult to perform, due to its cyclic and seasonal variations, as well as the irregular and seemingly random movements these data exhibit[3]. These factors, among others, have contributed to many machine learning techniques failing to demonstrate success in price prediction[4]. The advent of deep learning techniques, such as LSTM, led some researchers to investigate whether these could provide more success. While some researchers have claimed a degree of success in using RNNs with LSTM to perform market prediction[5], however the degree of this success is limited. By maintaining a small window size with which to train their RNNs, the models in these prior attempts react primarily to only very recent data, creating a predicted data set that while appearing to be accurate, in fact provides predictions only a very short time ahead. Our experimental goal was to use an RNN with LSTM to accurately predict the price of futures contracts, as well as to determine how far ahead in

time this technique can assist in prediction.

When analyzing time series datasets, there are two main goals. The first is in modeling the time series in order to gain an understanding of the underlying mechanisms at work to generate the data. The second is forecasting or predicting of future values based on current or previous values in the time series[6]. The main idea of using a learning algorithm in recurrent neural networks is to compute the gradient descent of a cost function regarding the weights of the network. This is particularly useful for the task of prediction with time series datasets. Time series data by their nature are often high-dimensional. It is useful, therefore, to attempt to reduce the dimensionality when analyzing time series datasets. There are two common approaches to this. The first, and most simple, is "sampling", and consists of simply taking the values of the data at regular intervals. The problem with this method is that it has the potential to distort the overall shape of the data unless the sampling rate is sufficiently high. However, high sample rates are what we are trying to avoid. The other common method of dimensionality reduction is to calculate the mean of each segment or bin[7]. The latter method is what we employed to produce single contiguous time series to feed to the RNN.

1.1 Definitions & Background

1.1.1 Recurrent Neural Network (RNN)

An RNN is a class of artificial neural network in which connections between units form a directed cycle. This allows a RNN to exhibit temporal behaviour. Unlike feedforward neural networks, RNNs can use their internal memory to process arbitrary sequences of inputs. This makes them applicable to tasks where each piece is dependent in some fashion on the one that preceded it, such as unsegmented, connected handwriting or speech recognition[8].

RNNs can be configured to accept one or more vectors of input to produce one or more vectors of output. Possible configurations of RNN input and output are one-to-one (one input following through to one output), one-to-many (one input resulting in many outputs), and many-to-many (many inputs resulting in many outputs with each input either resulting in its own output or in different or different numbers of outputs). For our experiments, we employed a many-to-one configuration, providing the RNN multiple vectors of input to produce a single output stream of data. Since we limited our investigations to examining the RNN's predictive ability on a single futures security this justified, it made sense to only consider that as a single output. We chose the many-to-one configuration to minimize the the risk of ignoring factors which affected the output vector of interest, but in a manner we could not foresee.

1.1.2 Long Short-Term Memory (LSTM)

Long short-term memory networks are a special kind of RNN, capable of learning long-term dependencies. They were introduced by Hochreiter and Schmidhuber in 1997, and were refined and popularized by numerous people in subsequent works. LSTM algorithms work very well on a wide variety of problems, and are now widely used in time series predictions. LSTM's are explicitly designed to avoid the long-term dependency problem, whereby a current value is in some way dependent on another that long preceded it. By default, they are designed to retain information for long periods of time. Because of this, they do not struggle in dealing with extended time periods[9].

All recurrent neural networks are in the form of a chain of repeating modules. In standard RNN's, this repeating module has a very simple structure, such as a single tanh layer.

In the detailed workings of a RNN with LSTM[9], X_t is the input at time t, and h_t is the output at time t. The repeating modules of the RNN are denoted by A. The sigma layers determine values to pass on, and their weighting. The tanh layer generates a vector of new candidate values that could be added to the state. The 'x' and '+' are gates that allow certain values through.

1.2 Additional Definitions

1.2.1 Machine Learning

The concept of machine learning has been around for well over half a century. However, it has been only within the past few decades that computing power and understanding has allowed for great advances in this area. The neural network is a part of the topic of machine learning. The design and implementation of neural networks was inspired by the study of naturally occurring biological systems, such as the human brain. The idea that there are nerve cells which process stimuli and communicate with neighbouring cells to produce a response was the foundation of neural network design[10]. Neural networks have shown to be extremely accurate at prediction of future values based on previous information, and have been successfully implemented in various fields from facial recognition to intrusion detection in computer security. Because of the wide applicability of neural networks, they continue to be a field of active research, and have been receiving much attention.

1.2.2 Deep Learning

Deep learning allows computational models that are composed of multiple processing layers to learn representations of data with multiple levels of abstraction. Deep-learning methods are representation-learning methods with multiple levels of representation, obtained by composing simple but

non-linear modules that each transform the representation at one level (starting with the raw input) into a representation at a higher, slightly more abstract level. The key aspect of deep learning is that these layers of features are not designed by human engineers — they are learned from data using a general-purpose learning procedure.

1.2.3 Time Series Dataset

A time series dataset is a dataset consisting of a series of values or readings taken at certain intervals over a period of time. Time series datasets are most often quite long, and are considered to be smooth, i.e. neighbouring values are within predictable ranges of each other, as opposed to being completely random[11]. Time series data, due to its nature, has some inherent difficulties that have to be overcome to be properly analyzed. First, they often contain quite a bit of signal noise and high degree of dimensionality. This needs to be accounted for when processing the datasets. Second, time series datasets may not contain enough information to properly understand the processes involved. In other words, there may be variables outside of the scope of the data that affect the values recorded. Especially when working with a complex system such as financial data, there can be nearly an unlimited number of outside factors that could affect the values.

Another difficulty with time series data is the time dependence. For example, a prediction using a specific value at a certain time will not necessarily provide the same result as a prediction using the same value at a different time. To combat this issue, it may be necessary to include more past data, or keep memory of inputs. This can lead to rapidly growing memory requirements, especially when the length of dependencies may not be known with any certainty. A related difficulty is that time series datasets are commonly non-stationary. This means that over time, the characteristics of the data may change or shift. This is often handled by treating the data in the frequency domain, rather than the time-domain, by treating them with wavelet or Fourier transforms.

Finally, most features used with time series data must be invariant with respect to translation in time: given the same set of inputs, we should not expect a different output, simply because the inputs occurred at a different time[12]. In the case of financial data such as with the futures market, this is not the case, as transactions occur at seemingly random intervals. This issue can be resolved during preprocessing by grouping or "binning" the data.

1.2.4 Prediction

In times series datasets, prediction is the use of the knowledge that data points in time series datasets are generally within predictable ranges of each other in order to determine the next few values of a series[1].

1.2.5 Futures

Futures contracts are legal agreements to either buy or to sell an item (most often a commodity of some sort) for a specified price at a future point in time. Whereas the stock markets trade stocks in individual companies, futures trading involves the trading of contracts. One thing to note is that futures contracts eventually expire (mature), at which time the holder of the contract could be obliged to fulfil the demands of the contract. This is an interesting quirk of futures trading that must be considered when making predictions or analyzing patterns in trading. As the expiration approaches, contracts are rolled over to the next time period, and trading generally decreases up until the contract is expired[13]. The contract then has a new expiry date. This leads to some preprocessing challenges in producing a single contiguous time series for use in an RNN.

1.2.6 TMX

The Bourse de Montréal, or Montreal Exchange (TMX), was founded in 1832 in Montréal, Québec, and is Canada's oldest exchange. It is the only derivatives exchange in Canada, with expertise in Financial Derivatives Markets, Clearing Services, Data Analytics, and Information Technology Solutions[14].

1.2.7 BAX

The BAX futures contracts are three-month Canadian Bankers' Acceptance Futures traded on the Montreal Exchange. Bankers' acceptances are short-term debt obligations that are backed by a major bank. Because they are backed, the payment of principal and interest on the debt is guaranteed. Investors can purchase these contracts at a discount based on yield, and collect face value at maturity. The maturity period of bankers' acceptance contracts generally ranges from 30 days to one year. Bankers' acceptances were first introduced in Canada in 1962, and BAX futures were the first interest rate contracts to be traded on the Montreal Exchange (BAX is a trademark of TMX[15]). The BAX futures contracts were the futures contracts analyzed for the purposes of this report.

2 Methods

2.1 Description of the Dataset

The dataset consisted of nine months' worth of event history from the Montreal Futures Exchange (TMX), from January to September 2015. This dataset comprised numerous different futures products, and all events related to these products. Examples of related events included bids, asks, trades,

Table 1: *Summary of trading records on TMX, Jan.–Sept. 2015*

Symbol	Records
BAX	1,114,781
CGB	4,922,494
CGF	6,880
CGZ	43
SCF	45
SXA	3
SXF	2,434,429
SXK	5
SXM	13,235
SXY	4
Total	8,491,919

and other specialized events, related to such activities as options and strategy trading. A bid event is defined as an offer to purchase one or more contracts of a specific future for a certain price. An ask event is defined as an offer to sell one or more contracts of a future at a specified price. A trade occurs whenever the prices of an ask and a bid match, and a transaction occurs.

The dataset was provided in 187 comma-delimited flat files, one containing all events for each of the 187 trading days that occurred between January 2 and September 30, 2015. These files, comprising some 80 million records were imported into a Microsoft SQL Server database table for easier filtering and processing. Since our analysis was concerned with the actual trading price of the futures contracts, we dealt only with actual trade events, filtering out all of the other events that did not result in futures contracts changing hands. Including only records that consisted of actual trades resulted in a dataset of about 8.5 million records, consisting of the trades that occurred over all futures products and contracts. A summary of these records can be seen in Table 1.

Many of these products were traded infrequently, with irregular intervals between each series of trades. Of the 10 futures traded on the TMX, only BAX contracts were traded in high volumes on each of the 187 trading days for which we had data. As a result, we decided to focus our study on this particular futures symbol. There were 1,114,781 BAX-symbol trades that occurred over the nine-month period.

Trades occur randomly and at irregular intervals, however RNNs require continuous time series data which is spaced at regular intervals. This was handled by placing the data from all trades into regularly-spaced bins, each of which contained the total volume of trades for a contract, as well as the average price at which it traded during the time interval of the bin. Trading occurs on the TMX from 6:00 a.m. to 4:00 p.m., Mondays to Fridays, excluding holidays. Since an RNN requires a high number of records in its training data set, we decided to maximize the number of bins that our data set could support. This goal was limited by the requirement to ensure that the bin size was large enough to ensure that all bins were populated with trading activity. By running SQL queries on the data set, we determined that while a half-hour bin size did not ensure all bins were populated, maintaining the bin size at 1 hour ensured that trades occurred for each front-end BAX contract in that bin interval.

Analyzing futures trading with an RNN is further complicated by the fact that futures contracts are time-limited, with one contract expiring every three months. For example, the BAXH15 contract matured in March 2015, while the BAXM16 contract expired in June 2016. A contract which is not delivered at maturity is automatically "rolled over" into the next-expiring contract. For example, anyone left holding the BAXH15 contract when it expired in March 2015 had their holdings automatically rolled over to the BAXM15 contract. As such, if we consider only the front-end contract (the one maturing next), it is possible to analyze the data as one perpetual time series, rather than a set of several shorter-duration time series, if we have a means to handle the rollover.

Several different techniques that can be employed to handle this problem[13], each with their own strengths and weaknesses. We chose to employ the perpetual time series model, to ensure as smooth as possible a transition between the front-end contracts during the rollover. This technique avoids the uncertainty associated with guessing at which point the activity of the new contract supersedes that of the expiring contract by treating the trading data as a weighted average of the two contracts[13]. This method suffers from the fact that, since it averages the price between two contracts, it does not necessarily provide the "real" price of any specific contract. That said, of all of the techniques used to splice futures trading data, this one provides the smoothest transition[13] and is thus best suited to provide input data to an RNN.

Rather than choosing an arbitrary smoothing period over which to average the two contracts (without any *a priori* knowledge of the optimal period to choose), we determined to average the two contracts with the nearest expiry dates on a sliding scale to produce a perpetual time series using the following algorithm:

1. On the expiry date (last trading bin), t_0, of the expiring contract, C_0, count the number of bins, N, to the expiry of the next expiring contract, C_1
2. For t_i, $0 \leq i \leq N$, using data from C_1 and C_2, calculate:
    a. $\text{volume}(t_i) = [\text{i}\times 7\text{volume}_{C2}(t_i) + (N - i)\times\text{volume}_{C1}(t_i)]/N$

b. $\text{price}(t_i) = [\text{i} \times \text{price}_{C2}(t_i) \times \text{volume}_{C2}(t_i) + (N - i) \times \text{price}_{C1}(t_i) \times \text{volume}_{C1}(t_i)] / [\text{i} \times \text{volume}_{C2}(t_i) + (N - i) \times \text{volume}_{C1}(t_i)]$

This algorithm was implemented in an SQL query. The resulting data set consisted of 1870 records (one for each hour of 187 trading days). Graphs of these data can be seen in Fig. 1 and 2.

While the volume data appear noisy and chaotic, the data for price appear to follow an orderly enough pattern for an RNN to predict. While the volume data may have been far too noisy to predict with any degree of accuracy, we observed that the large spikes in trading volume corresponded with accompanying increases and decreases in price. As a result, we determined to include the volume data as an input to the RNN (along with the price), to generate a predicted price.

2.2 Execution

When we try to decide the parameters we use for our training process, we need to choose them considering their trade-offs with respect to time and accuracy. For example, if the accuracy of our prediction did not improve significantly beyond a certain number of iterations, increasing them further serves to greatly increase processing time with diminishing returns. Our goal in experimentation is to find an optimum balance between these factors. In an RNN, the epoch refers to the number of iterations used to train our neural network. The RNN processes the data in batches, a certain number of records at a time. Once the RNN has processed the entire training data set (one epoch), it attempts to minimize the loss/error on each batch of records by updating the weights on each batch. The neural network tries to find the optimal value where the error of a batch of data can be minimal for all epochs.

The loss (error) remaining at the completion of each epoch of training is shown in Fig. 3. Following a steep reduction between 1 and 20 epochs, further reduction is greatly limited. Since almost no further reduction in loss was observed for several epochs leading up to epoch 100, this was the number of epochs we chose for our experiments.

Ideally, with unlimited processing power, we would analyze each line of the training dataset and find the perfect value to reduce the loss value to zero. However, this scenario, corresponding to a batch size of 1, would be incredibly inefficient. Further, such a scenario would also be exceptionally susceptible to noise in the dataset. Rather, to reduce the processing we require, while minimizing the impact caused by outlier data, the RNN processes the data in larger batches at once, updating the mean weights of the data points as it proceeds.

Plots of the processing time and final loss value determined for batch sizes of 16, 32, and 64 for runs of 30 epochs and 60 time step values per prediction point are shown in Fig. 4. We observe that processing time required declined consistently as the batch size was increased, as we would expect. Moreover, an excessively-large batch size resulted in an increased loss. The most interesting observation from Fig. 4 is that, beyond a certain point, a reduction in batch size does not result in an improvement in the final loss value.

Based on these results, we determined our optimum batch size to be on the order of 32. At this level, we optimize the accuracy of the prediction, while limiting the length of time required in processing the training data.

The number of time steps (records) used in the prediction is another parameter of the RNN. As with the previous parameters, the desired accuracy of the prediction must be weighed against the processing time required to achieve it.

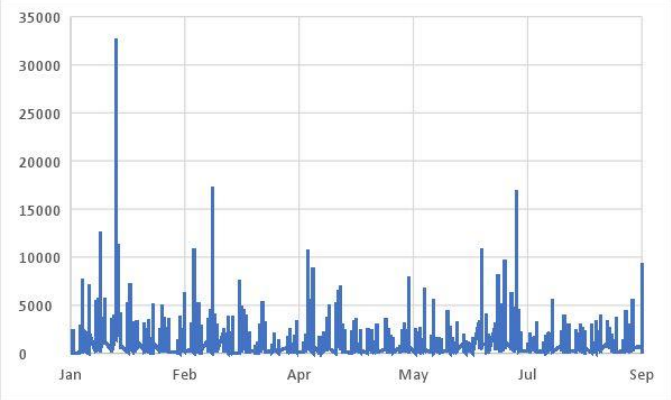

Figure 1: *Spliced BAX front-end contract trading volume data, grouped into 1-hour bins.*

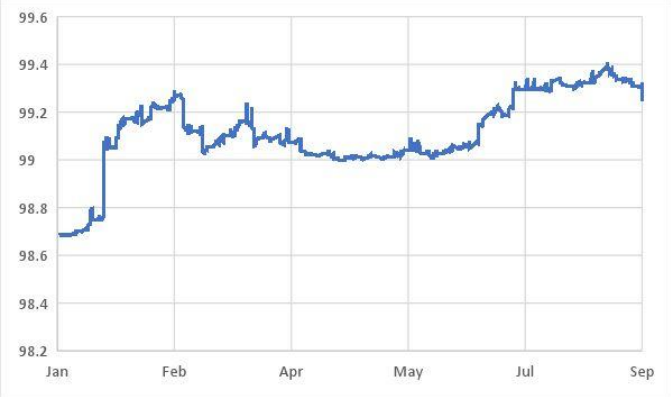

Figure 2: *Spliced BAX front-end contract trading price data, grouped into 1-hour bins.*

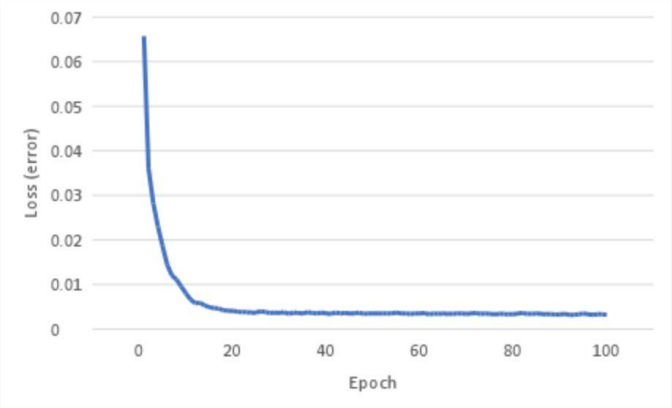

Figure 3: *The loss remaining after the completion of each epoch (batch size = 32).*

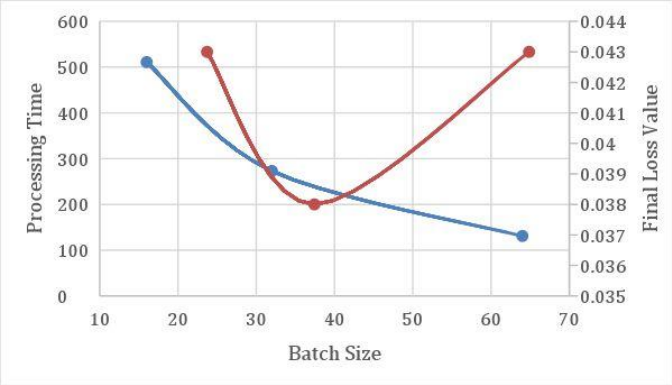

Figure 4: *Processing time required (blue) and final loss value (orange), by batch size.*

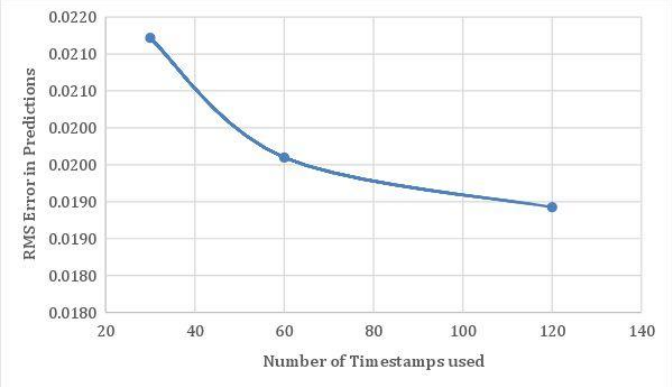

Figure 5: *Root-Mean-Square Error over all predicted values, 1 bin ahead, for 100 epochs and batch size of 32.*

Figure 5 shows the the RMSE over all predicted values for varying numbers of time steps used. As the figure shows, while increasing the number of time steps decreases the overall error, it does so with diminishing returns. Since we found using more than 120 time steps led to excessively long processing times, we used a time step value of 120 records for our experiments to minimize the error, while keeping the processing time manageable

An RNN with LSTM works by back-propagating the weightings between layers using an error-minimization strategy. The choice of which strategy to employ depends on the data we are trying to predict. One of the simplest, and most commonly-used error measurements is the root-mean-square (RMS) measurement. Since our aim for this experiment was to minimize the error in all predicted price measurements, we employed an RMS propagation LSTM, which keeps a moving average of the squared gradients for each weight and divides it by its mean squared to fix the learning rate.

Using these determined parameters for our RNN, our team decided to test the RNN's capability to predict the price of the front-end BAX contract, for each of 1, 3, 5, 10, 20, and 30 bin intervals in the future. For each of our experiments, our team fed the first 1700 records of the processed data set into the RNN as training data, using the remaining 187 records to perform the predictions and compare the results.

3 Results

The results of the experiments are shown in Fig. 6–11. As is demonstrated by these charts, in none of the experiments was the RNN able to pinpoint the price. Moreover, the accuracy of its predictions appear to decline considerably the further in the future its predictions are projected. Figures 10 and 11 in particular, demonstrate very little relationship between the predicted and actual price for predictions 20 and 30 bins in the future, respectively. Notably, since we were working with bin sizes of one hour for 10-hour trading days, this means the RNN showed little predictive capability only two and three days in the future.

4 Discussion and Conclusions

After conducting six experiments with varying numbers of time steps it was noticed that an RNN is not useful for predicting the price with any real accuracy, particularly for predictions of more than 10 bins (one day) ahead. While the RNN seems able to sense price changes as (or after) they occur, even the short-range predictions appear unable to reflect sudden or sharp price movements, tracing these as fluid curves rather than as they occur, as sharp spikes in price. This indicates that the RNN is merely reflecting the movement it is detecting in its most current data, rather than predicting future movements, based on its prior learning.

The accuracy of the predictions (predictably) decrease as the RNN is tasked to range its predictions even a couple days (20 bins) into the future. This, coupled with the lag observed between when price movements begin occurring and when

they are observed in the RNN's predictions suggest an RNN to be, at least as we have implemented it, of limited utility as a price-predictive tool. That said, the RNN's ability to sense and reflect trends and movement in the data suggest that it may show some promise as a tool to detecting patterns, but with the caveat that the time range it is analyzing not be extended too far beyond the data it is provided.

Given the RNN's sensitivity to fluctuations in the provided data, it would be an interesting extension to these experiments to see how the results would be affected by changing the bin size. Some sizes that may produce interesting results are two hours, up to a whole day. Grouping the data into larger bins would result in reducing the noise levels of the input data, potentially improving the RNN's performance.

Another interesting experiment that could be done is to run these experiments using an unsupervised learning neural network. The RNN used for the purposes of this paper was a supervised network, meaning that we needed to train the network on preselected data before feeding it the data used to make its predictions. It would be interesting to see the results of an unsupervised neural network could provide, particularly when provided more input vectors to analyze. Given the ability of RNNs to detect patterns, such an experiment could potentially reveal unforeseen relationships in trading between multiple contracts.

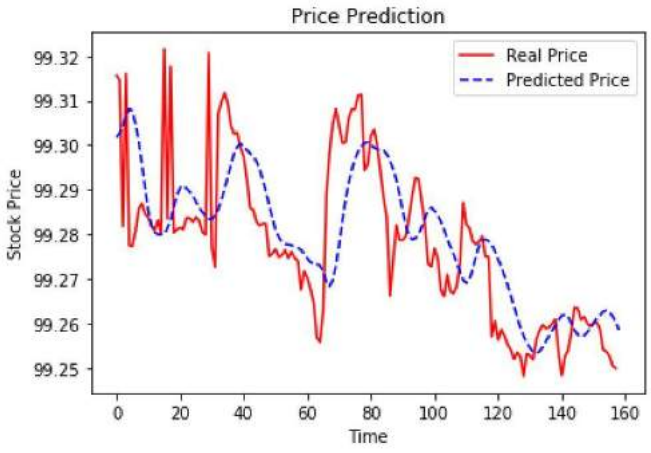

Figure 6: *Predictions 1 bin ahead.*

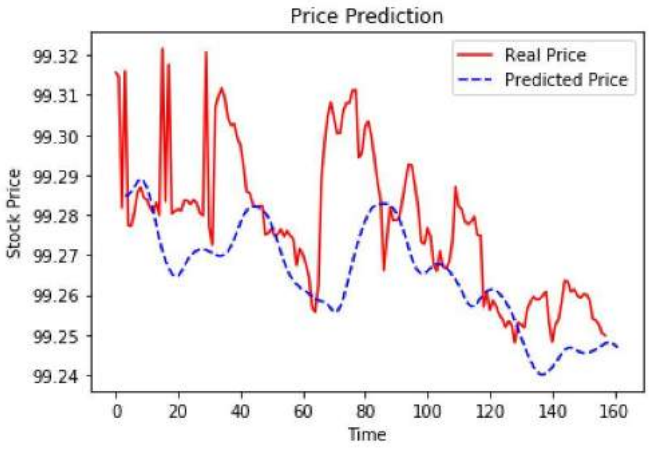

Figure 7: *Predictions 3 bins ahead.*

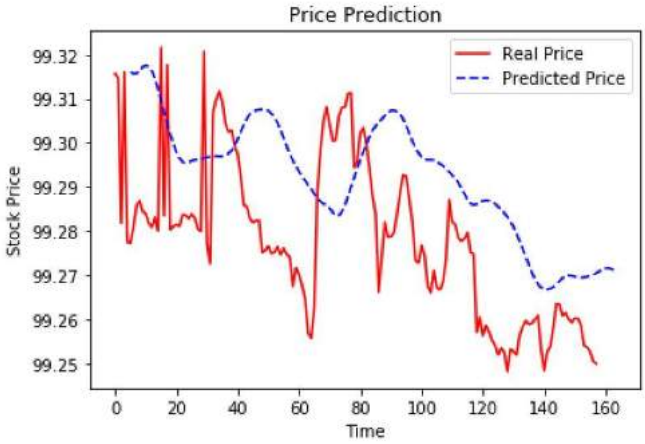

Figure 8: *Predictions 5 bins ahead.*

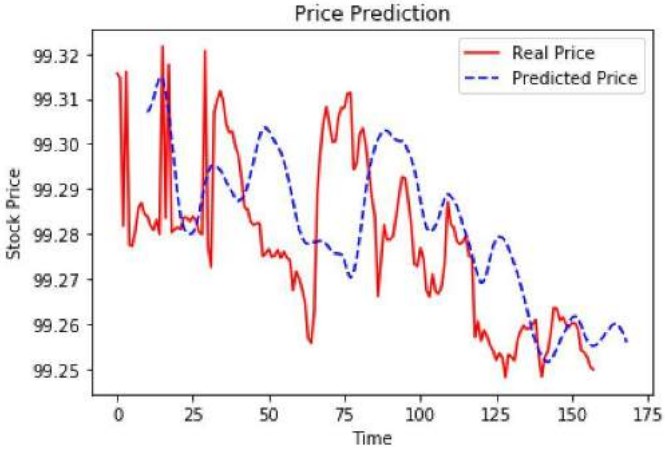

Figure 9: *Predictions 10 bins ahead.*

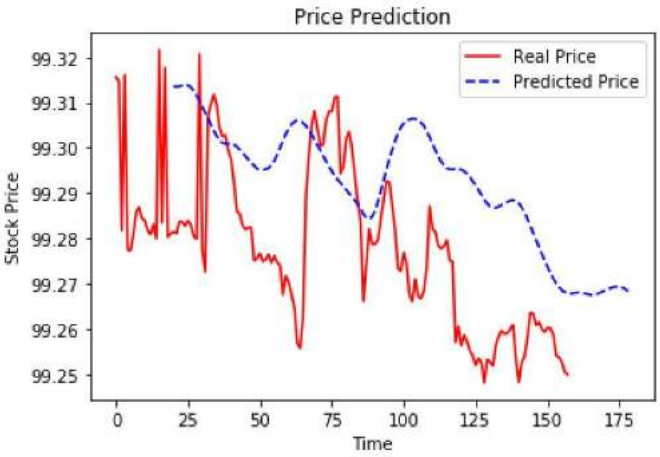

Figure 10: *Predictions 20 bins ahead.*

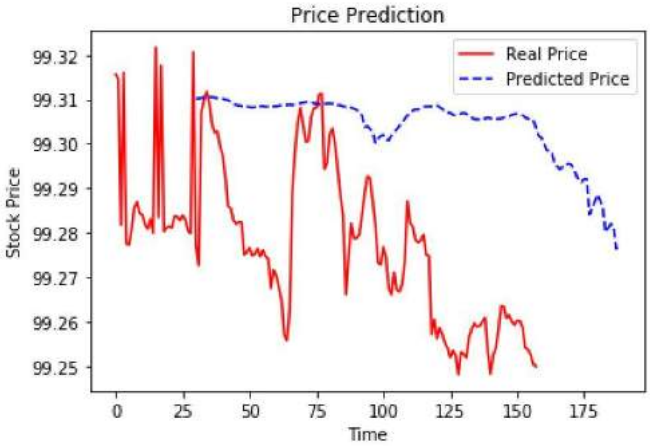

Figure 11: *Predictions 30 bins ahead.*

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Research Article

Impact of Drainage Ditch Construction and Subsequent Use on a Treed Bog Adjacent to a Peat Harvesting Operation, Southwestern Manitoba, Canada

Lindsay Edwards[1], Pete Whittington[1]

[1]Dept. of Geography, Brandon University, Brandon, MB, R7A 6A9

Corresponding Author: P. Whittington (whittingtonp@brandonu.ca)

Abstract

Manitoba has the most peatland by provincial area of any province in Canada and contributes ~11% of Canada's horticultural peatland production. Peat harvesting requires the lowering of the water table; this water is usually channeled to a fluvial system (e.g. a river) but in some cases must be actively pumped. The South Julius bog in Manitoba is an example where the pumped discharge was through an adjacent treed bog. The trees in the bog on one side of the drainage ditch were dead, but the trees on the other side were alive after nearly 10 years since the ditch was created. This study investigated possible hydrological causes by instrumenting three transects of wells that ran perpendicular to the drainage ditch and extended 20 and 50 m into the bog on the dead and live side, respectively. Average water tables on the live side were 15 cm lower than the dead side. The dead side water levels were similar to a natural fen located adjacent to the treed bog. Construction of the drainage ditch yielded a >20 cm high berm that ran alongside the live side, functionally isolating the live side from the surplus water in the drainage ditch. The berm helped with maintaining the lower water table treed bog vegetation requires. We recommend that future drainage ditches be constructed in such a way that berms on both sides are made, functionally creating a canal to the fen, where the excess water is less detrimental to the fen vegetation which is adapted to wetter average conditions.

Keywords: Peat Harvesting, Peatlands, Hydrology, Disturbance, Drainage

1 Introduction

Wetlands cover ~43% of Manitoba's land area with the majority of those being organic wetlands, commonly known as peatlands[1]. Within Manitoba, peatlands can be found across the province except for the southwest corner where part of the prairie pothole region (marshes and shallow open water wetlands) of North America is found[2]. Globally, peatlands represent a significant storage (16–33%) of the soil carbon (C) pool, despite covering only 3% of the Earth's surface[3]. Within North America, wetlands store 220 Pg C, most of which is in peat[4].

Peatlands are divided into two main categories: bogs and fens (some swamps are peatlands, but these are the minority in Canada)[5]. Bogs are ombrogenous (a water source from precipitation only), meaning that they are isolated from regional groundwater or surface flows. However, fens can receive water from various sources such as groundwater discharge or surface water (e.g. streams) inflows. Bogs typically have lower water tables (up to ~50 cm below the surface), whereas fens can have flooded conditions most of the year and act as conveyors of water in the landscape[6].

Bogs have a diplotelmic (two layer) soil structure[7]: the acrotelm and the catotelm. The acrotelm is the upper ~50 cm of alive and poorly decomposed *Sphagnum* mosses, which have very large pores and high hydraulic conductivity. These properties allow water to flow quickly and easily off of the bog in times of high water tables, such as spring melt or heavy rain. The catotelm is below the acrotelm and is typified by highly decomposed *Sphagnum* mosses (called peat), which has small pores and low hydraulic conductivity, which limits the lateral runoff of water from a bog. The catotelm varies in thickness, but 1-3 m is common in this area (unpublished data). Combined, these two layers allow the bog to remain wet (catotelm) but not too wet (acrotelm); permanently flooded conditions are detrimental to most bog vegetation[8].

Canada is one of the world's largest producers of horticultural peat[9] with ~11% of Canada's total coming from Manitoba peatlands. Bogs are the main peatland type used for peat extraction due to the presence of *Sphagnum* peat. Preparing a bog for peat extraction requires lowering the water table. This is achieved by digging ditches spaced 30 m apart to allow the catotelmic peat to drain more efficiently[10]. These drainage channels are connected in rectangular style drainage networks and, when possible, drained by gravity to a nearby fluvial system such as a stream or river. However, due to the low relief typical in peatland systems, sometimes the drainage water must be actively pumped into a nearby

fluvial system because gravity drainage alone is insufficient.

At the South Julius bog in South Eastern Manitoba, drainage water is actively pumped through an adjacent bog from the harvested fields into a nearby fen. A drainage ditch was constructed through the bog to facilitate the drainage. A site visit in May 2015 revealed that all of the trees on one side of the ditch were dead, whereas on the other side the trees were alive. A bog with dead vegetation is no longer a carbon sink, nor would offer suitable habitat for various species. Thus understanding the cause of the death may lead to recommendations for prevention for future developments.

The objective of this research paper was to determine what might be causing the tree death on one side of the drainage ditch, but not the other. We hypothesise that excess water from the pump would be too much water for the acrotelm to effectively shed, raising the water table too high for healthy bog vegetation growth.

2 Methods

2.1 Study Site

The South Julius bog (49.937778°N, -96.235249°W) is located ~20 km west of the town of Whitemouth, Manitoba (Fig. 1). The peat extraction area is ~250 ha and drains towards the middle of the east edge of the site. To the north/east of the site is the remaining treed bog that was not harvested, and north of that is a fen system that flows north west. Black spruce and tamarack contained in the bog make it heavily treed with a ground cover of *Sphagnum* hummocks and various *Ericaceous* shrubs.

The drainage ditch was constructed in 2007 (Tim North, personal communication) it runs ~500 m in a north-east direction and is ~2 m wide. During construction of the ditch, the extracted material was placed on the east side, forming a small berm (more details in the results) that runs along the length of the ditch. The north/west side of the ditch is called the "dead" side and the south/east side is called the "live" side.

Beausejour, ~22 km northwest of South Julius, is the closest Environment Canada weather station with 1981-2010 climate normals data. Mean January and July temperatures are -16.9°C and 19.2 °C respectively, with a mean annual temperature for the area of 2.8°C. Annual precipitation is 570.3 mm, with snow accounting for 20% of this total[11].

2.2 Methods

To determine the elevation profile across the ditch, a topographic survey using a differential global positioning system

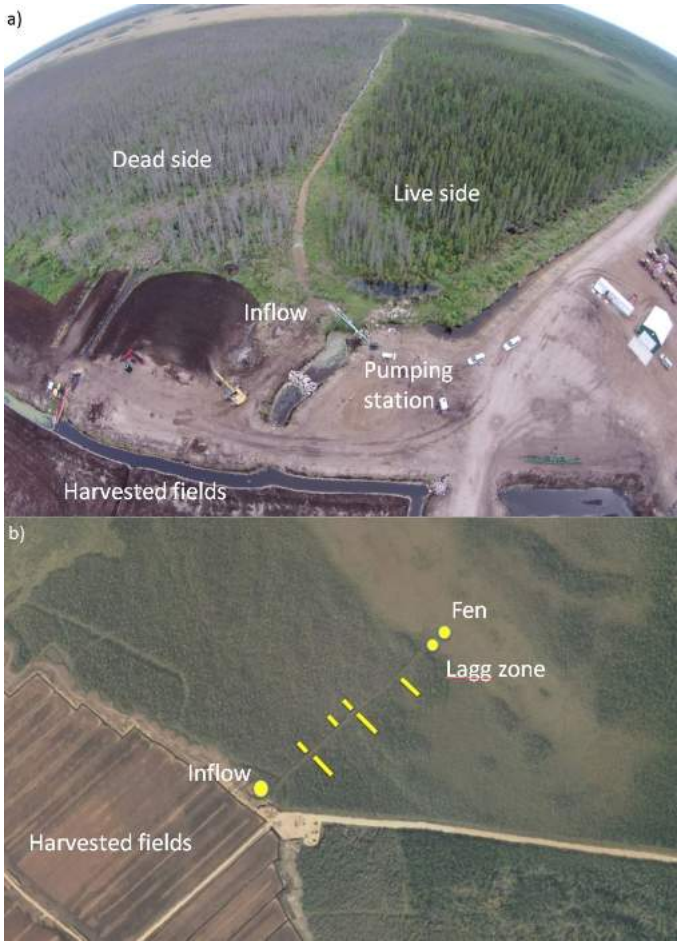

Figure 1: *Oblique air photo (drone) of the site looking northeast (a) and Google Earth satellite image of the surrounding landscape, showing the approximate locations of the transects and well locations (b). Distance from Inflow to Fen point in the lower figure is ~500 m.*

(DGPS) was conducted in June 2015 by KGS Group Consulting Engineers. They surveyed three transects (~80 m long) that ran perpendicular to the ditch at approximately 50, 200, and 350 m from the start of the ditch. The transects went into the bog on both the live and dead side (Fig. 1).

To determine meteorological inputs (rain) to the site, a simple weather station was installed (as part of another project at the South Julius site) approximately 500 m southwest of the start of the drainage ditch. The weather station consisted of a Campbell Scientific CR1000 data logger with a Texas Instrument (TE525) tipping bucket rain gauge and a Rotronic Instrument Corp (HC-S3) air temperature and relative humidity probe. The logger measured the instruments every minute, but recorded the total rain and average temperature every 30 minutes.

To determine water table positions on either side of the ditch, three transects of wells that ran perpendicular to the drainage ditch were installed approximately equidistance

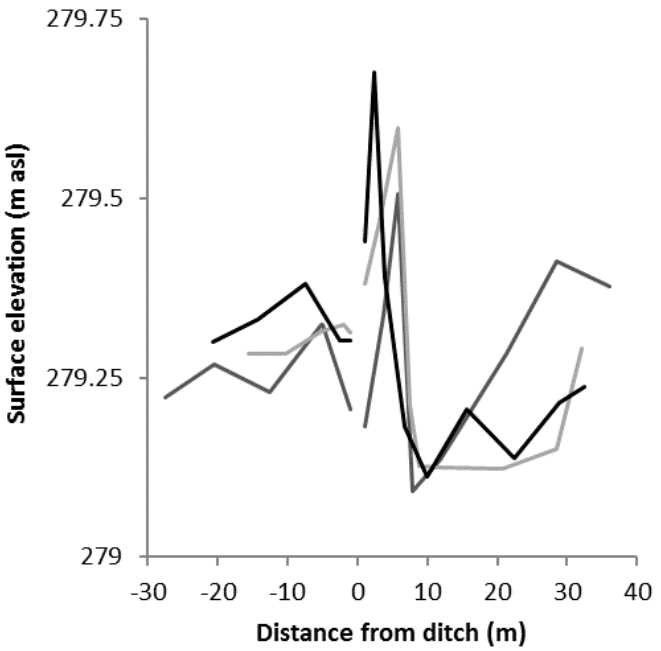

Figure 2: *Surface elevation of the three DGPS transects. Distances are reported from the end of the ditch, assuming a 2 m wide ditch (hence -1 and 1 m on the x-axis). The live side are the positive values. The distances from the start for the light grey, black, and dark grey lines were 50, 200, and 350 m, respectively.*

along the length of the drainage ditch (100, 200, and 300 m from the start of the ditch; except on the Dead side where the final transect was closer, due to the incredibly difficult walking conditions). Wells within each transect were located on the live side at 2, 5, 20, and 50 m intervals from the ditch edge. On the dead side, wells were located at 0, 5, and 20 m (Fig. 1). Wells were also installed at the inflow (south end of the ditch), in the lagg (transition between bog and fen) zone, and in the fen proper. Within the bog on the live side, the wells were typically installed in the hollow (low lying areas between the hummocks) as they represent a more consistent eleveation. Due to the flooded conditions, this was not possible on the Dead side (could not see the hollows). These 28 wells were measured bi-weekly in July and August. Wells were constructed from 2.54 cm diameter PVC pipe with 0.75 cm holes every 10-15 cm along the length of the pipe. The pipes were covered in a nylon stocking to prevent peat from entering the pipe and clogging the holes. An auger with a diameter slightly smaller than the pipe was used to pre-auger the hole to ensure a snug fit. Wells were measured using a calibrated blow stick to measure the water depth relative to the surface and were measured five times (roughly every other week) from late June to early September, 2015. Pumping rates for the water pumped into the ditch were obtained from SunGro Horticulture staff as the amount of time the pump was on. If the pump was on, it would be pumping at a rate of 200 imperial gallons per minute (909.2 L/min).

3 Results

The seasonal (May to September) precipitation was 199 mm higher than the 30-year climate normal[11]. Temperatures were generally slightly warmer with May to September average temperatures being +0.7, +0.7, +1.2, -0.1, and +3.6 °C compared to the 30-year average for that respective month.

The topographic survey revealed that construction of the drainage ditch yielded a small raised berm on the live side of the ditch (Fig. 2). Of the transects surveyed, the berm height ranged from 22 cm to 33 cm, but visual observations obtained by walking the entire length would suggest that the range was actually larger, with some areas of berm being > 60 cm higher than the surrounding landscape. The width of the berm also varied, but was typically between 6 and 8 m wide. Ground surface elevations on the dead side appeared to be ~15 cm higher than that on the live side immediately adjacent to the ditch. These differences became less pronounced by 25m away from the berm, where the dead and live sides were similar in elevation.

Water table depths were statistically significantly different between the dead and live sides of the ditch (Fig. 3, Table 1). Median water table depth (relative to the surface; positive being above and negative being below the surface) on the dead side ranged between 13 and 16.8 cm depending on the distance from the ditch. On the live side, these values ranged between -5.8 and 2.6 cm, with the 5, 20, and 50 m distances being within 1 cm of each other (3.4, 3.6, and 2.6 cm for 5, 20, and 50 m, respectively). Within the Lagg and Fen locations, median water tables were 21 and 13.4 cm, respectively, and not statistically significantly different (at 99%, but were at 95%) from each other, nor any of the Dead side locations (Fig. 3, Table 1).

From May to September the pump ran between 24 to 168 hours per week (i.e. never off), with monthly total discharge ranging from 6541 m^3 (May) to 30,526 m^3 (August). Total discharge for the May to September period was 88,669 m^3. With a surface area of the dead side of 0.6 km^2, the pumped water represented 153 mm of "runoff" (volume of water pumped / surface area of the bog) on the dead side.

4 Discussion and Conclusion

It is well documented that bogs and fens have different hydrology[2, 5]. Bogs are seen as storage features in the landscape, discharging water rapidly through the acrotelm in the spring when water tables are high, or after heavy rain events. Fens, however, are seen as conveyors of water, or the "rivers" of

Table 1: *Wilcoxon Rank Sum test p-values of water table depths between the Dead (D) and Live (L) sides for their given distance (m) from the ditch. For example, the wells located 20 m from the ditch on the dead (D20m) and live (L20m) sides were significantly different, but the D20m was not different from the Lagg, Fen, or the other Dead side locations.*

p-value	D20m	D5m	Dom	L2m	L5m	L20m	L50m	Lagg	Fen
D20m	-	0.14	0.53	<0.01	<0.01	<0.01	<0.01	0.12	0.76
D5m		-	0.49	<0.01	<0.01	<0.01	<0.01	0.54	0.76
Dom			-	<0.01	<0.01	<0.01	<0.01	0.22	0.23
L2m				-	<0.01	<0.01	<0.01	<0.01	<0.01
L5m					-	0.83	0.22	<0.01	<0.01
L20m						-	0.21	<0.01	<0.01
L50m							-	<0.01	<0.01
Lagg								-	0.015
Fen									-

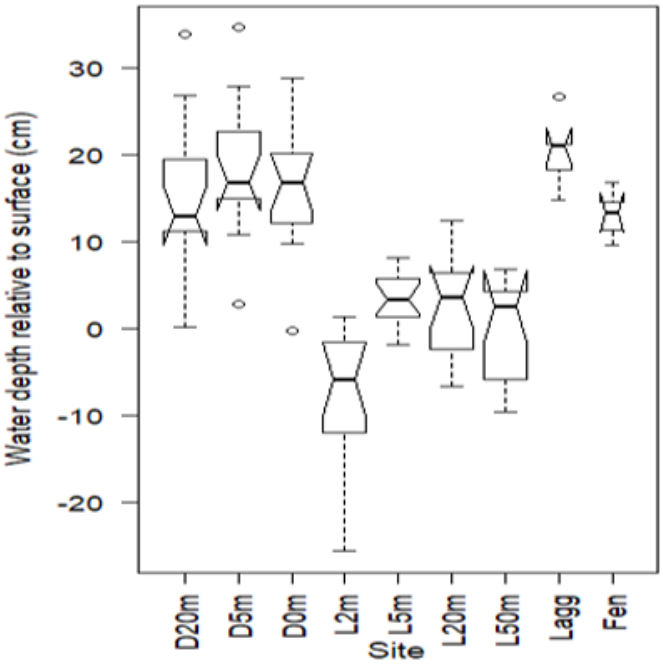

Figure 3: *Boxplots of median water table elevations relative to the surface (positive being above, or flooded, and negative being below the surface) on the dead (D) and live (L) sides. Distances follow the letter (e.g. D20m means 20 m from the ditch on the dead side). The notches (triangular indent) above and below the median (black line) can be used as a way to visualize statistically significant differences: where the notches (or the base of a triangle that would fill the notch) do not overlap on the y-axes they are significantly different at $\alpha = 0.05$, but where they do overlap, they are not significantly different*[12]*. For example, L50m has top and bottom notches at ~6 cm and -1 cm, and Lagg has top and bottom notches at ~22 cm and 18 cm. L50m's notch range of -1 to 5 cm does not overlap with Lagg's notch range of 18 to 22 cm, and thus they are statistically significantly different. However, L20m's notches are ~6 cm and 0 cm, and 0 to 6 cm overlaps with -1 to 5 cm (L50m), and thus they are not statistically significantly different. See also Table 1.*

peatland systems, and thus often have water tables at or above the surface. As such, vegetation indicative of each environment has adapted to the average water table conditions.

The 2015 field season was much wetter than average, with 199 mm of surplus precipitation in only 5 months (recall average annual precipitation is only 570.3 mm, with 374.1 mm for the same 5 months). As such, the discussion of the results must be considered with a wetter than normal field season for data.

We believe that the berm, being > 22 cm high, isolated the live side from the surplus water of the pump. The pump's "runoff" (volume of water pumped / surface area of the bog) was 153 mm of water, or 15.3 cm, which is below the height of the berm. Combined with the surplus precipitation, 352 mm of extra water needed to be shed from the bog, which likely exceeded the bog's ability to remove the water. The dead side water tables were 15 cm (150 mm) higher than the live side, suggesting that the flood waters remained well past the spring melt, as the pump acted as surrogate for continuous "heavy rain" that the bog was unable to shed in a timely fashion.

Complicating matters further, we were informed after our field season that there was a second, even larger pump that discharged water into the middle of the dead side for periods of the summer. We do not have the data for this pump, but would argue that its impact would exacerbate the problem of the main pump and not change the interpretation of our results.

Interestingly, there was no difference in water table between the Lagg and Fen locations and the dead side (Fig. 3, non-overlapping notches), suggesting that the fen would be more than capable of handling the surplus water of the pump. In fact, due to the increased area of the fen, the pumped water from the 2015 field season would be ~43 mm

of water (rather than the 153 mm reported above).

It is likely that the difference in water levels reported here between the dead and live sides is actually much less (i.e. we have under-represented how flooded the dead side is) due to the location of the wells. As noted in the Methods, wells in peatland studies are often installed in the hollows as the hollows have a much more consistent elevation within the landscape, despite hollows only covering roughly a third to a quarter of the area. On the live side, wells were installed in the hollows. If we considered the depth below the average hummock height (not measured, but a reasonable estimate would be 30 cm obtained from a nearby bog), water tables would drop a further 30 cm. The flooded conditions on the dead side meant that locating hollows was nearly impossible as we could not see the surface when the wells were installed. Visual observations of the live side would suggest that hummocks accounted for 75% of the surface area, thus is it quite likely that wells installed in the dead side were installed in hummocks. Installing a well in a hummock would automatically increase the depth to water table (or lessen the flooded depths found here) as hummocks rise up above the hollow surface. Thus, based on our well locations the live side would have had an average lower water table than reported here, and the dead side a higher average water table. This highlights just how much more standing water there is on the dead side than the live side.

Lowering of the water table in harvested peat fields is a necessary component of peat extraction, and, when possible, peat companies would much rather discharge to a natural fluvial systems (e.g. a river) as it can be done passively, without the costs of running and maintaining a pump. Given that river watershed areas are significantly larger than the peatlands noted here, the volume of water can easily be absorbed by the system with no "flooding" impact downstream. However, when no such natural fluvial system exists, pumps must be used. Discharging to a fen makes a lot of sense, given their higher water tables and natural "river" role in the landscape, and their ubiquity of being located next to bogs[13]. However, when discharging through a bog, we would strongly recommend that berms be constructed on both sides of the ditch so that the water may flow directly to the fen, bypassing the bog. This would allow the bog to remain as a carbon accumulating ecosystem with important habitat for various flora and fauna. As bogs are ombrogenous, the ditch with berms would have little impact to the hydrology of the bog, as evidenced by normal water tables and the healthy, live vegetation immediately adjacent to the ditch.

We acknowledge that this study represents only one field season at one field site and that the flooded conditions of this field season alone did not contribute to the death of the trees on the dead side (as they were dead when we arrived in May). However, it is very likely the surplus water discharged into the bog every summer from 2007 to the current study year continually raised the water level to maintain flooded conditions not conducive to continued bog vegetation growth, and hence their death.

5 Acknowledgements

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Research Article

Developing a Genotyping Scheme for *Mycobacterium abscessus* Complex Using Whole Genome Sequencing Data

Michelle Wuzinski[1], Meenu K. Sharma[2,3]

[1]Dept. of Microbiology, University of Manitoba, Winnipeg, MB, R3T 2N2
[2]Dept. of Medical Microbiology, University of Manitoba, Winnipeg, MB
[3]National Reference Centre for Mycobacteriology, National Microbiology Laboratory, Winnipeg, MB, R3E 3R4

Corresponding Author: M. Wuzinski (wuzinskm@myumanitoba.ca)

Abstract

Mycobacterium abscessus *complex is a rapid growing non-tuberculous mycobacteria (NTM) and a clinically significant pathogen capable of causing varying infections in humans. It is notoriously difficult to treat due to its inducible resistant state to clarithromycin and intrinsic resistant states to other drugs including rifampicin. Typing schemes for bacterial pathogens provide numerous applications including sourcing an outbreak, identifying cross contamination, chain of transmission and surveillance. However, they either lack or are limited for many NTMs including M. abscessus complex. The current publically available scheme on Pubmlst has not been updated in several years and was only able to apply a sequence type to less than half of tested isolates. This project was aimed at creating a workflow for the development of a multi locus sequence typing (*mlst*) scheme using whole genome data. A total of 104 genomes and 14 loci were used to build the scheme (including 3 type strains of each of the 3 subspecies). All 7 genes from the Pubmlst scheme were incorporated namely,* argH, cya, gnd, murC, pta, purH, *and* rpoB *and were expanded by 6, 9, 12, 8, 12, 10, and 8 alleles, respectively. Another 7 novel genes were added including* hsp65, erm(41), arr, rrs, rrl, gyrA, *and* gyrB *with 9, 14, 20, 7, 25, 24, 22 alleles, respectively, with 62 unique sequence types were identified among all isolates. This scheme can also differentiate* M. abscessus *complex to the subspecies level on the basis of 3 discriminatory genes and includes 6 genes related to antimicrobial resistance.*

Keywords: Whole Genome Sequencing, MLST, Mycobacterium abscessus Complex, NTM

1 Introduction

Mycobacterium abscessus is a rapidly-growing mycobacteria, commonly found in soil and water that is becoming a growing clinical concern[1]. It can cause a range of infections from pulmonary to soft tissue infections[2]. More concerning is its intrinsic resistant state to a large number of antimicrobials, making it one of the most resistant pathogenic rapidly growing mycobacteria (RGM)[3]. A particular challenge with M. abscessus is its controversial nomenclature. *M. abscessus* (also known as *M. abscessus* complex or *M. abscessus sensu lato*) has recently been divided into three subspecies, *M. abscessus* subspecies *abscessus*, *M. abscessus* subspecies *massiliense*, and *M. abscessus* subspecies *bolletii* (for simplicity each subspecies will be referred to as *M. abscessus*, *M. massiliense* and *M. bolletii*, respectively). Until 1992, *M. abscessus* was classified under the *M. chelonae* group and it was not until 2013 that it was split into the three subspecies that are now most commonly used[2, 4, 5].

Typically, mycobacteria are differentiated by their 16S rRNA sequences as it is well conserved within the genus[6]. However, this is not sufficient to differentiate the subspecies as they have been found to have identical 16S sequences[7]. While the subspecies are typically differentiated by PCR amplification and sequencing of the hsp65 or rpoB genes, with the growing trend of whole genome sequencing, it has become easier and less expensive to use whole genome data than ever before. A similar genotyping scheme reported that to obtain all the data for their analysis it both less expensive and less time consuming to use whole genome sequencing versus traditional Sanger sequencing. Bartels[8] Multi-locus sequence typing (MLST) has been a staple in molecular biology for the past 20 years. Its usefulness extends from an epidemiological tool to pathogenicity, evolution and surveillance[9].

Traditionally, MLST schemes are composed of seven house-keeping genes of 450–500 base pairs in length and each gene would be sequenced and analyzed (MLST-home, mlst.net) using an algorithm to assign allele numbers for each gene. However, with access to whole genome sequencing, this method is becoming outdated and whole genome data should be utilized in every way possible. It becomes much easier to use larger and a greater number of genes because individual gene sequencing is no longer required. With whole

Table 1: *Expansion of alleles from previously established loci as last updated by curator S. Kim on 2012/08/06, accessed 2018/04/10*

Alleles	argH	cya	gnd	murC	pta	purH	rpoB
Previous	6	5	7	8	11	7	4
New	6	9	12	8	12	10	8
Total	12	14	19	16	23	17	12

genome sequencing becoming more and more prevalent due to lowering costs, novel techniques are needed to be able to process and utilize more of the data and information that it brings. The publically available MLST database for *Mycobacterium abscessus* complex is split into two schemes, *M. abscessus* and *M. massiliense*, this paper and scheme is a modified and updated version of the *M. abscessus* scheme developed by Kim[10].

This project was aimed at creating a workflow for the development of a multi locus sequence typing (MLST) scheme using whole genome data.

2 Materials and Methods

2.1 Genome Collection

An original set of 16 genomes (subset 1; Appendix 1) were downloaded from the European Nucleotide Archive in fastq format. They were then uploaded to Galaxy[11] and run through the MentaLiST algorithm[12], an MLST allele caller that uses the publically available scheme on pubmlst.net as imported on the Galaxy and fastq formatted genomes. Two other MLST algorithms were tested for use, the first being the Centre for Genomic Epidemiology (CGE) and Stringmlst[13]. CGE was rejected due to taking over a day to type a single sample and often failed. Ultimately, MentaLiST was chosen over Stringmlst as it was faster and user friendly.

Reference genomes were obtained from ncbi for the type strains of the three subspecies, *M. abscessus* subspp. *abscessus*, *massiliense*, and *bolletii* under the accession numbers ATCC19977, CCUG48898 and CCUG50184, respectively. The genome size of each is roughly 5 Mb. An additional set of 97 genomes (subset 2; Appendix 2) (accessed from ncbi under the accession SRP127025) and 75 genomes (subset 3; Appendix 3) from the study ERP001039 were downloaded. All 198 genomes from the three subsets were subjected to quality filters on the following parameters: successfully be typed by MentaLiST, successfully assemble, have a coverage greater than 5 and at least 80% mapped to the reference strain. From the 198, 104 passed all parameters and were used to create the scheme.

2.1.1 Genome Assembly

The first 16 test genomes were assembled in irida (irida.ca) with default parameters. For simplicity and ease, the remaining genomes were assembled in Public Health Agency of Canada's Galaxy (developed by Bioinformatics core) using the SPAdes pipeline which provided the output of average coverages used as a parameter of greater than 5. While this coverage is generally considered low, due to the quality of available data it was decided to be sufficient in order to have a workable dataset. Sequencing reports were also run in Galaxy to ensure all were greater than 80% mapped to the reference of ATCC19977.

2.1.2 MLST Scheme Development

All 104 genomes were then run through a customized R script (known as "MasterBlaster") in R Studio (R Studio Inc., Boston, USA) initially developed in-house by Walter Demczuk (National Microbiology Lab, Winnipeg, MB) for genotyping of enteric pathogens. The script takes a user imported wild type gene and utilizes blast[14] to query a single genome (or several) against the wild type and identifies a match or calls it as not found and the user can input it as a novel allele. This was done for all seven genes of the previously established loci in the M. abscessus MLST scheme (pubmlst.com) as well *hsp65*, *erm(41)*, *rrs*, *gyrA*, *gyrB*, *rrl*, and *arr*. We developed an allele list specific for MAB incorporating essential genes for both identification genes and antimicrobial resistance genes. These genes are relevant in identification, clarithromycin resistance, aminoglycoside resistance, fluoroquinolone resistance (*gyrA* and *gyrB*), clarithromycin resistance and rifampicin resistance, respectively.

Wild type genes for the novel genes were arbitararily obtained from the genome of the ATCC19977 strain of *M. abscessus* (accession: NC_010397). The exception to this is the erm(41) where a partial sequence for the T28 sequevar was used as the wild type from accession HQ127365, the C28 sequevar was obtained from HQ127366, and the *M. massiliense* and *M. bolletii* alleles came from their respective type strains. All sequences from the M. abscessus database on Pubmlst were merged with the developing database with allele 1 from each respective loci becoming the 'wild type'.

The first part of the script (MasterBlaster) is known as the development stage. A wild type gene was selected (and became allele 1) and all genomes were blasted against this sequence. The script reports results for each genome on the basis of presence or absence of the gene (POS or NEG), if positive it proceeded to whether the allele matched one already in the database and it gave the allele number or reported it as not found (NF). If NF, the sequences were then opened in a sequence viewer, AliView v. 1.23[15], an alignment viewer and editor that can be downloaded for free from the internet.

Table 2: *Allelic characteristics of novel genes added to the scheme.*

	hsp65	erm(41)	arr	rrs	rrl	gyrA	gyrB
Alleles	9[1]	14	20	7	25	24	22
Length (bp)	424	360[2]	426	1504	3112	2520	2025

[1] An allele for *M. chelonae* was added in order to differentiate it from M. *abscessus* but not included in calculations in Table 3.
[2] The length for the allele correlating to *M. massiliense* is 283 base pairs.

Table 3: *Sequence diversity of each locus. Full similarity matrices of all alleles can be found in Appendix 4. erm(41) was excluded due to the large deletion in M. massiliense that skews results.*

Locus	Average Sequence Divergence	Average Percent Identity
argH	2.33	97.7
cya	1.82	98.2
gnd	2.58	97.5
murC	2.28	97.8
pta	1.47	98.6
purH	1.48	98.5
rpoB	2.67	97.4
hsp	3.33	96.8
arr	1.72	98.2
rrs	0.162	99.8
rrl	0.165	99.8
gyrA	1.25	98.8
gyrB	1.56	98.4

Table 4: *Allelic assignments for hsp65 with associated subspecies name and mutations. Mutation numbering based upon the M. abscessus type strain (ATCC19977)*

Allele #	Subspecies	Associated Mutations
1	*M.abscessus*	Wild type
2	*M.massiliense*	Wild type
3	*M. bolletii*	Wild type
4	*M. massiliense*	T293A
5	*M. bolletii*	T200C
6	*M. abscessus*	C200T
7	*M. abscesus*	C299A
8	*M. bolletii*	C173T and T200C
9	*M. chelonae*	Wild type

From this point, any duplicate sequences of an individual gene were identified in AliView and removed, leaving only unique alleles which were arbitrarily assigned a number and then used to build the allelic database for each individual loci. When the individual gene databases were completed, sequence types (STs) are defined by running all genomes with all loci to generate allele profiles through the MasterBlaster script. These allele profiles were unique to each of the 62 STs, in other words, each had different combinations of alleles at each locus. These were exported into Microsoft Excel and the "Remove Duplicates" function was used to filter out any of the 62 STs that may have been repeated among all isolates. After the definition of STs, the second and separate script, known as the MLST script, was used. This script reported allele numbers for a given isolate at each loci and reports the ST that corresponds with a given allele profile.

As it currently functions, the MLST script requires that alleles for a given gene must all be the same length in order for the algorithm to identify the sequence, due to this; multiple genes had to be trimmed. The last base pair of argH was deleted and the last codon from *gnd* and *gyrB* were removed. However, *erm(41)* posed a greater issue. Because *M. massiliense* has a truncated gene, it is significantly shorter than the others; when all alleles were trimmed to match the length of *M. massiliense*, multiple sequences were flagged as duplicates because their variation existed after the cutoff, thus introducing errors as these sequence were not actually duplicates. When samples are run through the MLST script, shorter sequences will be given an X (meaning no gene was found) and they can then be taken back to the MasterBlaster script and identified as containing the *M. massiliense* allele for *erm(41)*. When an allele was found that did not match one in the database, it was flagged with a question mark. This genome would then be taken back to the development stage (MasterBlaster script) and generate a new allele if applicable. This step is highly quality controlled.

Following completion of the scheme and establishment of sequence types, two clinical strains were extracted by a colleague using the InstaGene protocol (Bio-Rad, Hercules, California, USA) and assembled in irida. The two samples, 1800282 and 1800298, were used to test the final scheme in the MLST phase and ensure it was functioning as expected.

3 Results

The original *M. abscessus* MLST scheme that is publically available on Pubmlst is comprised of 7 genes (*argH*, *cya*, *gnd*, *murC*, *pta*, *purH*, and *rpoB*) which were compiled into 26

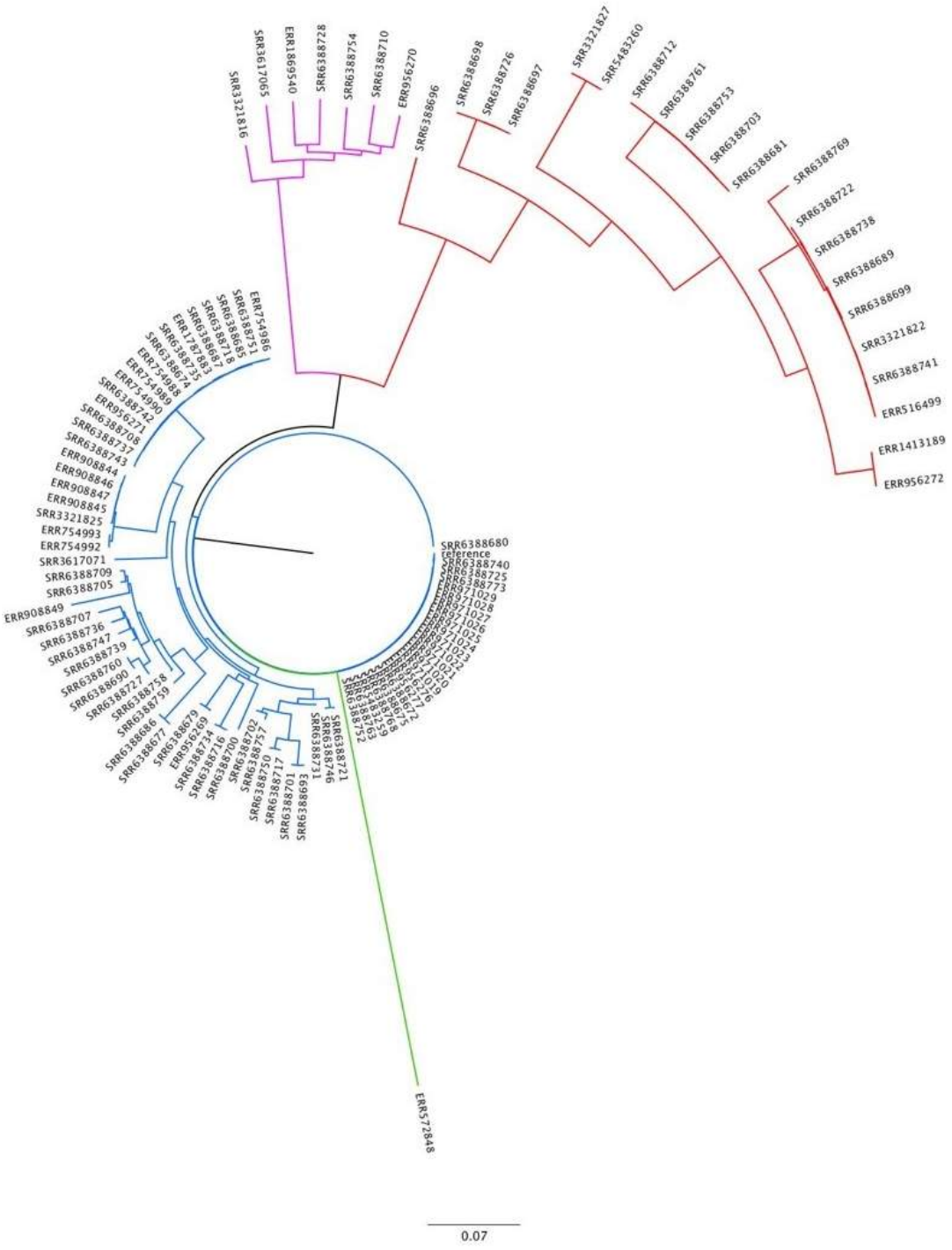

Figure 1: *Phylogenetic tree of all query genomes based on single nucleotide polymorphism analysis. Created as output of SNVPhyl pipeline (from Galaxy) analysis and visualized with FigTree v.1.3.4. Magenta branches represent M. bolletii, red is M. massiliense, blue is M. abscessus, and green is M. chelonae. Run with Galaxy's default parameters except the minimum coverage which was set to five.*

sequence types. A total of 7 new genes were added, all with capability to differentiate between the subspecies of *M. abscessus* complex or a gene involved in antimicrobial resistance. These novel genes are: *hsp65*, *erm(41)*, *gyrA*, *gyrB*, *rrs*, *rrl*, and *arr*. Similarity matrices for all alleles of all genes can be found in Appendix 4.

Table 5: *Characteristics of alleles used for erm(41).*

Allele #	Subspecies	Associated Mutations	Relevant Sequevar	Predicted Phenotype
1	*M. abscessus*	Wild Type	T28	Resistant
2	*M. abscessus*	Wild Type	C28	Susceptible
3	*M. massiliense*	Truncated/Wild Type	NA	Susceptible
4	*M. bolletii*	Wild Type	T28	Resistant
5	*M. abscessus*	G95T/T285C	T28	Resistant
6	*M. abscessus*	A246G/T285C	T28	Resistant
7	*M. bolletii*	G63A, C285T	T28	Resistant
8	*M. abscessus*	T285C	T28	Resistant
9	*M. bolletii*	A63G, C77T, T285C, A289G, T357C	T28	Resistant
10	*M. abscessus*	T198C, C285T	T28	Resistant
11	*M. bolletii*	A63G, T285C, T357C	T28	Resistant
12	*M. abscessus*	G294C	T28	Resistant
13	*M. bolletii*	A63G, A96T, C259T, T285C, A297G	T28	Resistant
14	*M. abscessus*	T154C, T285C	C28	Susceptible

Mutation numbering based on M. abscessus type strain (ATCC19977) as reference sequence. Due to a partial sequence being used, position 28 is 154 in this scheme.

3.1 MentaLiST Results

Using the MentaLiST algorithm, samples were first typed by the *M. abscessus* scheme from Pubmlst. Fastq files were not obtained for *M. massiliense* or *M. bolletii* type strains and thus did not undergo this typing. Of 102 samples, 45 were identified as a defined sequence type (ST), 23 belonging to ST5, 15 belonging to ST9 and 2 belonging to ST24. That leaves 56% of samples not matching a ST. While this can partially be explained by the fact that subspecies other than M. abscessus were included, only 26% of samples were identified by *hsp65* analysis as not belonging to the *M. abscessus* subspecies so many samples still fall outside of the defined sequence types.

Based on the correlation of sequences of their *rpoB*, *hsp*, and *erm* genes, 72 samples were found to be *M. abscessus*, 8 as *M. bolletii*, and 19 as *M. massiliense*. Three samples produced discrepant results (discussed below; Table 7) and one sample was identified as *M. chelonae* based on its *hsp65* and 16s RNA sequences (ERR572848). These identities align as expected with the clustering of the phylogenetic tree below (Fig. 1) where *M. abscessus* type strain was used as a reference.

3.2 Novel MLST Results

A total of 62 unique sequence types (STs) were identified among all samples with 41 duplicated STs. The STs were completely redefined in accordance with all loci and in no way relate to those on the published Pubmlst scheme. STs 1, 2 and 3 correspond to the type strains for *M. abscessus*, *M. bolletii* and *M. massiliense*, respectively. All remaining ST numbers were arbitrarily assigned based on unique allele profiles. However, at this point in time, due to the truncation in *erm(41)*, no *M. massiliense* STs can be automatically identified by the MLST script.

By increasing the amount of unique allele profiles from 26 to 62, the discriminatory power is increased by 4.2x. The amount by which each gene was added to in terms of alleles is displayed in Tables 1 and 2. Additionally, the allelic diversity of each gene is illustrated in Table 3. On the other hand, because there is double the number of genes involved in the scheme, it will become less likely for an isolate to have a 100% match to a given ST.

A total of 16 samples failed to be applied a specific ST. Of these, 24 individual errors within the 16 samples were identified. The reason for this is unclear because an allele was found for all genes and all genomes within the MasterBlaster script and a ST was assigned for all isolates in the scheme. Problems arose in alleles: *rrs24*, *rrs25*, *rrs1*, *rrs3*, *rrs7*, *purH12*, *pta16*, and *arr18*. All of these alleles with the exceptions of *arr18* and *purH12* were properly identified in other genomes. A possible explanation would be the assembly quality of the genomes as the parameters were set fairly

Table 6: *Allele information for rpoB.*

Allele #	Subspecies	Attributes
1	*M. abscessus*	Pubmlst original\Wild Type
2	*M. abscessus*	Pubmlst original
3	*M. abscessus*	Pubmlst original
4	*M. abscessus*	Pubmlst original
5	*M. bolletii*	Wild Type
6	*M. massiliense*	Wild Type
7	*M. massiliense*	
8	*M. massiliense*	
9	*M. massiliense*	
10	*M. massiliense*	
11	*M. massiliense*	

Table 7: *Gene Identities of Discrepant Genomes*

Accession #	rpoB Identity	hsp65 Identity	erm41 Identity
SRR5483260	*M. abscessus*	*M. massiliense*	*M. massiliense*
SRR3321827	*M. abscessus*	*M. massiliense*	*M. massiliense*
ERR908849	*M. abscessus*	*M. bolletii*	Non type strain

low due to the quality of available data. It is speculated that the whole genome sequencing (WGS) was missing data for some of these genes.

After competition of the scheme and establishment of STs, two clinical samples were analyzed as a test. While neither matched a previously defined ST, the first sample, 1800282, had 100% matches for 13/14 genes but lacked an exact match for *gyrB*. The second sample, 1800298 had 100% matches to 8/14 alleles including *hsp*, *erm*, *rrl*, *rrs*, *argH*, *gnd*, *murC*, and *purH*. Two new STs were added for these isolates.

3.3 Discrepant Genomes

Three samples presented with discordant results with differing identities at several identification loci, as seen in Table 7. According to the recommendations by Griffith[16] *M. massiliense* is that of the organism with a large deletion in the *erm(41)* gene making it non-functional, thus both SRR5483260 and SRR3321827 should be identified as *M. massiliense*. Furthermore, Macheras[17], isolated a strain that was identified as *M. abscessus* based on *rpoB* but *M. massiliense* on the basis of hsp65 which highlights the necessity of using multiple genes to identify the subspecies. Evidence of horizontal transfer of the rpoB gene has been reported and could explain this discrepancy[18]. Griffith[16], also recommends that *M. bolletii* be that which differs from *M. abscessus* and *M. massiliense* based on its *rpoB* sequence and has

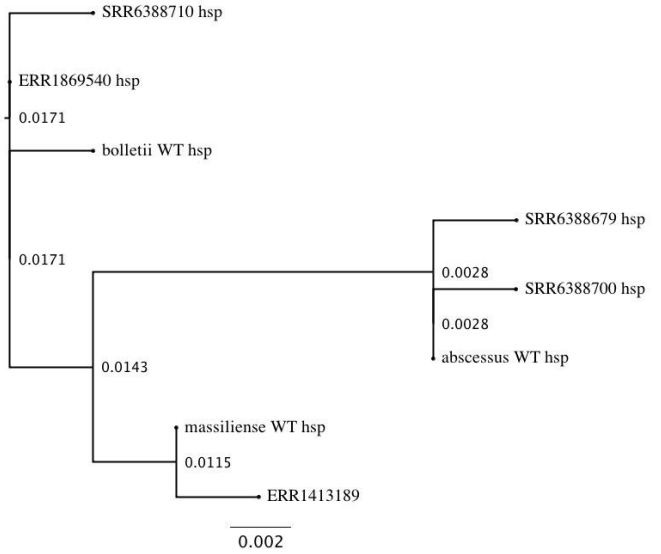

Figure 2: *Dendogram of all new alleles added to hsp65. Made with FastTree (Galaxy) and visualized with FigTree v.1.4.3.*

Figure 3: *Dendogram of sequences of new alleles added to rpoB. Made with fasttree, visualized with FigTree (v.1.4.3).*

a functional *erm(41)*. On closer analysis of ERR908849's *erm(41)* sequence, it is 4 SNPs (single nucleotide polymorphism) away from the *M. bolletii* type strain but only 2 SNPs from the *M. abscessus* type strain (one being position 28 making it a C28 sequevar). When looking at the phylogenetic tree (Fig. 1), it clusters with *M. abscessus* but significantly further away than other samples. Based on this, a clear identity cannot be assigned to ERR908849.

4 Discussion

A major advantage of MLST is the fact that it does not account for how many nucleotide differences there are be-

tween alleles so point mutations and recombination are given the same weight in terms of diversity. Additionally, MLST has a high discriminatory power due to the rarity of an isolate exactly matching a sequence type[19].

This modified MLST that was developed takes advantage of some of the information that whole genome sequencing has been able to provide and is a simple and easy-to-use way to build an MLST scheme. A single locus could be added in a matter of minutes as all that is required is a wild type copy of the gene. However, it has flaws associated with it. First, any sequence with gaps or shortened for any reason, such as a single base pair deletion, cannot be properly recognized by the MLST script. While they can be identified with no issue in MasterBlaster, errors arise when they are brought into the MLST script as it looks for an exact user-specified length. Additionally, issue arose with the blast program downloaded from ncbi. Genes had to be trimmed because the blast output would be missing a base pair or two or have extra base pairs. The technical glitch identified was sample SRR6388768 originally identified as allele 18 for *rrl* but MLST was unable to provide an output.

For a select few genes, some extra information was encoded to be outputted when the script was run. Genes commonly used for identification (namely, *hsp65*, *erm(41)*, and *rpoB*) will display the associated subspecies name and in addition, *erm(41)* will output the relevant sequevar (elaborated on below). However, this output only exists in the MasterBlaster script as the MLST only reports the allele number of a given locus and any additional output is not shown. A solution to this however, is knowing what allele number corresponds with what genotype.

4.1 hsp65

Hsp65 sequencing is a common method for differentiating not only the subspecies of the *M. abscessus* complex but also from the closely related *Mycobacterium chelonae*[20]. This gene was not included in the original MLST scheme and thus was the first one added in order to give the scheme a higher discriminatory power. By using the hsp65 sequences for each reference genome of the respective subspecies, the scheme now has the power to subspeciate any *M. abscessus* genome it is given. 89% of samples matched the allele from one of the given type strains. The numerical allele assignments can be found in Table 4.

There were two samples, ERR1869540 and ERR1413189, that did not have a 100% match to one of the reference genome alleles and based on their phylogenetic clustering of the *hsp65* gene (Fig. 2), they were identified as *M. bolletii* and *M. massiliense*, respectively. Additionally, when these sequences were blasted online against the National Centre for Biotechnology Information (NCBI) database, they both obtained multiple 100% matches to other strains, implying that these alleles were not errors in sequencing but true differentiation. When subset two was applied, three new alleles were found; their subspecies identification was confirmed in two ways. First, the tree was analyzed and the identity found was confirmed with the *rpoB* identity. SRR6388679 and SRR6388700 clustered with the *M. abscessus* wild type and had the matching *rpoB* sequence, each with one mutation from the wild type (C200T and C299A, respectively). SRR6388710 clustered with the *M. bolletii* wild type *hsp65* and contained the *M. bolletii rpoB* sequence but had two mutations: C173T and T200C.

4.2 erm(41)

The *erm(41)* gene is important for *M. abscessus* for its ability to induce resistance to macrolides (namely clarithromycin) as well as differentiating the three subspecies[21]. They can be differentiated based on the presence or absence of a functional *erm(41)* gene. *M. massiliense* has a large deletion in this region which produces a truncated and non-functional erm protein. On the other hand, both *M. abscessus* and *M. bolletii* have functional erm genes but have unique sequences. Perhaps most importantly, a functional *erm(41)* confers inducible resistance meaning that after the standard 3 day incubation period in minimum inhibitory concentration testing, isolates may appear susceptible to clarithromycin and other macrolides. However, in an incubation period of up to 14 days, induction of the gene can occur and generate resistance to the drug[16]. Furthermore, some *M. abscessus* complex isolates have a T to C mutation at position 28 (position 154 in this scheme) which also demonstrates a susceptible macrolide phenotype. All of these factors are included and recognized by the scheme. While no 100% matches to the wild-type C28 sequevar were found, the allele is present in the scheme. However, one C28 sequevar was found but was a single base pair away from the wild type of C28 (2 base pairs from the T28 wild type) and was identified in 10 different samples. All alleles included in the scheme can be found in Table 5.

4.3 rpoB

Encoding for the beta subunit of RNA polymerase, *rpoB* was included in the previously established scheme and is involved with rifampicin resistance[22]. However, because of its use in subspecies identification[18], it requires special attention and curation in this new scheme. Firstly, alleles for *M. massiliense* and *M. bolletii* from their respective type strains were added, and then 5 other new alleles were found from data

subsets 1 and 2. These 5 genomes that lacked a 100% match to an already established allele or an allele from a type strain were both blasted against the ncbi database and their phylogeny was compared against the type strains in order to assign a subspecies (Fig. 3). Furthermore, their identities were compared with the calls made by the other useful identification genes, *erm(41)* and *hsp65*. The alleles can be found in Table 6.

4.4 rrl

23s rRNA is encoded for by the *rrl* gene and, along with *erm(41)*, is involved in clarithromycin resistance[21]. While *erm(41)* is responsible for inducible resistance, both genes are important factors when looking at clarithromycin resistance. While 25 different alleles were identified among the samples, most differed by only a SNP or two from one another with only 25 variant positions found in 3112 total positions. Mutations known to be associated with resistance are 2058 (A to G, C or T) and 2059 (A to G, C, or T) (E. coli numbering)[23]. All alleles showed an A at position 2058 and a G at 2059 which implies that they are likely resistant to clarithromycin. As demonstrated by Bastian[21], *rrl* mutations are sufficient to produce a resistant phenotype in M. massiliense and C28 sequevars, genotypes that are typically associated with susceptibility to clarithromycin.

4.5 rrs

The *rrs* gene encodes the 16S ribosomal RNA. The least amount of variation was found within this gene with only 7 unique alleles identified in all samples and 6 variant positions. Resistance to 2-deoxystretamine aminoglycosides have been shown to be acquired through a single point mutation at position 1408 (*E. coli* numbering) from A to G[24]. This mutation was present in all 7 alleles in the scheme implying that they are all resistant to aminoglycosides. Nessar[2], showed that two other mutations (C1409T and G1491T) that are known to cause kanamycin resistance in *M. tuberculosis* also had the same effect in *M. abscessus*; thus, these mutations should be noted and added to the scheme.

4.6 gyrA and gyrB

Fluoroquinolone resistance is typically attributed to mutations in the *gyrA* and *gyrB* genes which encode for the A and B subunits of DNA gyrase, respectively. Resistance occurs through mutations within the quinolone resistance determining region (qrdr) which is a conserved region that interacts with the drugs[25]. As shown by Monego[26], the most prominent mutations in *gyrA* that confer resistance are in amino acid positions 90 and 94 (Mycobacterium tuberculosis numbering; 83 and 87 in *E. coli*). In the B subunit, positions 495, 516, and 533 (*M. tuberculosis* numbering, 426, 447, and 464 in *E. coli*)[25]. A susceptible phenotype codes for a serine at position 90 in *GyrA* while resistant organisms have an alanine to valine substitution.[26]. These particular mutations known to confer resistance were not added into the scheme simply due to time constraints. While they were not observed in the database, they would be useful for future analysis with additional genomes. However, the diversity among these genes was moderately low (Table 3), despite there being a large number of alleles.

4.7 arr

Rifampicin is a first line drug used to treat infection caused *M. tuberculosis*. The drug's activity is derived from its ability to binds to the beta subunit of the DNA-dependent RNA polymerase thereby inhibiting enzymatic activity. In most bacteria, rifampicin resistance is attributed to mutations in the *rpoB* gene (which encodes the beta subunit of RNA polymerase) that lower the drugs affinity for the enzyme. However, in addition to this mechanism, *M. abscessus*'s genome encodes a rifampicin ADP-ribosyltransferase (MAB_0591) which gives it intrinsic resistance to the drug. As shown by Rominski[27], when introduced into naturally susceptible organisms, the *arr* gene was enough to demonstrate rifampicin resistance. This gene was found in all strains that were analyzed and identified as belong to the M. abscessus complex. Interestingly, *arr* is not found in the closely related species *Mycobacterium chelonae*[27] and as expected, the program reported the gene as not found when the reference genome for *M. chelonae* was used (accession: CCUG47445). It was also lacking in the one test sample that was identified as *M. chelonae* (ERR572848).

5 Conclusion

In conclusion, the original goal of this project was to establish a whole genome multi locus sequence typing scheme. Once the desired genes are identified and genomes collected, the process is fairly straight forward, albeit labor intensive, from that point. The most significant advantage of this method is the ability to bypass the amplification and individual gene sequencing steps that come with traditional MLST scheme creation and use. All that is required is a collection of assembled genomes and a wild type allele which can easily be obtained from an online database such as ncbi. Additionally, commercial software such as Ridom SeqSphere's MLST+ Target Definer exist to identify genes to be used in a core genome

MLST (cgMLST) scheme and was used to create a cgMLST scheme for M. tuberculosis. An additional advantage over MentaLiST is that allele calls require a 100% match in order to be called that allele whereas MentaLiST (and Stringmlst) will simply assign the closest allele which could introduce inaccurate sequence typing but this issue is eliminated in the novel scheme.

It is a known problem in the science community that analytical tools for whole genome data produce a large block of results of which not everything is useful. We not only developed a novel scheme but an established a simple method for utilizing whole genome sequencing data. The script within R Studio is very user friendly and easy to use with little bioinformatics expertise required. Even if not used as a traditional MLST scheme, even looking at a single locus can provide numerous advantages from identifying a subspecies to detecting a resistant genotype.

Modifications can still be made in order to improve the scheme such as adjusting the script to allow for differing gene lengths and displaying subspecies name as part of the ST. Additionally, mutation information and predicted phenotypes could be applied to the other antimicrobial resistance genes as they were for *erm(41)* and for the identification genes.

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Research Article

Characterizing the Spatial Heterogeneity of Basic Physical Properties of Lake and Peat Soils as it Relates to the Moss Spur Peatland, Manitoba

Steven R. Patterson[1], Pete Whittington[1]

[1]Dept. of Geography, Brandon University, Brandon, MB, R7A 6A9

Corresponding Author: S. Patterson (pattersr41@gmail.com)

Abstract

Moss Spur (the study site) is a remnant vacuum-harvested peatland in south eastern Manitoba that has, with little intervention, revegetated on its own. As part of unraveling the mystery as to why, this study investigates the spatial heterogeneity of vegetation and underlying lake sediments at Moss Spur. Physical properties like hydraulic conductivity, bulk density and porosity relate to hydrology and the ability of water to flow, which are of importance in this study. This study looked at those properties and attempted to find a connection between the physical properties of the peat and underlying sediments and the heterogeneity of surface vegetation found at different study areas at Moss Spur. Peat cores as well as sediment cores were extracted from sub-locations within sites. Sample cores were tested via a variety of methods to establish their physical and hydraulic properties. Heterogeneity based on core samples was revealed between sites matching the general heterogeneity of surface vegetation at Moss Spur. This study presents some regionally key aspects to understanding groundwater relationships with respect to harvested bogs and Manitoba wetlands in general. The variability in lake sediment properties across even the relatively small site of Moss Spur suggests that lake sediment properties cannot be assumed to be the same at every location. Heterogeneity of the surface vegetation with respect to the spontaneous regeneration is found to be correlated with the underlying peat and lake sediments. It is expected that areas with lower bulk density and higher porosity and hydraulic conductivity K, *would be in the areas with the bog-like vegetation and as such with regrowth.*

Keywords: Peatland Restoration, Spatial Heterogeneity, Lake Sediments, Peat, Hydraulic Conductivity

1 Introduction

Peatlands cover over a third of Manitoba's land area[1], and globally, store twice as much carbon as all of the world's forests, despite covering only 3% of the Earth's surface[2]. It is generally well understood that peatland initiation and maintenance is governed by their hydrogeomorphic setting[3], that is, the combination of the area's hydrology (including climatology), hydrogeology, and geomorphology. There are two main types of peatlands in Canada: bogs and fens[4]. Bogs are ombrogenous, meaning that they receive water by precipitation only, isolating them from regional groundwater or surficial inputs of water. Bogs are the peatlands targeted for peat extraction to be used for fuel (more typically in Europe) or as a horticultural product,"peat moss", which is the main use in North America[5]. Manitoba contributes ~11% to Canada's peat production total, with the majority coming from Quebec and New Brunswick; Canada's is one of the world's largest producers of horticultural peat.

Harvesting a (bog) peatland requires digging drainage ditches to lower the water table so that machinery can be driven across the site to remove the upper ~50 cm of vegetation to get to the deeper, more decomposed peat. The surface is then tilled and allowed to dry so that only the upper ~1 mm of the peat surface is vacuumed up per pass of the harvester. Harvesting of a single site can last 30 years taking only 5–7.5 cm of peat per year and after harvesting is completed, restoration efforts begin. Despite being sold as a growing medium, the surfaces of the extracted peatlands are actually quite inhospitable to natural or spontaneous peatland vegetation regrowth. The inhospitality is due to the soil hydraulic properties of the peat that do not allow for the easy transmission of water, as well as the dark surface (low albedo) that promotes evaporation and thus desiccation of any vegetation that tries to establish. Therefore, active restoration of these peatland sites is required. The aim of restoration is to return the site back to a carbon accumulating ecosystem with vegetation similar to a natural bog (i.e., an abundance of Sphagnum moss). Much of the work done on peatland restoration in Canada has been completed in eastern Canada (Quebec and New Brunswick). However, the Moss Spur peatland in south eastern Manitoba has grown back wetland vegetatation without human intervention. Why? We suspect that the hydrogeomorphic setting is partly responsible.

The peatlands of the St. Lawrence Lowlands in eastern

Canada represent a different hydrogeomorphic setting than those peatlands in south eastern Manitoba. The Lowland's peatlands are located in the Boreal Shield ecozone and have Canadian Shield bedrock (an impermeable substrate) underlying them, which severely limits ground water flow[6]. This potential lack of water is made up for by the climate; Quebec has a much wetter climate (~1000 mm vs. ~570 mm precipitation totals at Moss Spur, annually)[7] and cooler growing seasons, which equate to less evaporation relative to Manitoba which possesses a hotter and drier climate[7].

Moss Spur is located within the Boreal Plain ecozone. Peatlands in the Western Boreal Plains (WBP) have been found to be more dependent on local and regional climate, bedrock and surficial geology because these regions experience decadal drought cycles[8, 9]. The combination of climatic and geologic characteristics of the Boreal Plains is unique in the Canadian boreal forest (Alberta, Saskatchewan, and Manitoba). The climate at each study site in the WBP region is characterized by average long-term annual precipitation that is equal to or lower than average annual open-water evaporation, yet peatlands still exist. Outwash, moraine and lacustrine deposits characterize the geology. The high density of wetlands, ponds, and shallow lakes in the Boreal Plains region reflects complex interactions with shallow-surface and groundwater flow systems[8], not typically found in Quebec. Study sites addressed by Devito et al.[8] include more than eight locations across the Boreal Plains in Alberta. It appears that when the local groundwater flow systems have higher hydraulic head than the bog (drought), water can actually discharge into the bogs until water levels are returned to normal and the head gradient driving recharge returns[10]. Groundwater flow reversals have been found to occur during periods of extreme drought. Water table drawdowns of 70 – 200 cm below normal conditions (drought-like condition) are enough to allow a flow reversal in the WBP peatlands[9]. Manitoba experiences decadal droughts[9] due to lower annual precipitation, and groundwater flow reversals have been known to supplement water to bogs in times of severe drought[11].

It has been hypothesized[12] that harvesting creates drought-like conditions due to the drainage ditches lowering the water table in the peatlands 1.5–3 metres. This lowered water table, like the droughts noted above, reduce the hydraulic head within the peatland and can reverse the direction of the gradient, allowing water from the mineral sediment below to come closer to the surface[11]. This is suspected to be what is happening at Moss Spur. Hawes & Whittington[12] found groundwater discharge zones in areas with better peatland vegetation establishment, and recharge zones in areas with poorer peatland vegetation re-establishment. However, they ignored the hydraulic properties of the underlying lake sediments in their study. The spontaneous revegetation at Moss Spur is not uniform, as some areas have better peatland vegetation regrowth, and other areas poorer regrowth (see Study Site for more details). Given that most post-glacial environments are far from homogenous, the different vegetation communities found at Moss Spur might be indicative of local differences in the physical properties of the peat and glacial lake sediments. Where soil bulk densities are lower and porosities are higher, one might expect groundwater upwelling to occur in greater amounts as lower bulk density and higher porosity could provide a less restricted flow channel. Less restriction enables higher hydraulic conductivity and thus more groundwater flow, thus assisting the vegetation to re-establish itself.

Therefore, the objective of this paper is to determine if the physical properties of the underlying peat and lake sediments is related to vegetation re-growth on the surface at the Moss Spur peatland. We expect that areas with lower bulk density and higher porosity and hydraulic conductivity K, would be in the areas with the bog-like vegetation. It is also expected to be the same outcome with vegetation regrowth; these parameters are outlined by Gagnon et al.[13]

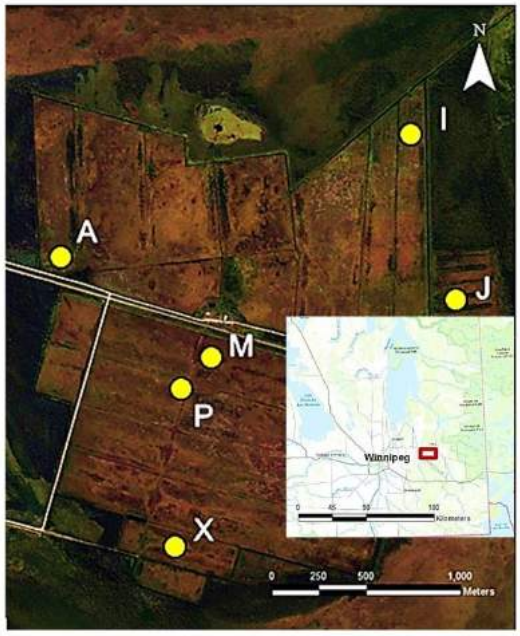

Figure 1: *This figure shows the study site area and where it exists in southeastern Manitoba. 1a: Sample locations within Moss Spur Manitoba harvest site. The yellow dots give an approximate location to core extraction sites and the letters refer to regeneration plots. b) A map of the geographic extent of Manitoba, Winnipeg has been emphasized for location reference and the red boxed area is expanded as Fig. 1a.*

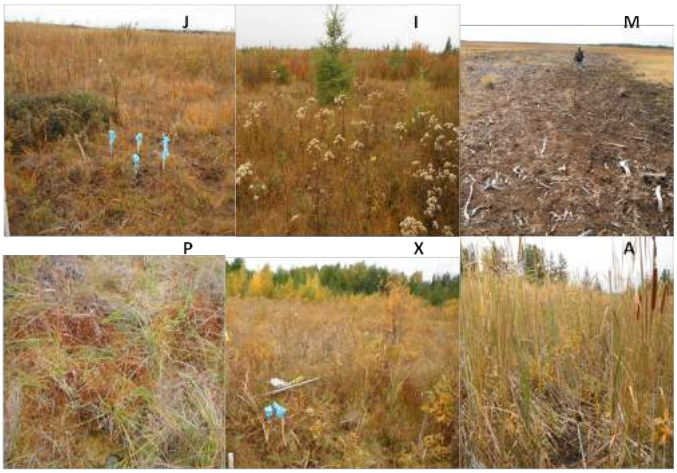

Figure 2: *Each of the labels in this figure corresponds to the location labeled in Fig. 1. This figure contains representative photos of each site (J, I, M, P, X, and A labelled accordingly).*

Bog--------J----------I----------M----P-X---------- A--- *Fen*

Figure 3: *The arbitrary scale for sample sites at Moss Spur between bog and fen based on vegetation observed*[13, 14].

2 Study Site

Harvesting ceased in 1999 and since that time Moss Spur has had very little assistance in its natural vegetation regeneration[15]. Moss Spur is located in south east Manitoba (Fig. 1) between the towns of Whitemouth and Beausejour on the Canadian Pacific Railway (49.99°N, 96.13°W). The closest Environment Canada station is Beausejour (~28 kilometers northwest of the site) and the 1981–2010 annual mean temperature was 2.8°C with January and July temperatures of -16.9°C and 19.3°C, respectively[7]. Annual precipitation is 570 mm (20% falling as snow).

Moss Spur is located in a part of the former glacial Lake Agassiz basin in Manitoba. Glacial and glaciofluvial deposits cover much of Manitoba, especially in the south eastern and eastern regions[16]. The major Lake Agassiz sedimentary basin covers the Moss Spur area and has contributed to peatland development due to the characteristics of the bedrock and lake sediments beneath the peatland and the possibility of groundwater flows.

Moss Spur underwent 53 years of peat harvesting beginning in 1936[17]. Moss Spur had a total harvested area of approximately 440 hectares which has been divided into 24 sections, mostly separated by large remnant drainage ditches. These sections were labeled alphabetically starting at the northwest corner of the site (Fig. 1 a). Since abandonment, beavers have dammed many of the major drainage ditches effectively re-wetting much of the site[18].

At the time of data collection, the remnant bog had limited vegetation regeneration at site M, and good to very good regeneration[13, 14] at sites J, I, P, X and A (Fig. 2). Site M demonstrates what a typical post-harvest peatland would look like, with minimal vegetation regeneration. These sites were chosen to study because they embodied distinct assemblages of vegetation that seemed representative of the communities across the entirety of the site. The assemblages were obvious from imagery acquired by a drone showing distinct patterns across the site. Previous studies[12] instrumented 6 locations within these assemblages (J, I, M, P, X and A) with various hydrological instrumentation (wells and piezometers) thus the current study took advantage of these locations to characterise the peat and lake sediments.

The vegetation found at each location was placed on an arbitrary gradient (Fig. 3) between a natural bog to fen[13, 14], but based on dominant vegetation found in the various wetland classes present in Canada[4]. Site M was not able to be classified as it is just the old remnant peat surface (no plants present). Site J contained *Sphagnum* spp. mosses and was classified as the most bog-like of all the sites where data were collected. Site I, in the north sector, appeared to contain little or no *Sphagnum* spp. mosses but mostly *Polytrichum strictum* mosses. There was a good coverage of ruderal spp. as well as black spruce (*Picea mariana*), classified as more of a bog than a fen. Site P classified closer to a poor fen with presence of Labrador tea (*Rhododendron* spp.). Similarly to P, site X represented a poor fen as well, however with presence of cattails (*Typhaceae* spp.) and willows (*Salicaceae* spp.). Site A was a different site all together; with *Typhaceae* spp. and sedges (e.g., *Cyperaceae* spp.) in high standing water it would be considered some kind of marsh rather than a fen.

3 Methods

3.1 Field

Peat cores were taken with a Russian peat corer (Aquatic Research Instruments, Hope, Idaho, USA) which is a side-filling soil sampler and measures 5.08 cm in diameter with a 50 cm length and removes a maximum volume of 645 cm^3 of peat. Peat cores were taken starting at the surface in 50 cm increments up to 250 cm, or until the underlying mineral soil (lake sediments) were reached, usually between 100 and 150 cm, but in some cases less than 100 cm. Lake sediments were sampled with an Oakfield Model T soil auger (Oakfield Apparatus, Fond du Lac, Wisconsin, USA), with a sample removing end, and were retrieved at varying depths following peat core removal. The augers corer end has a length of 30 cm and a sample diameter of 1.905 cm.

At each main site (A, X, P, J, M) 3 sub-locations were sampled within each site, in a cluster sampling formation (section I had only 1 as the site was difficult to traverse). In an attempt to better characterise either the homogeneity or heterogeneity of each site's samples, soil auger cores were taken directly beneath the peat core samples via the Russian cores pre-established hole. All cores (peat/lake sediment) were wrapped in plastic wrap and then placed in sealable plastic bags to contain all of the material, and stored in a refrigerator (4°C) to slow decomposition of organic materials, until laboratory analysis.

Sediment cores were retrieved only from sections I, J, M, and X, at 3 sub-locations where the peat was cored. The reason for this was lack of equipment during site visitation during the season. Each sediment core sample was wrapped and stored in the same manner as the peat samples.

Hydraulic conductivity (speed of the bulk movement of water in the ground) was measured using three roving piezometer nests in roughly the same locations as the peat/lake sediment samples, which were all near the previously established nests of another larger study[12]. Each roving piezometer nest contained three piezometers (with 20 cm slotted intakes) at 50, 100 and 150 cm, and one well (approximately 100 cm long) using 2.54 cm inside diameter PVC pipe. Each site location (A, J, etc.) then, had four nests tested for hydraulic conductivity. The depths of the deeper piezometers varied with sample site location due to the peat depth at that location, but ranged 50 to 250cm. The Hvorslev[19] method [Eq. 1] was used to estimate the hydraulic conductivity and requires removing a volume of water from the pipe and measuring the rate of the return of the water, where K is hydraulic conductivity (m/s), r is the inside radius of the tube (m), L is the slotted length of the pipe (m), R is the outside radius of the pipe (m), T_0 is the basic lag time parameter.

When the recharge was too quick to measure manually, a Schlumberger level logger was used, which recorded measurements every 0.5 seconds.

3.2 Lab

Four parameters were determined for each lake sediment soil sample: hydraulic conductivity, particle density, porosity, and bulk density. Porosity (n) [Eq. 2, 3, 4] is also the empty volume (V_w) of the soil not occupied by solid particles (expressed as a proportion or percentage). Calculating porosity takes advantage of the known density of water as when the core is completely saturated (M_s), all of its empty pore space is occupied by water and the dry mass (M_d) is the mass of the core with no water present; the mass difference is the mass of the water, and as water has a density of 1 g/cm^3 the mass can be converted to a volume (V_w). Bulk density (ρ_b) [Eq. 5] is the mass of dry soil per total soil volume (V_t) including the air space (g/cm^3). Particle density (ρ_p) [Eq. 6] is the dry mass of soil (M_d) per unit volume of the soil particles (V_s)(g/cm^3). Particle mass was determined by grinding the sample with a mortar and pestle and weighing a subsample on a scale. Particle volume was determined by adding a known mass of soil (typically 10–15 grams) to an empty 200 ml volumetric flask. A second container of water with a known mass (and therefore volume) of water was poured into the volumetric flask until the water level in the flask reached the volume line in the narrow neck. The difference between the initial mass of water and the remaining mass would then be equal to the volume of the soil. Peat samples were only measured for bulk density.

$$K = \frac{\left(r^2 \times \ln\left(\frac{L}{R}\right)\right)}{2LT_0} \tag{1}$$

$$n = \frac{M_s - M_d}{V_s} \tag{2}$$

$$n = 1 - \frac{\rho_b}{\rho_p} \tag{3}$$

$$V_t = V_s + V_w + V_a \tag{4}$$

$$\rho_b = \frac{M_d}{V_t} \tag{5}$$

$$\rho_p = \frac{M_d}{V_s} \tag{6}$$

$$K = \frac{d_t^2 L}{d_c^2 t} \ln\left(\frac{H_0}{H}\right) \tag{7}$$

For hydraulic conductivity measurements each lake sediment core was sealed by wrapping the core in dry wall webbing to provide structural stability and then the core was repeatedly dipped in liquid paraffin wax[20] until a 4–5 mm thick wax "shell" was formed. The wax helps to ensure that preferential flow paths do not occur along the outside of the core[20].

A falling-head test was used to measure hydraulic conductivity (K). We assumed, *a priori*, that the cores would be low K cohesive sediments which requires smaller water volumes to run through the sample. Equation 7 (with falling-head hydraulic permeameter apparatus[21]) was used to calculate hydraulic conductivity (K), where time (t) is recorded

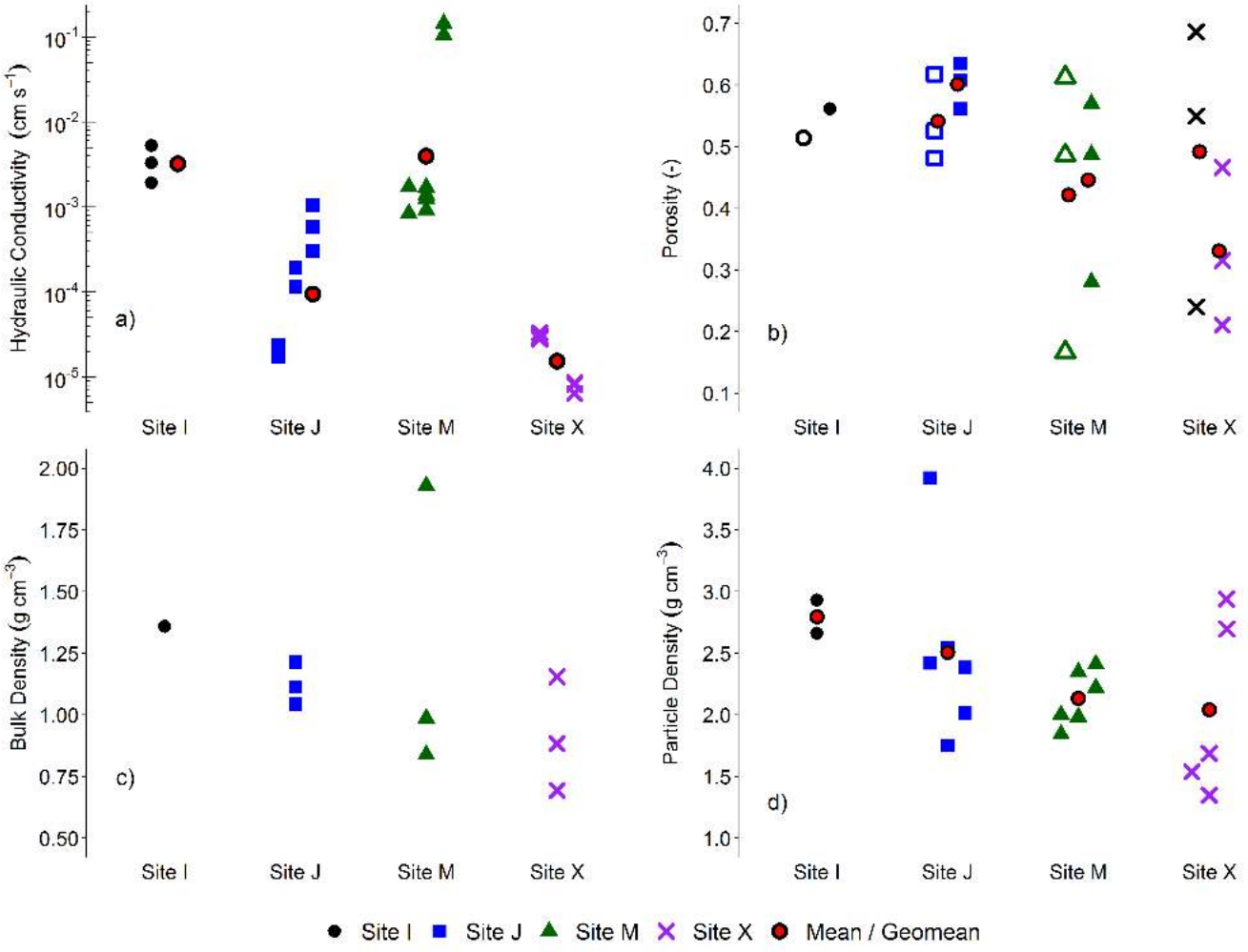

Figure 4: *a) Hydraulic conductivity of lake sediment samples, each tested a minimum of 2 times (excluding site M sample C). The points that are vertically stacked are repeated tests of the same sample core to increase the confidence of the test (e.g., J1 test 1, J1 test 2). Those points of the same symbol, but separated horizontally from the geomean are the different sub-sites (e.g., J1, J2, J3). The geomean is of all the tests (repeats and sub-sites). b) Porosity results from Eq. 2 and Eq. 3. Plotted points with a solid fill are results from the physical test of porosity [Eq. 2]. Points with a hollow fill are results from the mathematical check done to test accuracy in the study [Eq. 3]. c) Bulk density values for each sub-location at each site. d) Particle density values for each sub-location, each tested twice (excluding Site X sample B). Note y-axes do not start at 0 to preserve the scale require to display the data*

beginning at the point where the water drains from the initial height (H_0) until the water reaches a final height (H); the natural logarithm (ln). This parameter, then, is the change in head; it is affected by the diameter of the falling-head tube ($2r$) and the diameter ($2r_s$) and length (L) of the core.

The soil sample must be fully saturated before any measurement is taken, as under the principle of continuity, the volume of water entering the sample chamber must equal the volume draining from it[22]. If the sample is not saturated before the test starts, the results will be inaccurate. Each sub location sample was completed up to four times to assess repeatability.

4 Results

4.1 Lake Sediments

Hydraulic conductivity values for each location are shown from the falling head permeameter tests (Fig. 4 a). Hydraulic conductivity of all lake sediment samples spanned 6 orders of magnitude between 6.4×10^{-6} and 1.7×10^{-1} cm/s. Site I had the smallest range (1.9×10^{-3} to 5.2×10^{-3} cm/s)

Table 1: *Sites ranked based on parameters and potential flow. Each parameter (e.g. porosity) is normalized and scaled equally (i.e. low, med, high), a qualitative rank for each site followed this process to generate the table*[13, 14].

Site	Porosity	Bulk Density	Particle Density	Hydraulic Conductivity	Potential Flow (sediments)	Peat Depth (cm)	Peat K	Peat Bulk Density	Classification (dominant flora)
A						80	Low	High	Marsh-like (sedges & standing water)
M	High	Low	Medium	High	Highest	250	High	Low	Unclassified (little - no plants)
P						160	Medium	Medium	Poor fen (Labrador tea)
X	Medium	Low	Low	Low	Low	100	Highest	High	Poor fen with cattails
I	High	High	High	High	High	135		Low	More bog than fen (little *Sphagnum* spp.)
J	High	Med-High	Med-High	Medium	Med-High	185	High	Low-Med	Most bog-like (*Sphagnum* spp.)

whereas site J had the largest (1.7×10^{-5} to 1.0×10^{-3} cm/s). Sub-locations are shown slightly offset and had a fairly tight grouping, showing the repeatability of the laboratory methods, but still showed heterogeneity within the site's location. Across the entire study area heterogeneity is quite apparent. The geometric mean of sites I and M were similar, and were both larger than J and X (Fig. 4 a). Geometric mean was used to show the central tendency of each site location.

Porosity of the lake sediments spanned from 0.16 to 0.68 across all sites. Sites I and J showed a small range relative to sites M and X. The range for site J is 0.48 to 0.64 whereas the range for site M is 0.17 to 0.61. Porosity values based on Eq. 2 and Eq. 3 are shown (Fig. 4 b). Each site contained 3 sub locations, as specified in the Methods excluding site I (only one sub location).

Bulk density (Fig. 4 c) of the lake sediments showed a small range across each site as well as within each site. Data ranged from 0.69 to 1.93 g/cm^3. Site J showed the smallest range (1.04 to 1.21 g/cm^3) and site M showed the largest range (0.84 to 1.93 g/cm^3). The high value at site M (1.93 g/cm^3) may be an error in sample collection resulting in sediment compaction. Most of the samples show values between 0.8 and 1.2 g/cm^3 indicating a high organic content in the sediments. Bulk density trended I > J > M > X.

Particle density (Fig. 4 d) of the lake sediments span values ranging from 1.39 to 3.92 g/cm^3. Site I showed the smallest range (2.66 to 2.93 g/cm^3) and site J showed the largest range (1.75 to 3.92 g/cm^3). The average for each site showed a smaller range of values between sites, the averages for sites M and X were similar and sites I and J were similar. Each sample was tested twice for accuracy (excluding site X sample B as it was a small sample, there wasn't enough material to run a second test). Even if we exclude the outlier value from site J (3.92 g/cm^3), these values range from 1.35 to 2.93 g/cm^3; this large range demonstrates the heterogeneity at each site as well as between sites.

4.2 Peat

Bulk density values are shown across every field site at varying depths up to 250 cm and was calculated using Eq. 5 (Fig. 5). Bulk density of peat ranged from 0.019 to 0.134 g/cm^3. Each site showed a close range of values at each sampled depth. The largest range was at site X at 50 cm depth (0.021 to 0.06 g/cm^3), whereas the smallest range was at site A at 50 cm depth (0.019 to 0.027 g/cm^3). The data shows that in most cases (all by X) as the sample depth increases so does the peat bulk density across each location.

Hydraulic conductivity values are shown across each field site (excluding I) at varying depths up to 170 cm measured using the Hvorslev[19] method (Fig. 6). Hydraulic conductivity of the peat data spanned 5 orders of magnitude ranging from 7.0×10^{-3} to 1.02×10^{3} cm/s. Site J had the smallest range in data from 8.3×10^{-1} to 2.24×10^{1} cm/s whereas site X had the largest range from 7.0×10^{-3} to 1.02×10^{3} cm/s.

K generally decreased with depth at sites A, X and P, but remained fairly consistent (range within a 1.5 orders of magnitude) with depth for sites J, and M.

5 Discussion

Peatlands in the western boreal plains, due to their hydrogeomorphic setting, have the potential for groundwater upwelling in times of drought when surficial hydraulic head is lower than the regional hydraulic head. However, the physical properties of underlying lake sediment materials as well as bedrock, can limit the amount of groundwater discharge. It was hypothesized that areas with lower bulk density and higher porosity and hydraulic conductivity K, would be in the areas with the bog-like vegetation[13,14]. Our findings indicate that areas with higher relative bulk density but similar porosity and will be most bog like among the sites.

The lake sediments that have been tested for hydraulic conductivity (K) in this study range from a very sandy soil at 0.1 cm/s to a typical clay or compacted soil substrate at 1×10^{-5} cm/s[23] (Fig. 4 a). The difference in K values across sites show the local heterogeneity at Moss Spur. High K values corresponds with bog like vegetation at site J and I along with low K values corresponds with poor fen-like vegetation at site X[13,14] (Table 1). Unexplained is site M having no real surficial vegetation, but similar K values to site I.

Related to the hydraulic conductivity, porosity values can indicate available pore space for water flow. According to Brady & Weil[23], ideal medium textured soil will have a porosity value of roughly 50% and may range between 25% and 60%. Soils of near 25% porosity are considered compacted soils (very little pore space) whereas those with a value of 60% (sufficient pore space) are well agitated and/or high in organic matter. It is important to note that clay typically has a very high porosity, despite a low K which is due to clay having many very small pores. Locations X and M cover the widest range of values while I and J (Fig. 4 b) are more consistent by comparison and have higher average values which are consistent with higher clay content or compacted soils[23]. This range in values illustrates the importance of understanding heterogeneity of the soil throughout the sub locations, especially for sites M and X. As shown in Table 1 , high porosity values corresponding with higher relative bulk densities are found at the more bog like sites J and I[13,14]. These results are shown to be different than site X with relatively lower porosity and bulk density outlined as a poor fen[13,14].

Where bulk densities are lower and porosities are higher, and the hydraulic gradients are that of groundwater discharge, one might expect greater amounts of groundwater

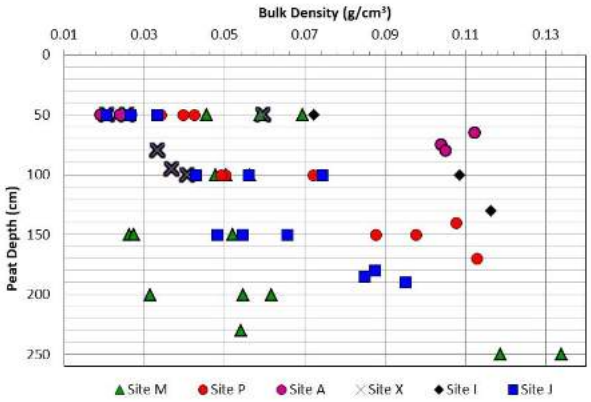

Figure 5: *Bulk density of peat samples (one point per sample) taken at each sub-location of each site.*

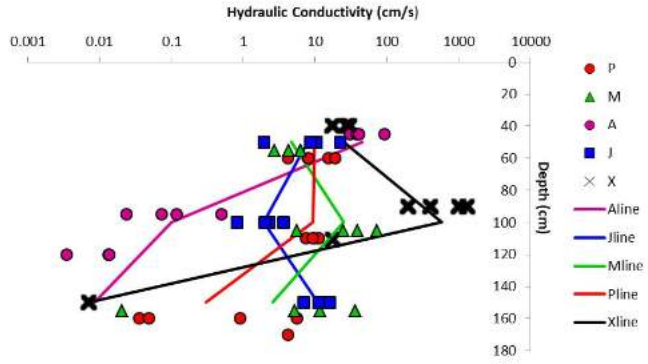

Figure 6: *Hydraulic conductivity values from roving nests at each sub-location and the main nest.*

upwelling due to increased flow. Based on bulk density values from Skopp[24], soils ranging from 1.3 to 1.9g/cm^3 will be considered more sandy, silty or clay-like. Organic matter and compactive history influence these values[24]. Sediment samples below 1 g/cm^3 have higher organic compounds[23]. Values in this study were typically low, indicating higher clay or organic content amounts. If the gradients are such that discharge is possible, one might expect more water, or less obstruction to flow.

The particle density value for quartz (the dominant component of sand) is 2.65 g/cm^3 because most soils are made up of the colloidal silicate minerals[23]. Values below 2.6 g/cm^3 are consistent with higher organic content which have particle densities as low as 0.9 g/cm^3. Values higher than 3 g/cm^3 are consistent with higher density minerals. All but one of the particle densities reported here (Fig. 4 d) fall below 3.0 g/cm^3 with the majority below 2.6 g/cm^3. This makes sense as natural bogs are groundwater recharge areas (i.e., water moves from the bog down). Organic carbon would be carried with that water, which would increase the organic content in the lake sediments beneath the peat, decreasing the particle density. The particle density values for sites I, J and M (omitting the obvious outlier for J), show some variation,

typically about 0.5 g/cm^3 between the most and least dense. Site X showed the greatest range where the most dense was more than double that of the least dense sample. Porosity calculated from particle density [Eq. 3] had a much higher range (0.24 to 0.69 g/cm^3) compared to direct porosity measurements [Eq. 2] (0.21 to 0.47g/cm^3) respectively (Fig. 4 b), suggesting that direct volumetric measurements of porosity are perhaps more reliable.

5.1 Peat

A lower bulk density (Fig. 5) typically equates to higher overall porosity [Eq. 3] which would restrict flow less and result in higher hydraulic conductivity (Fig. 6). Based on the results found for this study site, peat bulk density and hydraulic conductivity correlate fairly well, as depth increases so does the bulk density. According to Boelter[25], bulk density values for peat range between 0.01 g/cm^3 and 0.25 g/cm^3 and the values from this study fall between those ranges. The range in bulk density values between the subsites also underlines the importance of increasing sample sizes, as site M showed little variation with depth until the 250 cm layer, but a lot of variability at each depth.

Hydraulic conductivity decreased with depth at some sites (A. X, and P), but at at sites J and M remained fairly consistent with depth. At sites A and X, K decreased 4–5 orders of magnitude over the ~100 cm vertical distance. The range in K values at any one depth was usually varied by about an order of magnitude, but at the deeper depths at M and P ranged ~4 orders of magnitude. However, site P at 100 cm, varied less than 10% between the nests. This again highlights the importance of capturing the spatial heterogeneity of K in the profile, because even within a site (e.g., site P) K can be consistent at one depth (100 cm) and range considerably only 50 cm deeper.

Each site (excluding A and P, see Methods) was ranked from low to high for each parameter measured in the study (Table 1). From this list and ranked data we can determine the flow potential rank for each site and correlate the results against the classification[13,14]. Based on these results it appears that strictly based on sediment samples for these sites, the highest flow results in a marsh like setting with standing water. Those with medium or high flow potential were most bog like in nature (sites I and J) and with low flow potential where found with a poor fen (Table 1). Based on this table, the ideal parameters for bog vegetation to accumulate are higher relative bulk density as well as high relative porosity and hydraulic conductivity. This would mean that decreased flow potential with respect to site M and an increase in flow potential with respect to site X is ideal. The literature that was reviewed stated that higher hydraulic conductivity and porosity values would equate to less restricted flow and more upwelling. Based on the reviewed literature combined with the results found, there is a so called 'goldilocks' situation where too much flow does not resemble bog-like vegetation, nor does too little flow [13,14].

The other important finding of this work highlights the need for increasing sample size. Often the number of piezometer nests in a study is limited due to cost as well as the increased time required for installation and measurement. The system we proposed here, where a roving nest was used, reduced the cost but increased the installation time. That said, the range in values underscores the importance of recognizing potential errors in studies with only 1 nest in a single location. When K varies by over an order of magnitude within a ~30m lateral distance (Fig. 6), caution should be exercised when considering the veracity of the results reported.

6 Conclusion

The study attempted to link the underlying lake sediments and the properties of the peat to the vegetation patterns at the surface. While the results are not definitive, studies in natural environments are generally on going and added to. The "goldilock" zone of not being too wet, or too dry shows promise that a more rigorous assessment of these properties is warranted, in particular, going much deeper into the lake sediment profile, and potentially into the bedrock. This was beyond the scope and budget of this project. An additional finding to the project was that while the spatial heterogeneity within a site was not 0, sites did tend to clump together such that their hydraulic properties appeared different than the other sites. So, while caution must still be exercised in interpreting results from studies with little to no replication, the overall results of such studies are likely correct.

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Research Article

Interaction Between Reed Canary Grass and Purple Loosestrife in a Replacement Series

Zijing Guo[1]

[1]Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2

Corresponding Author: Z. Guo (guoz3457@myumanitoba.ca)

Abstract

*Both reed canary grass (*Phalaris *spp.) and purple loosestrife (*Lythrum salicaria*) are common invasive plants in Canadian wetlands that can erode biodiversity of native plants. A replacement series study was conducted in a conservatory greenhouse to examine effects of replacement ratio and watering regimes on competitive ability between reed canary grass and purple loosestrife. The ratio of reed canary grass to purple loosestrife was in a 4:0, 3:1, 2:2, 1:3, 0:4 sequence based on the final number per pot. The dry weight of plants was used to quantify their competitive ability. The results showed that the plants in waterlogged and mesic treatments had similar biomass, indicating watering regime did not have a significant impact on competition. Different replacement ratios had a significant impact on biomass accumulation. The 1:3 reed canary grass: purple loosestrife treatments had the highest total biomass, the highest reed canary grass biomass, and the lowest purple loosestrife biomass. Reed canary grass always had higher dry weight per plant than purple loosestrife in intercropping treatments. The per plant biomass of reed canary grass increased as more reed canary grass was being replaced by purple loosestrife in replacement series, suggesting growth of reed canary grass was more affected by intraspecific competition than competition with purple loosestrife. These results indicate that reed canary grass is more competitive than purple loosestrife and the attempt of supressing growth of purple loosestrife using slightly elevated water level is not viable. If we want to maintain a high level of biodiversity in wetland ecosystems, we should consider control of reed canary grass and purple loosestrife simultaneously.*

Keywords: Purple Loosestrife, Reed Canary Grass, Replacement Series, Wetland Invasive Species, Plant Interaction

1 Introduction

Purple loosestrife is an emergent perennial invasive weed introduced from Eurasia that can erode biodiversity of wetland and floodplain habitat in the United States and Canada[1]. The oldest records of purple loosestrife in America can be found in the *Flora of North America* in the early 1800s[2]. It is believed that purple loosestrife first appeared in North America in the 1800s in ballast heaps. The trading ships at that time usually took moist sand as ballast and unloaded it on North American shores or shoals upon arrival. Alternatively, purple loosestrife may have been deliberately introduced by European immigrants to grow as a medical herb[2].

After the 1930s, purple loosestrife began to spread aggressively by invading wetland habitats and floodplains[3]. Typically, purple loosestrife infestation is associated with wetland disturbance. It is believed that rapid expansion in the range of purple loosestrife was related to agricultural settlement and highway and canal construction. Because of prolific seed production and phenotypic plasticity, it can also be a strong competitor once established[1]. Shipley et al.[4] studied the relative competitive ability of purple loosestrife in a controlled experiment and found purple loosestrife has an competitive advantage against most native wetland species in North America. Moore et al.[5] suggested that purple loosestrife is more likely to invade infertile wetlands which have higher species richness and more rare species than more fertile wetlands. Since infertile wetlands are also more vulnerable to eutrophication and human disturbance than fertile wetlands, purple loosestrife may be a further annoyance on this fragile ecosystem.

Changes to wetland plant communities can affect how animals acquire food and shelter. Compared with native wetland species, purple loosestrife provides little food value and offers relatively poor cover and nest material[2]. Dense patches of purple loosestrife can block the gateway to open water and provide a cover to pradotors, such as foxes, potentially increasing the predation risk of waterfowl.

Reed canary grass is the common name for most grasses in the genus *Phalaris*. It is a long-lived perennial grass which can produce dense crowns and vigorous rhizomes to spread vegetatively[6]. Reed canary grass was repeatedly introduced from Europe to North America after 1850 on many independent occasions. Not all reed canary grasses are invasive; there are still some native *Phalaris* species documented before

Figure 1: *Left: first batch of purple loosestrife in silica sand. Right: Second batch of purple loosestrife.*

European settlement[7]. But these native *Phalaris* species are considered not aggressive.

Similar to purple loosestrife, reed canary grass can also alter wetland plant community composition, with potential of long-lasting environmental effects[8]. Reed canary grass reduces biodiversity by reducing variation in environments: it can trap silt and constricting waterways, restrict tree regeneration in riparian areas by crowding out seedlings, and decrease retention time of nutrients and carbon deposited in wetlands by accelerating turnover cycles.

Reed canary grass commonly cohabitates, in the same environment, with purple loosestrife. In a survey of 12 random purple loosestrife habitats in Manitoba, reed canary grass was a frequent associate (Manitoba purple loosestrife project unpublished data). Both reed canary grass and purple loosestrife are perennials, growing in similar marshy habitats, and forming monospecific stands[1]. Despite the fact reed canary grass and purple loosestrife may occupy similar niches in the wetland ecosystem, competition involving purple loosestrife and reed canary grass has received little attention. For most plant interactions, a successor in competition means an advantage in resources utilization and a better fit to their environment. Purple loosestrife usually prefers moist soil with good aeration[9], while reed canary grass can grow in a greater range of soil moisture conditions[8]. So different watering regimes may have an impact on the biomass accumulation in these two species. We performed a replacement series experiment using different ratios of purple loosestrife and reed canary grass to assess the effects of intra and interspecific interactions, as well as effect of atering regimes, on biomass accumulation with the following hypotheses:

H_{00}. Reed canary grass and purple loosestrife have a similar pattern of biomass accumulation.

H_{01}. There is no difference in the dry weight of reed canary grass and purple loosestrife among different replacement ratios.

H_{02}. Biomass accumulation in mesic and waterlogged treatment is similar.

2 Methods

A replacement series experiment was conducted in the conservatory greenhouse in Faculty of Agricultural and Food Science at the University of Manitoba in fall 2017 and lasted for 32 days. For this experiment, purple loosestrife was propagated from vegetative tissue that was collected at the ditch along Harte trail near Assiniboine Forest Winnipeg, Manitoba (49.844505°N, 91.255031°W) (first batch collected September 22) and the artificial pond in Linden Woods Winnipeg (49.832976°N, 97.191227°W) (second batch collected October 1). After harvest, purple loosestrife stems were cut into pieces with sanitized surgery blades with at least four axiliary buds in each piece. The first batch was planted in silica sand inside plastic vials (Fig. 1, left). In order to increase survivorship of purple loosestrife, the second batch was planted in a mixture with a thin layer of peat moss on the bottom, soil in the middle, and vermiculite on top (Fig. 1, right). After visible root growth, purple loosestrife plants were transplanted into growth trays with soil as growth medium in moisture chamber watered every weekday. On November 3, all purple loosestrife plants were trimmed and plantlets with exactly four leaves attached to stems and a similar weight were selected for the replacement series experiment.

Fresh seed of reed canary grass was obtained from the perennial crop breeding lab. To stimulate germination, a pre-sowing treatment was used. Specifically, reed canary grass seeds were soaked in 0.2% KNO_3 in a Petri dish[10] on October 17. On October 18, these seeds were placed in a dark box for 24 hours. Thereafter, the seeds were exposed to a two-hour period at 12°C followed by a two-hour period at room temperature and this was repeated three times. During these three cycles, the seeds were exposed to 16-hour light periods and eight-hour dark periods. On October 20, excessive reed canary seeds were sown into four-inch pots filled with a growth medium mix comprised of peat moss, clay, and sand at a ratio of 1:2:1 and were placed in the conservatory greenhouse.

One week after transplanting, waterlogged groups were placed in a plastic bucket. The water table in waterlogged replacement series was maintained at about 2 cm the below soil surface so the soil medium in this treatment was continuously saturated throughout the experiment. The water in the plastic bucket was replenished every weekday, while the mesic treatment was watered on a daily basis. The position of the pots was re-randomized once during the experiment to eliminate possible confounding variables. ABS sa-

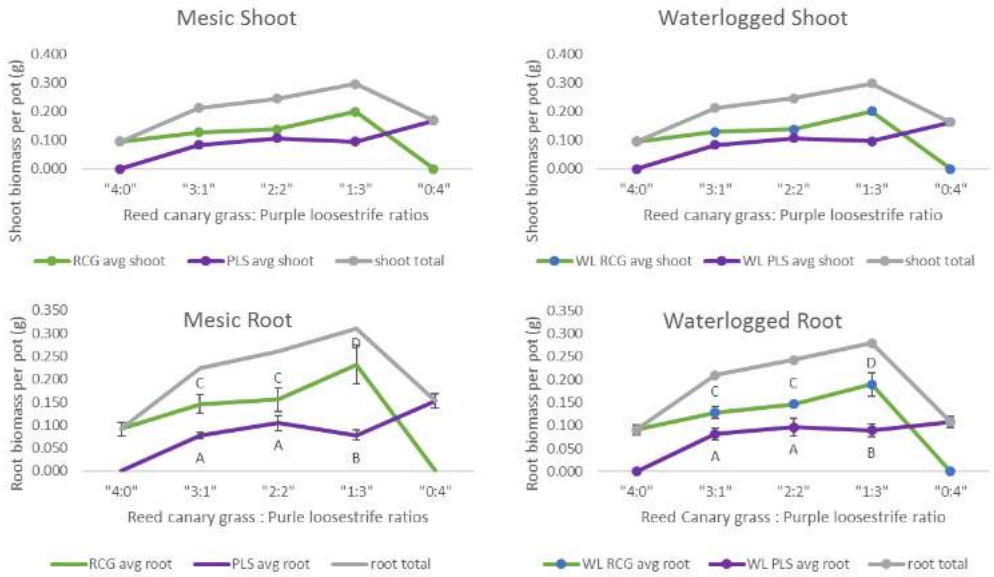

Figure 2: *Average above ground and below ground per pot biomass of purple loosestrife (PLS) and reed canary grass (RCG) in waterlogged (WL) and mesic conditions at various density ratios in a replacement series. Horizontal axis indicates the RCG to PLS ratio in each treatment and vertical axis is the dry weight of root/shoot tissues per plant. Letters on the figure is the results of Fisher's protected least significant difference test on plant aggressivity. Error bar in the figure is the standard error of the mean.*

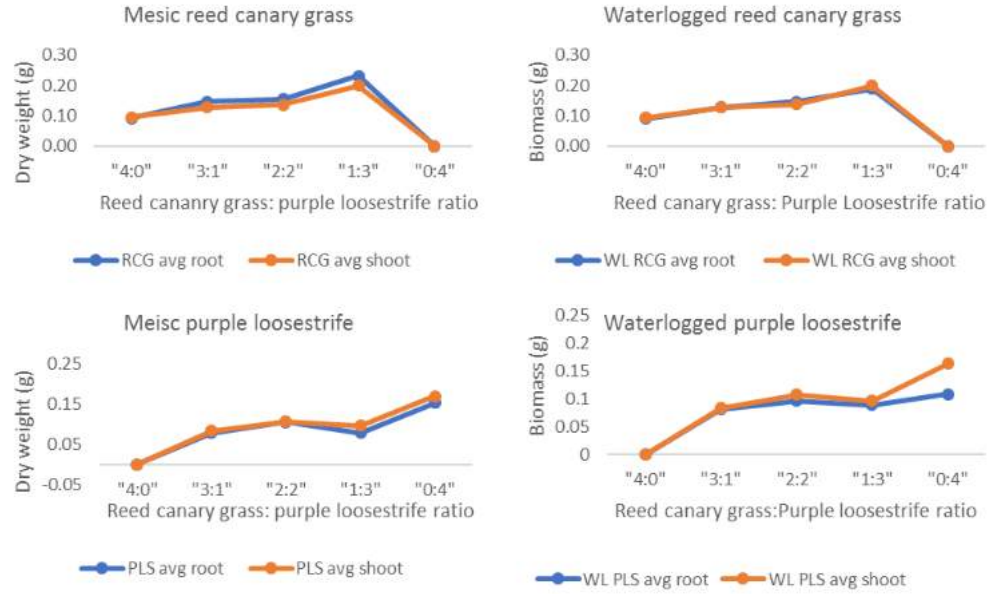

Figure 3: *Biomass (dry weight per pot) partition in purple loosestrife (PLS) and reed canary grass (RCG) roots and shoots in waterlogged (WL) and mesic conditions at various density ratios in a replacement series.*

chets (biobest — *Amblyseius cucumeris*), Sulphur powder, and Kontos (systemic pesticide that kills aphids; applied as a soil drench) were used to treat aphids and other pests.

On December 4, 32 days after transplant, the effects of competition on biomass partitioning were analyzed by comparing the above ground and below ground biomass accumulation in purple loosestrife and reed canary grass. Plant tissue was harvested and rinsed with water to remove adhering soil. After cleaning, plants were clipped at the imaginary soil surface (based on the colour of stem) to separate below ground and above ground biomass. Plant tissue was dried in an oven for 24 hours at 55°C and biomass was determined

Table 1: *Species, water content, and replacement ratio effect on root biomass.*

Effect	Biomass (least mean square)
Species	
Purple lossestrife	0.08129
Reed canary grass	0.1595
Water	
Mesic	0.1270
Waterlogged	0.1138
Ratio (RCG:PLS)	
4:0	0.05200
3:1	0.1103
2:2	0.1259
1:3	0.1453
0:4	0.1684
Effect	**P-value**
Species (S)	<0.0001
Water (W)	0.1497
Ratio (R)	<0.0001
S × W	0.2508
S × R	0.0063
W × R	0.5602
S × W × R	0.6893

using a four-digit balance (Mettler, AE100).

Because of a mistake in measuring scheme, I did not get enough information to calculate the standard deviation of the biomass. Specifically, this unfortunate error combined all replicates into one measurement and did not give me enough data to conduct a statistical analysis. To fix this, I tried to separate the plant tissue by assigning them to different replicates one month after the initial measurement. The separation of root tissue was a success, but the separation of shoot tissue failed because some leaves were shattered and could not be assigned to any replicate. Therefore, a statistical analysis could only be conducted on the biomass accumulation of roots in both species.

Competitiveness in intercropping treatments was quantified using plant aggressivity (AGGR) with the following formula[11]:

$$\text{AGGR} = \frac{W_{ab}}{W_{aa}} - \frac{W_{ba}}{W_{bb}}$$

W_{aa} and W_{bb} in the formula are the weights per plant of species a and species b when grown in monoculture. W_{ab} and W_{ba} are the per plant weights of the species in mixture with each other.

Root biomass was examined in the following two ways (no statistical analysis can be made in shoot biomass because of pooling error). The statistical significance level was set at $\alpha < 0.05$ for all tests. SAS was used for to compute 3-way ANOVA (analyses of variance) tests with Proc MIXED procedure in which the effects of replacement ratio, watering regime, type of species, and their interaction on the accumulation of root biomass were compared. Treatment differences were deemed significant if $p < 0.05$ using Fisher's protected least significant difference (LSD) test for multiple comparisons.

3 Results

3.1 Effects of the watering scheme on plant biomass

In Fig. 2, we can see that the per plant dry weight of both shoot and root tissues in the waterlogged treatment varied from about 0.05g to 0.23g. Meanwhile, the dry weight in the mesic treatment had a similar value. There was no statistical difference in the biomass of reed canary grass and purple loosestrife between waterlogged and mesic treatments ($p>0.05$), suggesting biomass accumulation by these plants is not driven by water regimes (Table 1).

3.2 Effects of species replacement in biomass accumulation

The ratio of purple loosestrife to reed canary grass had a significant impact on biomass ($p > 0.05$) (Table 1). Reed canary grass always had higher per plant dry weight than purple loosestrife in intercropping treatment. In fact, the highest total dry weight, the highest reed canary grass dry weight, and the lowest purple loosestrife dry weight always occurred in the 1:3 purple loosestrife: reed canary grass combination (Fig. 2).

The aggressivity of both species in both waterlogged and mesic treatments at 1:3 reed canary grass:purple loosestrife planting ratio was significantly different from any other intercropping ratios (Table 2, Fig. 2). The purple loosestrife in the 1:3 treatment was assigned with B characteristic while all other purple loosestrife intercropping treatment have A characteristic. Similarly, the reed canary grass in 1:3 treatment was assigned with D characteristic and all other reed canary grass intercropping treatment have C characteristic. Specifically, reed canary grass in 1:3 planting ratio was significantly more competitive than reed canary grass in any other planting ratios; while the purple loosestrife in this ratio was significantly less competitive than purple loosestrife in other

Table 2: *Aggressivity of purple loosestrife in various replacement ratios. Due to the reciprocal nature of aggressivity in two competing species, only the value of purple loosestrife is included in this table, reed canary grass has exactly the same value with opposite signs. The less competitive species will have negative aggressivity number.*

Water treatment	Reed canary grass : purple loosestrife ratio	Aggressivity of purple loosestrife
mesic	3:1	-2.025
mesic	2:2	-1.012
mesic	1:3	-1.084
waterlogged	3:1	-1.267
waterlogged	2:2	-0.724
waterlogged	1:3	-0.662

planting ratios. For reed canary grass, the root biomass gradually decreased from the highest value of 0.146 g per plant in waterlogged treatment and 0.232 g/plant in mesic treatment at density ratio of 1:3 (reed canary grass: purple loosestrife) to monoculture treatment (4:0) with a dry weight of 0.090g/plant and 0.092/plant respectively (calculation derived from Fig. 2). As it suggested in aggressivity, purple loosestrife has an opposite trend for biomass accumulation than reed canary grass; the highest root biomass was observed in monocultural pots at 0.11g/plant in waterlogged treatment and 0.15g/plant in mesic treatment, and declined to 0.081 g/plant and 0.078g/plant in at the density ratio of 1:3 (reed canary grass: purple loosestrife) in waterlogged and mesic treatments, respectively. The root biomass of both species in 3:1 and 2:2 pots had a similar intermediate weight which were slightly higher than the lowest dry weights but much lower than the highest dry weights of each species (Fig. 2).

Although we were unable to measure per plant variability in shoot biomass, the total biomass of each species followed a similar pattern to that of root biomass. Biomass of reed canary grass was always greater than that of purple loosestrife in intercropping treatments for both waterlogged and mesic scenario. Moreover, the highest total biomass, the highest reed canary grass biomass, and the lowest purple loosestrife biomass in waterlogged and mesic conditions were also found in 1:3 planting ratios; these characteristics were consistent with what we found in root biomass.

3.3 Monocultures

In general, reed canary grass in monoculture assimilated less biomass than their peers in intercropping treatment. In fact, the lowest reed canary grass per pot root biomass in both waterlogged and mesic conditions occurred when interspecific competition was absent i.e. at 4:0 (reed canary grass: purple loosestrife) planting ratio. In contrast, purple loosestrife from the monoculture pots produced more biomass than their counterparts growing in replacement series. As pressures from intraspecific competition in purple loosestrife gradually being substituted by interspecific pressures from reed canary grass, aggressivity and biomass of purple loosestrife in both mesic and waterlogged treatments decreased accordingly (Fig. 2, Table 2).

3.4 Biomass partition

No consistent pattern can be found in reed canary grass root:shoot biomass allocation. In waterlogged intercropping treatments, there was only a minor difference between the dry weight of root and shoot tissues (Fig. 3). In mesic pots, the shoot biomass of reed canary grass was higher than the root biomass in intercropping ratios and the gap between root and shoot biomass is slightly larger than that in waterlogged scenarios. In particular, as more reed canary grass was replaced by purple loosestrife in mesic replacement series the gap of root and shoot biomass in reed canary grass became bigger gradually (Fig. 3). In both waterlogged and mesic monocultural treatments, shoots comprise a greater proportion of purple loosestrife biomass. Although the small sample size and lack of standard deviation prevents a clear interpretation of these results, it does seem that purple loosestrife biomass allocation strategy was affected by replacement ratios (Fig. 3).

3.5 Abnormality

About three weeks after transplantation, some leaves of purple loosestrife felt extra soft when being touched and turned into reddish-yellow (Fig. 4). This discolouration symptom first showed up at shoot tips in a few waterlogged plants and eventually spread to most of the purple loosestrife in this study during the fourth and fifth weeks after transplanting. The detailed records are listed in Table 3. Because this symptom can be caused by many reasons, such as nutrient defi-

Table 3: *Number of red purple loosestrife plants in each treament. Plants with at least three reddish-yellow-green leaves were labelled red.*

Treatment	Mesic (watered daily)	Waterlogged
1 PLS: 3 RCG	0/3	3/3
2 PLS: 2 RCG	6/6	4/6
3 PLS: 1 RCG	7/9	3/9
4 PLS: 0 RCG	5/11	4/12

Figure 4: *Reddish leaves in purple loosestrife.*

ciency (Mg, Ca, K, P, and N) or elevated levels of anthocyanin, no conclusion could be made without further examination (Pioneer Agronomy Science, 2009).

Mortality occurred in one of the mesic purple loosestrife monocultural replication, probably caused by damage in transplanting or damage during maintenance activities such as pot rotation. Although plant death will reduce total density and thus impose an impact on the plant interactions, the average dry weight for this replication did not appear to be abnormal as indicated by Student's t-test. So, pots with plant death were still used in data analyses.

4 Discussion

Our results highlight the impact of replacement ratio on biomass accumulation in early stage interaction between purple loosestrife and reed canary grass. Not enough support for the water saturation hypothesis were found in this study (H_{02}). Few studies have investigated biomass accumulation of purple loosestrife and reed canary grass in various circumstances[12, 13, 14, 15].

Plant biomass is a complex response variable that incorporates factors like resource availability, light exposure, and disturbance as well as biotic interactions[16]. Few studies have been conducted on using a water gradient as an influencing factor in plant competitions. In theory, plant roots need oxygen to respire, water saturated soil could hinder root oxygen up-take efficacy in most terrestrial pln a field study[14], *Phalaris arundinacea* (reed canary grass) ranked first in mean percentage ground cover at 33.3% in the third year after vegetation removal in drier sites and purple loosestrife ranked at sixth place with 1.7% ground cover. In contrast, the flooded site was codominated by two native wetland species. Purple loosestrife ranked seventh in terms of ground cover and reed canary grass was not found at this site in natural colonization treatments. Fowler and Antonovics[12] also found that the dominance hierarchy in a grassland community varied with water availability.

In this experiment, we found no effects of water regimes on plant biomass accumulation. This finding does not fit the trends in aforementioned literature. Lack of disparity between waterlogged and mesic treatments may the cause of this disagreement. In another study, when seven water depth treatments (-6, -4, -2, 0, $+2$, $+4$, and $+6$ cm relative to the soil surface) were incorporated, 12 species (including purple loosestrife and reed canary grass) had their lowest biomass and lowest survivorship at water depth greater than 0 cm[17]. From my observation, the soils in the mesic treatment were usually moist. In other words, the mesic treatment in this experiment was essentially waterlogged but in a lesser extent. Nevertheless, the watering regime in my experiment was set on purpose; to induce early competition, the smallest pots were used, but they cannot hold much water. All pots in the mesic treatment needed to be watered every day to prevent severe dehydration damage.

Another factor that may influence plant interference is soil fertility. Especially in wetlands where productivity is not limited by either moisture or sunlight availability[18]. Day et al.[18] also found soil fertility to be the predominant aspect that explains variation in species composition along riverine areas on the Ottawa River. In a study using purple loosestrife as a phytometer to compare the competitive ability of 40 common wetland plants, soil organic matter, P, N, Mg, and K were strong drivers of plant biomassn[13].

The purple loosestrife in this experiment had some reddish-yellow leaves (Table 3, Fig. 4). The discolouration symptom can be causedby many reasons, such as nutrient deficiency (Mg, Ca, K, P, N) or elevated level of anthocyanin, no conclusion could be made without further examination(Pioneer Agronomy Science, 2009). On the contrary, reed canary grass had a normal colour and shape in both waterlogged and mesic treatment.

Under the conditions of this experiment, the measures of plant biomass in the replacement series demonstrated suppression of both species in the presence of reed canary grass. The biomass accumulation of both purple loosestrife and reed canary grass were negatively related to the density of reed canary grass in all conditions. This suggests that reed canary grass is more sensitive to intraspecific competition, whereas purple loosestrife is more sensitive to interspecific competition.

The previous research on the comparison of competitive ability between reed canary grass and purple loosestrife had mixed results. Mal et al.[2] developed a regime that incorporated a policy of repeated mowing, plowing, and subsequent seeding with reed canary grass to successfully suppress purple loosestrife in the highly infested Great Meadows near Concord, Massachusetts. Gaudet and Keddy[13] found that intraspecific competition within purple loosestrife populations reduced 96% of its biomass, while interspecific competition with reed canary grass suppressed 89% of purple loosestrife biomass in an additive design conducted at various shorelines in eastern Canada. This finding suggests that purple loosestrife was more affected by competition from itself than competition with reed canary grass. Fraser and Karnezis[17] found that purple loosestrife had the greatest standing crop among 14 various wetland species while reed canary grass ranked between fourth and 10th in total biomass, indicating purple loosestrife was more competitive than reed canary grass in their microcosm greenhouse study. In another field survey of 24 wetlands in the Pacific Northwest, the abundance of purple loosestrife and reed canary grass were negatively correlated but the hierarchy of competitive ability was not fixed and was likely determined by various environmental factors[15].

These inconsistencies may be caused by differences in scales among those experiments. Various studies have demonstrated that properties or processes emergent at one level or scale of interaction may not be predictable at different scales of interaction[13, 19]. Environmental differences that originated from different site locations might also be a reason for these inconsistent results. All aforementioned results were either from a greenhouse study with a large sample size[17, 2] or field plant survey[17, 15], while this experiment took place in a conservatory greenhouse with a relatively small sample size.

Although reed canary grass was more competitive than purple loosestrife in this experiment, using reed canary grass to crowd out purple loosestrife in Canadian wetlands may not lead to enhanced biodiversity. Previous studies have shown that reed canary grass is capable of out-competing and displacing other native wetland plants as well[6, 15]. Reed canary grass creates a thick layer of litter that impedes the growth of other species whereas purple loosestrife cannot produce such a dense litter layer[15]. The replacement of one invasive weed by another one is not likely to have many benefits for biodiversity.

The ultimate practical value of understanding competitive mechanisms of invasive species is to find a management strategy. Current efforts to control purple loosestrife in Manitoba have focused on introducing its natural enemies from Eurasia i.e. purple loosestrife beetle (*Galerucella calmariensis L*)[1, 20, 21]. Reed canary grass can outcompete purple loosestrife in some studies[2]. Purple loosestrife in the aforementioned natural enemy predatory experiment suffered from plant competition and herbivore predation simultaneously. The presence of plant competition could be a confounding factor in evaluating the efficacy of biocontrol agents. Future studies on separating the effects of plant competition and herbivore predation might be needed to demonstrate the true efficacy of biocontrol agents.

The distribution of purple loosestrife and reed canary grass overlap in Manitoba wetlands[21]. Since these two species are competing with each other and both species have been demonstrated as being more competitive than other native wetland species[15], removing one will likely result in the increase of the other, and targeting one while ignoring the other is not likely to lead to an increase in biodiversity. In order to truly restore wetlands in Manitoba, I recommend integrating purple loosestrife and reed canary grass control programs.

There are some caveats to this study that may limit its ability to extrapolate to larger, more complex systems. Firstly, using biomass to infer competitive ability may not be very accurate in a short-term experiment. Regardless of the limiting resource involved in a plant competition, the result is usually associated with a critical age or stage of development[1]. Grace et al.[22] demonstrated biomass measurements were correlated with initial sizes of plant in the first two years. With the fact that reed canary grass was grown from seeds and purple loosestrife was propagated from vegetative cuttings in this experiment, it is not possible to eliminate the impact of initial plant size. Secondly, there are some concerns on the accuracy of replacement series design. Marshall and Jain[23] suggested that the competitive ability in a replacement study may be dependent on the total density chosen. Tilman[24] found that the effects of intraspecific and interspecific competition cannot be easily separated in replacement series. Thirdly, due pooling plants for biomass measurements, we lost statistical power and could not perform robust models. Consequently, the results and findings in this experiment might be eroded by these flaws.

5 Conclusion

In conclusion, I found the density ratio of reed canary grass and purple loosestrife has an impact on the biomass accumulation. While the watering regimes do not have a significant impact on competitive ability, reed canary grass appeared to be more competitive than purple loosestrife in this experiment. However, using reed canary grass to crowd out pur-

ple loosestrife is not likely to enhance biodiversity in Canadian wetlands. According to the results in this experiment, using slightly elevated water levels to suppress the growth of purple loosestrife is not practical, and I recommend integrating purple loosestrife and reed canary grass control programs to increase the biodiversity in wetlands. There are some inconsistencies with previous research, possibly caused by differences in scales of study, influences of initial biomass, and errors of pooling plants for biomass measurements.

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Research Article

Construction of A-optimal Designs for Linear Models

Xiang Zheng[1], Saumen Mandal[1]

[1]Dept. of Statistics, University of Manitoba, Winnipeg, MB, R3T 2N2

Corresponding Author: X. Zheng (zhengx34@myumanitoba.ca)

Abstract

For estimating parameters of a statistical model, it is important to minimize the variances of the estimators. Efficiency of an estimator increases as its variance becomes smaller. Sometimes instead of minimizing the variances of the individual parameters, it is important to minimize the total or average variance of all the parameter estimators. This refers to A-optimality in the context of optimal experimental design. Motivated by this fact, we construct A-optimal designs for some regression models using a class of algorithms, indexed by a function which depends on the derivatives of the criterion function. We also develop strategies for constructing A-optimal designs and investigate techniques for improving convergence rates by using the properties of the directional derivatives of the criterion function. Computational studies show that convergence of the algorithm improves a great deal when amended by the properties of the directional derivatives. We explored the design construction through some examples including one practical problem arising in chemistry.

Keywords: Average Variance, Directional Derivatives, Multiplicative Algorithms, Optimal Designs, Parameter Estimation

1 Introduction

Optimal designs are constructed according to a statistical criterion for a specific statistical model. The objective is good estimation of the parameters of the model. There are a variety of criteria defining good estimation, the popular ones being D, G, A and linear optimality. In D-optimality, we minimize the determinant of the covariance matrix of the parameter estimators. That is, in this optimality, the generalized variance of the parameter estimators is minimized. Note that because of the reciprocity property of the covariance matrix and the information matrix, minimizing the determinant of the covariance matrix is equivalent to maximizing the determinant of the information matrix. In G-optimality, we minimize the maximum standardized variance of the predicted response over the design space. This optimality may be useful when a researcher is interested in predicting the outcome variable as efficiently as possible over the design space. In this present work, we focus on A-optimal criterion and construct such designs for some models of interest. An A-optimal design seeks to minimize the sum of the variances of the parameter estimators or their average variance. Some motivations for A-optimality are given at the end of this section. A full description of this criterion is given in Section 2.

As the present work is based on optimal design theory, we start with a brief introduction to this area. We first assume a probability model. A probability model is a mathematical representation of a random phenomenon. It is defined by its sample space. A sample space for a probability model is a collection of all possible outcomes of a random experiment. A sample space could be discrete or continuous. In optimal design context, we first assume a probability model of the type

$$y \sim \pi(y|\boldsymbol{x}, \boldsymbol{\theta}, \sigma)$$

where y is the response variable; $\boldsymbol{x}$ are design variables, $\boldsymbol{x} \in \boldsymbol{\mathcal{X}} \subseteq \mathbb{R}^m$, $\boldsymbol{\mathcal{X}}$ is the design space; $\boldsymbol{\theta} = (\theta_1, \theta_2, ..., \theta_k)^\top$ are unknown parameters; σ is a nuisance parameter; and $\pi(.)$ is a probability model. The objective of an optimal design is good estimation of the parameters of the model. As discussed earlier, there are a variety of criteria defining good estimation. When the model is linear in the parameters, we further assume that $y(\boldsymbol{x})$ has an expected value of the explicit form $E(y|\boldsymbol{v}) = \boldsymbol{v}^\top\boldsymbol{\theta}$, where $\boldsymbol{v} \in \mathcal{V}$, $\mathcal{V} = \{\boldsymbol{v} \in \mathbb{R}^k\colon \boldsymbol{v} = \eta(\boldsymbol{x})\}$ with $\eta(\boldsymbol{x}) = (\eta_1(\boldsymbol{x}), \eta_2(\boldsymbol{x}), \ldots, \eta_k(\boldsymbol{x}))^\top$, a vector of k real valued functions defined on the design space $\mathcal{X}$. The space $\mathcal{V}$ is called the induced design space (or design locus) because $\mathcal{V}$ is the image under a set of regression functions η of $\mathcal{X}$. We discuss how we obtain $\mathcal{V}$ from the original design space $\mathcal{X}$ in the following.

In optimal design theory, an approximate design is characterized by a probability measure, say p, defined on the design space $\mathcal{X}$ and hence on $\mathcal{V}$. In practice we must discretize these spaces. Suppose that we discretize the design space $\mathcal{X}$ into J distinct points, say, $\boldsymbol{x}_1, \boldsymbol{x}_2, \ldots, \boldsymbol{x}_J$. Thus, $\mathcal{V} = \{\boldsymbol{v}_1, \boldsymbol{v}_2, \ldots, \boldsymbol{v}_J\}$, where $\boldsymbol{v}_j = \eta(\boldsymbol{x}_j)$, $j = 1, 2, \ldots, J$.

At this point we specify p by a set of weights or proportions p_j satisfying $p_j \geq 0$, $j = 1, 2, \ldots, J$, $\sum p_j = 1$. We assign weight p_j to $\boldsymbol{v}_j$. We wish to choose the vector $\boldsymbol{p} = (p_1, p_2, \ldots, p_J)^\top$ optimally. If $\hat{\boldsymbol{\theta}}$ is the least squares estimator of $\boldsymbol{\theta}$, then the covariance matrix $cov(\hat{\boldsymbol{\theta}}) \propto \boldsymbol{M}^{-1}(\boldsymbol{p})$, where $\boldsymbol{M}(\boldsymbol{p})$ is the per observation information matrix. The matrix $\boldsymbol{M}(\boldsymbol{p})$ is given by

$$\boldsymbol{M}(\boldsymbol{p}) = \sum_{j=1}^{J} p_j \boldsymbol{v}_j \boldsymbol{v}_j^\top = \boldsymbol{V} P \boldsymbol{V}^\top \tag{1}$$

where $\boldsymbol{V} = [\boldsymbol{v}_1\, \boldsymbol{v}_2\, \ldots\, \boldsymbol{v}_J]$ and $\boldsymbol{P} = \text{diag}(p_1, p_2, \ldots, p_J)$. In order to ensure good estimation of $\boldsymbol{\theta}$, we wish to choose the proportion p_j of observations taken at $\boldsymbol{x}_j$ by optimizing some criterion, say $\phi(\boldsymbol{p})$. There are many useful books in optimal design [1, 2, 3, 4, 5].

The aim of this paper is to construct A-optimal designs in linear regression models. In A-optimality, we minimize the sum of the variances of the parameter estimators or their average variance. This criterion was introduced by Elfving[6]. There is an extensive literature available for this criterion. The alphabetical nomenclature for different design criteria was introduced by Kiefer[7]. As the trace is the sum of the main diagonal elements of a matrix, the A-optimal criterion minimizes the trace of the covariance matrix of the parameter estimators. A-optimality is important in the sense that we always try to minimize the variances of the parameters of a statistical model. Instead of minimizing the variances of the individual parameters, this optimality minimizes the total or average variance of all the parameter estimators. We construct A-optimal designs using a class of multiplicative algorithms, indexed by a function which depends on the derivatives of the A-criterion function. The function is positive, increasing and may depend on a free positive parameter. The goal is also to develop strategies for constructing A-optimal designs and investigate techniques for improving convergence rates by using the properties of the directional derivatives of the criterion function.

We take the criterion function $\phi(\boldsymbol{p})$ to be the A-optimality criterion. We maximize $\phi(\boldsymbol{p})$ subject to $p_j \geq 0$ and $\sum_{j=1}^{J} p_j = 1$. So we consider an example of the following general problem.

$$\text{Maximize } \phi(\boldsymbol{p}) \text{ over } \mathcal{P} \equiv \{\boldsymbol{p} = (p_1, p_2, \ldots, p_J) : \tag{2}$$

$$p_j \geq 0, \sum_{j=1}^{J} p_j = 1\}$$

The equality constraint $\sum p_j = 1$ renders the problem a constrained optimization problem. Note also that $\mathcal{P}$ is a probability simplex. The probabilities are nonnegative and sum to one. The set is closed and bounded. By the definition of convexity, the full constraint region is convex.

2 Methods

Our general problem is to maximize a criterion $\phi(\boldsymbol{p})$ subject to $p_j \geq 0$, $j = 1, 2, \ldots, J$ and $\sum p_j = 1$. In order to solve this problem, we first need to determine the optimality conditions.

2.1 Optimality Conditions and a Class of Algorithms

We determine the optimality conditions in terms of point to point directional derivatives. We use differential calculus and exploit the directional derivative of Whittle[8]. The directional derivative $F_\phi\{\boldsymbol{p}, \boldsymbol{q}\}$ of a criterion function $\phi(.)$ at $\boldsymbol{p}$ in the direction of $\boldsymbol{q}$ is defined as

$$F_\phi(\boldsymbol{p}, \boldsymbol{q}) = \lim_{\varepsilon \downarrow 0} \frac{\phi\{(1-\varepsilon)\boldsymbol{p} + \varepsilon \boldsymbol{q}\} - \phi(\boldsymbol{p})}{\varepsilon}. \tag{3}$$

The derivative $F_\phi\{\boldsymbol{p}, \boldsymbol{q}\}$ exists even if the criterion function $\phi(.)$ is not differentiable. If $\phi(.)$ is differentiable, (3) can be simplified as: $F_\phi(\boldsymbol{p}, \boldsymbol{q}) = (\boldsymbol{q} - \boldsymbol{p})^\top \partial\phi/\partial\boldsymbol{p}$. Let $F_j = F_\phi(\boldsymbol{p}, \boldsymbol{e}_j)$, where $\boldsymbol{e}_j$ is the j^{th} unit vector in $\mathbb{R}^J$. So, F_j can be simplified as

$$F_j = d_j - \sum_{i=1}^{J} p_i d_i \tag{4}$$

where $d_j = \partial\phi/\partial p_j$, $j = 1, 2, \ldots, J$. As F_j is the directional derivative of the criterion function $\phi(.)$ at $\boldsymbol{p}$ in the direction of the extreme vertex $\boldsymbol{e}_j$, we call F_j the vertex directional derivative of the criterion $\phi(.)$ at $\boldsymbol{p}$.

Now, if $\phi(.)$ is differentiable at an optimizing distribution $\boldsymbol{p}^*$, then the first-order conditions for $\phi(\boldsymbol{p}^*)$ to be a local maximum of $\phi(.)$ in the feasible region of the problem are

$$F_j^* = F_\phi\{\boldsymbol{p}^*, \boldsymbol{e}_j\} \begin{cases} = 0 & \text{for } p_j^* > 0 \\ \leq 0 & \text{for } p_j^* = 0. \end{cases} \tag{5}$$

If the criterion $\phi(.)$ is concave on the feasible region, then the first-order conditions (5) are both necessary and sufficient for optimality, a result known as the general equivalence theorem in optimal design [8, 19].

It is typically not possible to evaluate an optimal solution explicitly. So, we often require an algorithm in order

to construct the optimizing distribution. A class of algorithms which neatly satisfy the basic constraints of the optimal weights take the form

$$p_j^{(r+1)} \quad \propto \quad p_j^{(r)} f(d_j^{(r)}) \tag{6}$$

where $d_j^{(r)} = \partial\phi/\partial p_j$ at r^{th} iterate $\boldsymbol{p} = \boldsymbol{p}^{(r)}$ and the function $f(.)$ satisfies the conditions that it's positive and strictly increasing. The function $f(.)$ may depend on a free positive parameter δ. Torsney[9] first proposed this type of iteration by taking the function $f(d) = d^\delta$, $\delta > 0$. Silvey, Titterington, and Torsney[10] studied the choice of δ when the function $f(d)$ takes the same form as above, i.e., d^δ. Torsney[11] considered the choice $f(d) = e^{\delta d}$, where the partial derivatives could be both positive and negative. Torsney[12] explored the monotonicity property of particular values of the free parameter δ. Titterington[13] describes a proof of monotonicity of $f(d) = d$ for constructing D-optimal designs. Chowdhury, Chen, and Mandal[14] used the above algorithm and considered a class of optimization problems on minimizing variance based criteria in respect of parameter estimators. Mandal, Torsney, and Carriere[15], and Mandal and Torsney[16] further developed the algorithm based on a constrained optimization problem and a clustering approach, respectively. Mandal, Torsney, and Chowdhury[17] used a Lagrangian approach and constructed optimal designs by minimizing a covariance criterion. They established optimality conditions for a non-standard criterion function. The conditions are given in terms first and second order conditions.

2.2 Optimizing Distribution and the A-optimality

Our general problem is given in (2), where $\phi(\boldsymbol{p})$ is a criterion function of interest. In the present work, we take $\phi(\boldsymbol{p})$ as the A-optimality criterion. We mentioned earlier that instead of minimizing the variances of the individual parameters of a model, it is also important to minimize the total or average variance of all the parameter estimators. In order to minimize the total or average variance of all the parameter estimators, we need to minimize the A-optimality criterion. In Section 1, we have seen that the covariance matrix of $\hat{\boldsymbol{\theta}}$ is actually the inverse of the information matrix $M(\boldsymbol{p})$. Because of this reciprocity property, minimizing the variance corresponds to maximizing the information. In terms of a maximization problem, the A-optimality criterion is defined by

$$\phi_A(\boldsymbol{p}) \quad = \quad \psi_A\{\boldsymbol{M}(\boldsymbol{p})\} \quad = \quad -Trace\{\boldsymbol{M}^{-1}(\boldsymbol{p})\}. \tag{7}$$

The above criterion has some properties. The criterion is concave and an increasing function over $\mathcal{M}$, where $\mathcal{M}$ is the set of all positive definite symmetric matrices. The criterion is differentiable whenever it is finite, and the first derivative is given by

$$\frac{\partial\phi_A}{\partial p_j} \quad = \quad \boldsymbol{v}_j^T\, \boldsymbol{M}^{-2}(\boldsymbol{p})\, \boldsymbol{v}_j. \tag{8}$$

The A-optimality criterion was considered by Elfving[6] and Chernoff[18] and subsequently studied [1, 2, 3, 5, 19, 20, 21]. Recently Chowdhury, Chen, and Mandal[14] considered a class of optimization problems on minimizing variance based criteria in respect of parameter estimators of a linear model. They did not consider the A-optimality directly. Instead of considering all the parameters, they considered minimizing the total variance of the estimators of some parameters of interest.

It can be easily shown that A-optimality is a special case of Linear optimality in which we minimize the criterion $\phi_L(\boldsymbol{p}) = \text{Trace}\{\boldsymbol{M}^{-1}(\boldsymbol{p})\boldsymbol{L}\}$, where $\boldsymbol{L}$ is a $k \times k$ matrix of coefficients. Suppose the matrix $\boldsymbol{L}$ is of rank s, where $s \leq k$. Then $\boldsymbol{L}$ can be expressed as: $\boldsymbol{L} = \boldsymbol{A}^\top\boldsymbol{A}$, where $\boldsymbol{A}$ is a $s \times k$ matrix of rank s. Then the criterion $\phi_L(\boldsymbol{p})$ can be written as $\phi_L(\boldsymbol{p}) = \text{Trace}\{\boldsymbol{M}^{-1}(\boldsymbol{p})\boldsymbol{A}^\top\boldsymbol{A}\} = \text{Trace}\{\boldsymbol{A}\boldsymbol{M}^{-1}(\boldsymbol{p})\boldsymbol{A}^\top\}$. When $\boldsymbol{A} = \boldsymbol{c}^\top$, where $\boldsymbol{c}$ is a $k \times 1$ vector, $\phi_L(\boldsymbol{p})$ corresponds to the c-optimality criterion. When $\boldsymbol{A}$ or $\boldsymbol{L}$ is an identity matrix, $\phi_L(\boldsymbol{p})$ corresponds to the A-optimality criterion.

2.3 Construction of A-optimal Designs

As we discussed earlier, problem (2) has a set of constraints on the design weights, namely, $p_j \geq 0$ and $\sum_{j=1}^J p_j = 1$. An iteration which neatly submits to these constraints is given in (6). As we mentioned, this type of iteration was first proposed by Torsney[9]. The function $f(.)$ in the algorithm may depend on a positive parameter δ. The full form of the algorithm is given by

$$p_j^{(r+1)} \quad = \quad \frac{p_j^{(r)} f(x_j^{(r)},\, \delta)}{\sum_{j=1}^{J} p_j^{(r)} f(x_j^{(r)},\, \delta)} \tag{9}$$

where $x_j^{(r)} = d_j^{(r)}$, the partial derivatives evaluated at $\boldsymbol{p}^{(r)}$. The function $f(\cdot,\cdot)$ is positive and strictly increasing in x. The function depends on a free positive parameter δ. When the partial derivatives are positive, a typical choice of $f(\cdot,\cdot)$ is d^δ[9]. When the partial derivatives are both positive and negative, a choice of $f(\cdot,\cdot)$ is $e^{d\delta}$[11]. Mandal and Torsney[16] considered the choice of $f(\cdot,\cdot)$ as d^δ, and further developed the algorithm based on a clustering approach. Algorithm (9) possess several nice properties. Any iterate $\boldsymbol{p} = \boldsymbol{p}^{(r)}$ is always feasible. An iterate $\boldsymbol{p}^{(r)}$ is a fixed point of the iteration if the

derivatives $\partial\phi/\partial p_j^{(r)}$ corresponding to nonzero $p_j^{(r)}$ are all equal.

However, convergence of the algorithm could be slow if we do not choose the function $f(\cdot,\cdot)$ in an objective way. We need to develop strategies for better convergence of the algorithm for constructing designs that optimize the A-optimal criterion. We attempt to improve the convergence by considering the first argument of the function $f(\cdot,\cdot)$ as the vertex directional derivatives of the A-optimal criterion function. Recall that from equation (5) the first order conditions for optimality are $F_j = 0$ for $p_j^* > 0$, and $F_j \leq 0$ for $p_j^* = 0$. Recall also that from equation (4) we have $F_j = d_j - \sum_{j=1}^{J} p_j d_j$. So the vertex directional derivatives are both positive and negative. Using equation (4), we can prove that $\sum_{j=1}^{J} p_j F_j = 0$. This suggests that we can improve the convergence of the algorithm if we choose a function which is centred at zero and also changes quickly about the value $F = 0$. It is also important that we should treat positive and negative directional derivatives symmetrically. One choice of $f(x,\delta)$ with the potential to satisfy these requirements is the normal cumulative distribution function. That is, $f(x,\delta) = \Phi(\delta x)$. This function changes quickly at $x = F = 0$. If we take x as the partial derivatives of the A-optimal criterion, this choice of $f(x,\delta)$ could be bad because the partial derivatives of A-optimal criterion are not centred at zero. The convergence of the algorithm will also depend on the choice of the parameter δ. Depending on the numerical values of the partial and directional derivatives, we need to choose the values of δ carefully.

3 Examples, Results, & Discussion

3.1 Example 1 — Quadratic Regression

We first construct A-optimal design to the quadratic regression model. This is a polynomial regression model in one variable. In polynomial regression, the regression function $E(Y|x)$ is nonlinear in the design variable x. However, the regression function is linear in the parameters. Therefore polynomial regression is considered to be a special case of multiple linear regression. Quadratic regression is a polynomial regression of order two. The model is given by

$$E(Y|x) = \theta_1 + \theta_2 x + \theta_3 x^2$$

with the design interval [-1, 1]. The design space is continuous. So we discretize the design space to be in some form of uniform grid on the continuous design space. In particular, we approximate the design interval by a grid of points equally spaced at intervals of 0.01. We report the performance of algorithm (9), by taking the first argument of the function $f(x,\delta)$ as both the partial and directional derivatives of the A-optimal criterion. As discussed earlier, we take $f(x,\delta) = \Phi(\delta x)$. We record, for $n = 1, 2, \ldots, 6$, the number of iterations needed to achieve $\max_{1\leq j\leq J}\{F_j\} \leq 10^{-n}$, where F_j are the vertex directional derivatives. We take the initial design to be $p_j^{(0)} = 1/J$, $j = 1, 2, \ldots, J$. Results are reported in Table 1 for $x = d$ and in Table 2 for $x = F$. Results for the best choices of δ are given in bold numbers. Note that the best choices of δ are determined by the least number of iterations at $n = 6$. Note that, for $x = F$ (Table 2), the design did not converge beyond the value of $\delta = 0.15$. This is why the best choice is given in the last row of this table. The algorithm converges to a solution having three support points, namely -1, 0 and 1 with corresponding weights (0.25, 0.50, 0.25). The directional derivatives corresponding to the above three support points are zero and are negative towards all zero weighted remaining design points. Therefore the design satisfies the first-order optimality conditions (5).

Table 1: *Number of iterations needed to achieve* $\max_{1\leq j\leq J}\{F_j\} \leq 10^{-n}$ *for the Quadratic Regression Model with* $f(x,\delta) = \Phi(\delta x)$, $x = d$.

δ	$n=1$	$n=2$	$n=3$	$n=4$	$n=5$	$n=6$
0.05	162	1755	17729	64702	106506	147568
0.10	130	1352	13565	49469	81419	112802
0.11	**131**	**1354**	**13567**	**49470**	**81418**	**112799**
0.12	134	1373	13736	50073	82408	114169
0.20	237	2166	21310	77542	127568	176705

Table 2: *Number of iterations needed to achieve* $\max_{1\leq j\leq J}\{F_j\} \leq 10^{-n}$ *for the Quadratic Regression Model with* $f(x,\delta) = \Phi(\delta x)$, $x = F$.

δ	$n=1$	$n=2$	$n=3$	$n=4$	$n=5$	$n=6$
0.10	56	616	6241	22781	37500	51957
0.12	46	514	5201	18984	31250	43298
0.14	39	441	4459	16273	26786	37113
0.15	**59**	**412**	**4162**	**15189**	**25001**	**34639**

As we discussed earlier, we attempt to improve the convergence of the algorithm by using the directional derivatives of the A-optimal criterion as the first argument of the function $f(x,\delta) = \Phi(\delta x)$. Results in Tables 1 and 2 clearly illustrate that convergence is improved considerably. For example, with $x = d$, $\delta = 0.11$ and $n = 6$, the number of iterations needed to converge to the A-optimal design is 112799 (Table 1), whereas by using $x = F$ and $\delta = 0.15$, this number reduces to 34639 (Table 2).

3.2 Example 2 — A Practical Problem in Chemistry

We now consider a model which is used in a practical problem arising in Chemistry. The regression model describes the relationship between the viscosity y and the concentration x of a chemical solution. Viscosity is the response. The model is given by

$$E(y|x) = \theta_1 x + \theta_2 x^{1/2} + \theta_3 x^2$$

with the design interval restricted to (0.0, 0.2]. This model was considered[22] for constructing designs for minimally dependent observations.

Note that there is no intercept in this model. The design space is continuous. As we discussed in Section 1, we discretize the design space to be in some form of uniform grid of points equally spaced at intervals of 0.01. We report the performance of algorithm (9), by taking the first argument of $f(x, \delta)$ as both the partial and directional derivatives of the A-optimal criterion. We record the number of iterations needed to achieve $\underset{1\le j\le J}{max}\{F_j\} \le 10^{-n}$. We take the initial design to be $p_j^{(0)} = 1/J, j = 1, 2, \ldots, J$. Results are reported in Table 3 for $x = d$ and Table 4 for $x = F$. Note that, for this particular model, both the partial and directional derivatives are numerically very large, so we needed smaller values of the parameter δ compared to the previous model. The algorithm converges to a solution having three support points, namely 0.01, 0.12 and 0.20 with corresponding weights (0.413419, 0.380949, 0.205632). Here also the directional derivatives corresponding to the above three support points are zero and are negative towards all zero weighted remaining design points. Therefore the design satisfies the first-order optimality conditions (5).

In this model, with $x = d$, $\delta = 7 \times 10^{-6}$ and $n = 6$, the number of iterations needed to converge to the A-optimal design is 9927 (Table 3), whereas by using $x = F$ and $\delta = 1.005 \times 10^{-05}$, this number reduces to 2863 (Table 4). Thus we see that convergence of the algorithm is improved considerably by using the directional derivatives of the A-optimal criterion function.

Table 3: *Number of iterations needed to achieve* $\underset{1\le j\le J}{max}\{F_j\} \le 10^{-n}$ *for the Viscosity Model with* $f(x, \delta) = \Phi(\delta x)$, $x = d$.

$\delta \times 10^{-6}$	$n=1$	$n=2$	$n=3$	$n=4$	$n=5$	$n=6$
3	5918	7458	8998	10537	12077	13615
6	4357	5489	6622	7754	8886	10018
7	**4319**	**5441**	**6562**	**7684**	**8806**	**9927**
8	4410	5555	6699	7844	8989	10133
10	4947	6230	7513	8796	10078	11360

Table 4: *Number of iterations needed to achieve* $\underset{1\le j\le J}{max}\{F_j\} \le 10^{-n}$ *for the Viscosity Model with* $f(x, \delta) = \Phi(\delta x)$, $x = F$.

$\delta \times 10^{-05}$	$n=1$	$n=2$	$n=3$	$n=4$	$n=5$	$n=6$
1.0	1277	1610	1943	2277	2609	2942
1.004	1233	1547	1921	2263	2597	2927
1.005	**1491**	**1763**	**2037**	**2311**	**2589**	**2863**
1.006	1919	2273	2629	2983	3337	3693
1.008	4723	5637	6553	7467	8385	9309

4 Conclusions

In the present work, we addressed an important problem of optimal design and statistical inference. The objective was good estimation of the parameters. For a statistical model, it is important to estimate the parameters with minimum variance.

We considered A-optimal designs and minimized the total or average variance of all the parameter estimators. In order to solve this optimization problem, we minimized the trace of the covariance matrix of the parameter estimators. Because of the reciprocity property of the covariance matrix and the information matrix, minimizing the variance corresponds to maximizing the information. We determined the optimality conditions in terms of point to point directional derivatives. In particular, we expressed the optimality conditions in terms of vertex directional derivatives of the criterion function. We constructed the A-optimal designs by using a class of algorithms which neatly fit the basic constraints of our optimization problem. We then developed techniques for improving convergence rates by using the properties of the directional derivatives of the criterion function. Computational studies show that convergence of the algorithm improves a great deal when amended by the proposed approach based on using the properties of the directional derivatives. We constructed optimal designs for some models including one practical model which describes the relationship between the viscosity and the concentration of a chemical solution.

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Mini-Review

Physiological and Behavioural Adaptations of Arctic Fish to Their Aquatic Habitat

Émilie Ferguson[1]

[1]Clayton H. Riddell Faculty of Environment, Earth and Resources, University of Manitoba, Winnipeg, MB, R3T 2N2

Abstract

Arctic fish have evolved different physiological and behavioural mechanisms to survive the frigid waters of the Arctic Ocean. They synthesize antifreeze glycoproteins (AFGPs) that coat and inhibit the growth of ingested ice crystals and lower the freezing point of fish fluids. In addition, certain species of Arctic fish express different types of hemoglobins depending on the temperature of the water and oxygen availability. Some Arctic fish have also adapted anadromous life-history strategies to take advantage of the high summer primary productivity of the Arctic Ocean, and have modified their horizontal and vertical migration patterns based on the season. These physiological and behavioural adaptations have enabled Arctic fish to thrive in northern waters; however, the effect of climate change may induce further adaptations to their rapidly changing environment.

Keywords: Arctic, Cold Adaptation, Antifreeze Glycoproteins, Hemoglobin Multiplicity, Migration

Cold adaptations refer to physiological and behavioural changes that an organism has developed to survive in polar climates over evolutionary time[1]. Physiological changes occur when an organism alters its normal body functions to cope with environmental changes to maintain homeostasis. Organisms will alter their behaviour, the way they act in response to a stimulus, to ensure their survival. The Arctic Ocean is characterized by its extreme winters, wide range of temperatures that occur throughout the year[2,3], and high summer primary productivity[4]. Therefore, the ichthyofauna of the Arctic have developed cold adaptations that ensure their survival in the sub-zero and wide-ranging temperatures of the northern waters. Certain physiological adaptations such as, the presence of antifreeze glycoproteins in fish fluids[5] have allowed Arctic fish to cope with the freezing waters of the Arctic Ocean. For example, an average teleost fish will freeze at -0.8°C[5], but an Arctic fish can withstand water temperatures of -1.9°C[5]. Furthermore, some species have hemoglobin multiplicity[6], which enables fish to withstand the wide-ranging temperatures of the Arctic. Finally, some Arctic fish have developed anadromous life-history strategies and migratory patterns to take advantage of favourable ecological conditions[4,7]. Therefore, these behavioural adaptions have enabled these species to thrive and successfully reproduce.

Ice crystals can enter Arctic fish through their gills and intestinal tract[8], potentially damaging internal organs[9]. Therefore, they have developed antifreeze glycoproteins (AFGPs) to inhibit the growth of ice crystals in their systems[10]. AFGPs are synthesized in the liver and pancreas of fish and distributed in their blood and gastrointestinal fluids (Fig. 1)[9]. As ice crystals enter their body, they are coated with AFGPs, inhibiting ice crystal growth[11]. AFGPs are continuously recycled throughout the circulatory system and only excreted with feces (Fig. 1)[9]. Therefore, fish have a readily available supply of AFGPs to cope with the presence of ice crystals in their system.

AFGPs also lower the freezing point of the blood of Arctic fish due to their non-colligative properties[5]. This phenomenon is known as thermal hysteresis, which means that the freezing point of a substance is inferior to its melting point[10,5]. As AFGPs coat the surface of ice crystals, they lower the freezing point of the fish's blood and allow it to withstand waters with freezing temperatures. The winter flounder (*Pseudopleuronectes americanus*) is an example of a fish that was found to experience thermal hysteresis to cope with sub-zero water temperatures during the winter months[10]. In the winter, the freezing point of this species' serum is -1.37°C, and its melting point is -0.75°C[10].

AFGPs are a vital adaptation that have allowed Arctic fish to cope with the ingestion and formation of ice crystals within their system and prevent the freezing of their fluids. Recent studies have shown that there are five distinct antifreeze proteins (AFPs), namely ATGPs, Type I, Type II, Type III, and Type IV that are found in different species of fish[12,13]. These proteins have different structures and sequence compositions, but all have the same antifreeze functions[12]. Therefore, these proteins have convergently evolved the same antifreeze properties to survive freezing water temperatures.

Hemoglobin multiplicity has enabled Arctic fish to cope

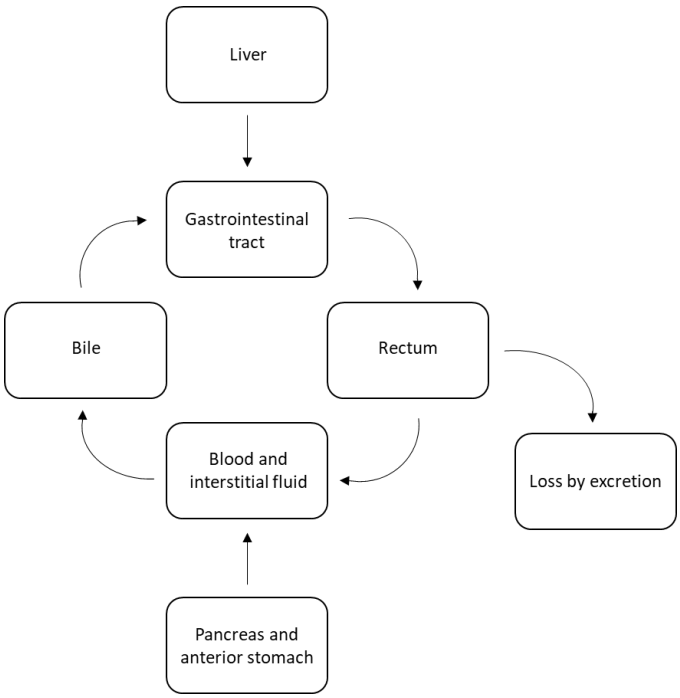

Figure 1: *Representation of antifreeze glycoprotein synthesis and recycling cycle in Arctic gadids (*Boreogadus saida*). The antifreeze glycoproteins are formed in the pancreas and liver. From the liver, they are released into the gastrointestinal tract. Those that are not excreted with the feces are resorbed by epithelial cells and transferred into the blood. Some are transferred into the bile and stored in the gall bladder, while others are released back into the gastrointestinal tract.*

with the wide-ranging temperatures of the Arctic Ocean[14]. Hemoglobins are proteins that are found in the blood of fish that increase the transportation of oxygen from the gills to the tissues[15, 6]. Hemoglobin multiplicity refers to the ability of hemoglobins to display different oxygen affinities depending on the temperature of the water[3]. For example, three major hemoglobins (Hb 1, Hb 2, and Hb 3) were found in the blood of the spotted wolffish *(Anarhichas minor)*[14], and three species of cods: the polar cod (*Arctogadus glacialis*); Arctic cod (*Boreogadus saida*); and the Atlantic cod (*Gadus morhua*)[6]. Hb 1 and 2 displayed a low Bohr effect, or low oxygen affinity, while Hb 3 displayed a high Bohr effect[14]. In other words, these species have the ability to acclimatize to waters with high and low concentrations of oxygen, and therefore waters of different temperatures.

Furthermore, hemoglobin multiplicity is often associated with fish that have a dynamic life-history[15]. For example, the Arctic charr (*Salvelinus alpinus*) and the Atlantic salmon (*Salmo salar*) are species that migrate to different tempered waters throughout their lives[4, 15]. Ten different hemoglobins were identified in the blood of the Arctic charr[16], whereas the Atlantic salmon is known to possess more hemoglobin genes than any other teleost[15]. Therefore, hemoglobin multiplicity has allowed these species to cope with the different levels of oxygen present in their migratory waters.

Arctic fish species have developed behavioural adaptations that allow them to best utilize the environmental conditions of the Arctic Ocean. For example, certain populations of Arctic charr have developed an anadromous life-history[17, 4], which means they migrate from salt water to freshwater to spawn. More specifically, they overwinter in northern freshwater lakes, migrate to the Arctic Ocean to feed in the nutrient rich waters during the spring and summer months, and then return to their freshwater habitat to spawn[4]. Arctic charr that have developed an anadromous life-history can grow to be more than 70 cm in length at maturity, while those that occupy freshwater year-round only measure up to 20 cm[4]. Hence, it is likely that the anadromous life-history strategy of the Arctic charr is advantageous because the ocean offers a richer supply of food than the freshwater habitat, which allows the fish to grow to larger sizes.

Furthermore, recent research has identified the horizontal and vertical movements of the migratory pattern of the Arctic charr[7]. They were found to occupy higher latitudes in the summer and fall months, than during the winter and spring[7]. The study also revealed that they mostly occupied surface waters during the winter and early spring, and deeper waters during the summer and fall[7]. It is likely that the migratory behaviour of the Arctic charr reflects its preferred water temperatures and the availability of nutrients in the water column.

Conclusion

Fish have adapted to the extreme winters and wide range of temperatures that characterize the Arctic Ocean. Physiological adaptations include the presence of antifreeze glycoproteins that allow fish to prevent the growth of ice crystals and lower the freezing point of their body fluids, and hemoglobin multiplicity, which allows fishes to cope with the fluctuating oxygen concentrations of different tempered waters. Behavioural adaptions include migratory strategies that allow the ichthyofauna to take advantage of the nutrient rich Arctic waters during the summer months, as well as horizontal and vertical displacements that allow them to best utilize their preferred aquatic environments. These adaptations have allowed species to survive and thrive in northern waters; however, with the warming climate Arctic fish may need to further adapt to their changing environment. The Arctic Ocean is experiencing warming temperatures and consequently, longer periods of stratification and lower levels of

available oxygen[18]. In addition, marine species and boreal fish are expanding their distributional ranges[19, 20]. Therefore, the warming climate is disrupting normal ecological interactions[18], which will greatly influence population sizes and the overall health of Arctic fish species[18].

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Mini-Review

Cryogenic Hibernation: A Review of Overwintering Mechanisms in the North American Wood Frog (*Rana sylvatica*)

Codey Dueck[1]

[1]Dept. of Biological Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2

Abstract

The North American wood frog, Rana sylvatica*, can inhabit extremely cold climates utilizing a variety of adaptations. This review identifies the driving mechanisms behind the overwintering response of* R. sylvatica*. The major response factors instrumental to survival include environmental and behavioral adaptations, internal freezing point depression by increasing blood and tissue concentration of glucose and urea, and reduced metabolic activity by increasing blood and tissue concentration of urea. These factors were contrasted between the Alaskan and Ohioan variants to explore and explain the relationships between their overwintering response factors and their geographical positioning.*

Keywords: Rana sylvatica, Cryoprotectant, Glucose, Hypometabolism, Urea

Of the amphibians known to be native to North America, the wood frog (*Rana sylvatica*) is the only one that has been found to inhabit the Arctic Circle[1]. Amphibians are ectothermic and cannot generate their own heat. Consequentially, they face a serious challenge living in a habitat with temperatures that drop below freezing for extended periods of time[2]. *Rana sylvatica* respond to these extreme conditions by undergoing an overwintering process that allows them to withstand temperatures of -16°C and below[3]. This review highlights the major environmental and physiological response factors of the overwintering process in Alaskan and Ohioan *R. sylvatica*. Specifically, behavioral adaptations, environmental insulation, urea-induced metabolic inhibition, and usage of both urea and glucose as cryoprotectants are explored.

The internal body temperatures of *R. sylvatica* during hibernation must be kept from declining too low to survive through winter. The habitat of wood frogs is generally limited to woody ecosystems bordered by the Appalachians, Pacific Ocean, northern tree-line, and southern tree-line[3]. Alaskan wood frog populations in the north-west of the geographical range are subject to an average annual snowfall of 174 cm and average January lows at -28°C. Meanwhile, Ohioan wood frog populations in the south-east experience milder conditions, with an average annual snowfall of 35 cm and average January lows of -5°C. In the winter, wood frogs utilize leaf litter and snow as an insulated barrier to maintain a temperature range adequate for survival[1]. Additionally, *R. sylvatica* tend to group close together and reside in depressions in the soil, which is hypothesized to be a behavioral adaptation to maximize insulation during hibernation, similar to other overwintering amphibian species found in Michigan[4]. However, the specialized adaptations *R. sylvatica* have for winter survival limits the maximum temperature they can survive and thus in the south, they are limited to temperate forests[1]. Despite the large habitable temperature range, there does not seem to be differences in behavior between the two geographical variants based on current literature, and thus warrants further study.

During winter, *R. sylvatica* accumulate glucose in their bloodstreams and organs to act as a cryoprotectant[5], depressing the freezing point of water in the organism[6]. This specifically leaves the heart, liver, and kidneys unfrozen but inactive as the muscles freeze and the body cavity fills with ice[5]. Under experimental pre-, during, and post-freeze conditions, plasma glucose concentration does not significantly vary between Alaskan and Ohioan wood frogs. However, under the same conditions, the glycogen reserves drop significantly less in the Alaskan variant during freezing conditions[3]. This is likely due to their large geographic and climate differences which require the Alaskan frogs to withstand colder and longer winters, thus needing a larger energy reserve.

The geographic differentiation of liver glycogen levels between Alaskan and Ohioan frogs is expanded when comparing their relative concentrations of GLUT2. GLUT2 is a major glucose transporter that is especially active in liver and pancreatic β-cells[7]. The Alaskan frogs have an overall GLUT2 concentration 1.8 times greater than Ohioan frogs, and to exhibit an increase in GLUT2 protein concentration in response to freezing stress[8].

Increased expression of GLUT2 will only significantly change the glycogen concentration in liver tissue[5]. This indi-

cates that enzymatic catabolism of hepatic glycogen appears to be *R. sylvatica*'s sole source of glucose for the body during their overwintering response to freezing temperatures. Moreover, it seems that the increased transport of glucose from the liver in the Alaskan *R. sylvatica* allows it to exhibit higher reserves of glycogen for longer hibernation while maintaining similar levels of plasma glucose as in the Ohioan variant.

Similar to glucose, urea acts as a cryoprotectant in *R. sylvatica*[3]. Compared to the Ohioan wood frogs, the Alaskan variant has a greater concentration of total cryoprotectants and compensates their overall slightly lower glucose concentration with a much higher overall concentration of urea[3]. Again, this follows with the geographic differences of Alaskan frogs needing to withstand much longer and extreme temperatures than Ohioan frogs.

Secondly, urea is capable of inducing hypometabolism in wood frogs[9, 10]. The sedentary nature of hibernation inhibits the ability of the wood frog to obtain nutrients, and thus a lowered metabolic rate is important to conserve maximal energy for winter survival. This is accomplished by increasing urea concentration in the plasma which induces hypometabolism, but direct ureic metabolism inhibition is only active in frogs that have undergone desiccation indicative of the winter seasons[10, 11]. Tissues with a high urea concentration exhibit a decrease in metabolic activity, but even non-urea-loaded tissues have a decrease in metabolic activity under desiccation, albeit slower[10]. This appears to be an adaptation of *R. sylvatica* allowing them to selectively reduce their metabolism with urea-loading tissues which decreases the amount of time spent active in unfavorable conditions. However, the effect of urea-loading is inconsistent between tissues and season which could result from differences in tissue-specific enzymes and heat or desiccation effects (which varies depending on the season) on enzymes[11]. Tissue-specific hypometabolism selectivity is a possible contributor to ensuring the vital organs survive with enough energy to support life after the thaw.

Conclusions & Future Study

Rana sylvatica is a remarkable amphibian that owes its success as a species to many factors. They have behaviorally adapted to survive harsh winters by burrowing. However, the more significant factor is their physiological shifts which allow them to conserve maximum energy by depressing their metabolism and freezing parts of their bodies while leaving some organs unharmed[1, 10, 3].

A lack of behavior differences between the Alaskan and Ohioan wood frogs requires confirmation to attribute the geographical survival variance mainly to physiological differences. Additionally, there is lacking literature on wood frogs outside of Alaska and Ohio to support climate adaptations and exclude genetic differences. Further research on the mechanisms which allow *R. sylvatica* to seamlessly exit cryogenic hibernation is recommended as the current literature is lacking. This could provide insight on preventing or assisting in the medical recovery of frostbite and hypothermia if we can understand these mechanisms. Alternatively, elective medical cryogenic hibernation is gaining popularity as a possible future aid to treatment. Small scale applications at the tissue or organ organization level would allow for a widening of the human organ transplantation window with a better-preserved tissue. Larger scale applications could allow whole body freezing to suspend animation in terminally ill patients while a cure is developed. However, a major hurdle is that there is currently no known method of recovering tissues from cryogenic hibernation. Mimicry of the wood frog's cryogenic hibernation mechanisms in humans could be one avenue to overcome this challenge.

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Mini-Review

Adaptive Radiation in Antarctic Notothenioids

Laura Alsip[1]

[1]Dept. of Environment & Geography, University of Manitoba, Winnipeg, MB, R3T 2N2

Corresponding Author: L. Alsip (alsipl@myumanitoba.ca)

Abstract

With the formation of the Antarctic Polar Front 30-35 million years ago, the Antarctic notothenioids have undergone adaptive radiation in order to survive. Many of the traits were evolved with respect to their frigid, sub-zero environment. This paper explores the mechanisms currently used by the Notothenioidei to survive the frigid Antarctic waters. With climate change threatening to warm their formerly stable environment, the phenotype-environment correlation that has allowed them to dominate the Southern Ocean may become their downfall in a changing environment.

Keywords: Antarctic, Notothenioidei, Southern Ocean, Adaptive Radiation, Climate Change

All around the world, numerous species will need to adapt to changes in their environment as the effects of climate change, such as warmer temperatures, become increasingly apparent. Species that live in thermally stable environments, such as deep in the Southern Ocean near Antarctica, are vulnerable to minute changes in temperature and the rates at which they occur[1, 2]. The Antarctic Polar Front (APF), an oceanographic barrier running between 50°S and 60°S, has thermally and geographically isolated the Antarctic waters for 30 to 35 million years[3]. The APF has made the Antarctic waters a more constant, extreme, and isolated environment than the Arctic waters, which has led to distinct differences among the marine fauna[4]. Of the 25,000-28,000 fish species globally, there are only 322 recognized species of fish that live in the Southern Ocean, due to the extinction event caused by the formation of the APF[5, 6]. Of these, 101 species are from five of the eight families under the suborder Notothenioidei, and they compose 92% of the fish abundance in Antarctica[5, 6]. These bony, perch-like fish have undergone adaptive radiation in the Antarctic, where many distinct species with a common ancestor rapidly diversify to fill the ecological niches left by an extinction event[5, 7]. Another major component of adaptive radiation are the features a species develops as a result of interactions with their environment[7]. For the Notothenioidei, this is seen through the development of antifreeze glycoproteins, and the loss of various cells in blood, heat shock protein (HSP) expression, and swim bladder to survive in the sub-zero Antarctic environment.

The most revolutionary mechanism the Notothenioidei evolved is the creation of antifreeze glycoproteins (AFGPs), which prevents internalized ice by lowering internal freezing points[8, 9]. Ice enters the fish via ingestion or absorption through the skin, then internal AFGPs can adsorb to the ice crystals in the gastrointestinal tract and spleen, likely excreting the complex in the feces[6]. The hyposmolarity of the stomach, intestinal, and pancreatic fluids determines the difference between melting and freezing points, which, if large, indicates a large presence of AFGPs in the organs[8]. Many larval Notothenioidei do not have AFGPs, instead relying on physical barriers from undeveloped gills and intact outer layers to prevent ice crystals entering the body in the first place[10, 3]. The eight types of AFGPs are now known to be constantly synthesized in the exocrine pancreas at approximately three months post-hatching, evolving from a trypsinogen-like protease[11, 8, 3, 9]. AFGPs are crucial to survive in a subzero environment, but as water temperatures warm, they will eventually no longer be needed.

Another adaptation of the circulatory system is the absence of erythrocytes, myoglobin, and haemoglobin (Hb) in the most derived Notothenioidei family, Channichthyidae, or "icefish"[12, 13]. Channichthyidae compensate for this loss through increased blood volumes and larger hearts[14]. The other families within Notothenioidei have reduced levels of Hb with low oxygen affinities and reduced levels of erythrocytes[3, 13]. These are energy-saving changes required by the increased viscosity of blood at cold temperatures, and are feasible due to the high solubility of oxygen at low temperatures[3]. Most of the Notothenioidei are known to have reduced metabolisms and slower heart rates from the frigid environment, which would reduce their oxygen requirements, further enabling this adaptation[15, 13]. With the potential for climate change warming the waters and decreasing the oxygen solubility, the loss of oxygen-carrying blood

cells would become detrimental. Antarctic fishes also have enzymes that are more efficient due to the flexibility of the proteins and membranes from changes in the intramolecular bonds at low temperatures[16, 17]. This would further aid in cold-adaptation at the cellular and tissue-levels.

In addition to losses within the circulatory system, most notothenioids do not see expression of HSPs[18]. HSPs were believed to have been lost from the notothenioids, Antarctic fish are now known to have retained these genes, and are unable to upregulate the gene in response to increased temperatures as they are extremely stenothermal, only being able to tolerate a very small range of temperatures, resulting from living in constant conditions[19]. The inability to respond to heat stress will be damaging with the imminent threat of climate change and its increasing temperatures. This is particularly documented in the emerald rockcod, *Trematomus bernacchii*, as this organism is highly acclimatized to its environment and is consequently under constant cold-stress, likely causing constant upregulation of the stress protein, hsp70[18, 19, 20]. In the non-Antarctic notothenioids, hsp70 is an inducible protein, typically seeing upregulation due to increases in temperature[19]. This protein assists in repairing thermally denatured proteins, and may be permanently activated in the Antarctic specimens in order to maintain appropriate protein levels due to cold denaturing the proteins, among other environmental stressors[19, 20].

Antarctic fishes also see adaptations to the environment at the tissue and organ level. All notothenioids have lost the swim bladder, a characteristic likely derived from their benthic ancestors[5, 6]. Some notothenioid species, for example the Antarctic silverfish, *Pleuragramma antarcticum*, have achieved neutral buoyancy in their role as secondarily derived pelagic sit-and-wait predators, eliminating the need for continuous uplift to stay in the suspended in the water column as an energy-conserving measure[21, 5, 6]. Neutral buoyancy is achieved in these species by high lipid contents, and reduced ossification[6]. Some species, for example *P. antarcticum*, have additional specializations for buoyancy, such as lipids sacs instead of adipose cells, reduced mineralization of scales, and the retention of larval characteristics, a feature which may also provide additional benefits such as camouflage in the pelagic environment[22, 21, 6]. As climate change causes sea ice and glaciers to melt, the ocean will experience a dilution effect, causing the water to become less dense, making neutral buoyancy challenging[1]. Other organ specializations in Notothenioidei, excluding the basal family Bovichtidae, are aglomerular kidneys, where the lack of capillaries likely evolved to prevent the loss of AFGPs through urine and to minimize the energetic costs of resynthesizing these glycoproteins[23]. Bovichtidae have a largely temperate distribution, with only one species in Antarctica[13]. They do not have AFGPs or aglomerular kidneys, which suggests that these are derived characters in the Antarctic notothenioids[23].

Given the unique Antarctic environment created by the APF, the Notothenioidei have had many diverse adaptations arise within the past 30 to 35 million years[3]. This suborder is a prime example of adaptive radiation, with the majority of the group rapidly diversifying and modifying its phenotype to survive in this extreme environment[23, 6]. However, with warmer water temperatures in the future, many of these once-crucial adaptations will become disadvantageous. The production of AFGPs will become unnecessary and a waste of energy, the loss of oxygen-carrying blood cells would become detrimental due to increased metabolisms and decreased oxygen solubility in warmer water, the inability to respond to heat shock would likely be lethal, and the loss of a swim bladder would be energetically inefficient with less dense water. When the Southern Ocean became hostile to most species with the formation of the AFP, the Notothenioidei rose to the challenge. When the effects of climate change hits, will the Notothenioidei be able to survive or will the ecosystem of Southern Ocean once again change drastically?

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Mini-Review

Trophic Interactions of Gray Wolves (*Canis lupus*), the Keystone Species in Yellowstone National Park

Christie D. Lavallée[1]

[1]Dept. of Biological Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2

Corresponding Author: C. D. Lavallée (lavallec@myumanitoba.ca)

Abstract

*The gray wolf (*Canis lupus*) is an apex predator described as a keystone species in Yellowstone National Park. Their importance in this habitat was unknown until they were anthropogenically extirpated in the 1920s. The 75-year absence of gray wolves in Yellowstone led to declines in biodiversity, and habitat quality, all of which is gradually returning upon wolf reintroduction in 1995. Trophic interactions are the driving forces behind the gray wolves' ability to directly and indirectly provide benefits for almost all species of fauna and flora within Yellowstone National Park. This paper reviews how wolves' trophic interactions have helped in reshaping the dynamics of Yellowstone National Park.*

Keywords: Gray Wolf, Apex Predator, Keystone Species, Trophic Cascade, Trophic Interaction

Species diversity comes in many forms yet varies between habitats. Few species have the ability to significantly alter or affect (both directly and indirectly) the environment in which they live[1]. These organisms are known as keystone species and through their presence or absence they have an impact on biodiversity, species richness, and the landscape and niches they occupy[2]. Keystone species' effects and interactions within the environment are exponentially greater than their population size[3]. Keystone species are most often predatory animals able to maintain stable population sizes of lower trophic levels, which leads to increases in biodiversity and changes in resource abundance and distribution. Without these species, the habitats they maintain would be radically different or nonexistent, and ecosystem productivity and biodiversity would decline[4].

Gray wolf (*Canis lupus*) of Yellowstone National Park are a prime example of an organism that is considered to be a keystone species. Through their trophic interactions and their status as an apex predator, wolves are able to both successfully limit populations of large herbivorous animals and force behavioural changes on smaller carnivores, such as avoidance and altered foraging/hunting strategies[1] .

Yellowstone National Park (Yellowstone) is an 8992 sq. km wilderness recreational park located on the state borders of Wyoming, Montana and Idaho, USA. It was established in 1872 making it the world's first national park[2]. The forests and meadows of this pristine habitat provide shelter and food for the species that inhabit Yellowstone[5]. Some of the more famous species of the park are the carnivorous, predatory mammals like gray wolves and coyotes (*Canis latrans*), the omnivorous grizzly bear (*Ursus arctos horribilis*), and herbivores like elk (*Cervus canadensis*) and beaver (*Castor canadensis*). The balance of predator-to-prey animals is critical for ecosystem productivity and Yellowstone is a testament to the significant role of gray wolves in the park[2].

Prior to the early 20th century, wolves were an integral part of Yellowstone. As settlement began in the early 1800s, the fear of wolves preying on livestock resulted in a cull and subsequent extirpation by the 1920s[2]. The extirpation resulted in major ecosystem changes through the loss of trophic interactions in a top-down trophic cascade[1]. A trophic cascade occurs when an apex predator, such as the wolf, changes the behaviour of their prey, the elk in this case, through population reduction and decreased foraging time, releasing plant species from foraging pressure and allowing them to recover. This cascade had great effects on species such as the elk, grizzly bears, and beavers as well as habitat quality[6, 2]. With the absence of the wolves, elk overgrazed on plants such as the willow (*Salix* spp.) stands along riparian systems, causing a decrease in available food for grizzly bears and beavers[7, 2]. The loss of plants and root structures along the riparian system resulted in sediment erosion along the banks. This led to further loss of plant diversity and the widening and change of river flow[7, 2].

Wolves were reintroduced into Yellowstone after a comprehensive environmental impact assessment looked at the possible implications[6]. Wolf reintroduction took place in 1995 and 1996[7], with 31 wolves coming from Canada. The population quickly grew to 270 by the end of 2002[1]. As the various wolf packs settled into their new environment, a change in Yellowstone's habitat slowly emerged. Their ecological importance as a keystone species was becoming more

apparent as the habitat slowly returned to a pre-wolf extirpation state. Willow stands along the riparian zones were re-establishing, beavers and grizzly bears were returning to their native home range, and elk and coyote foraging behaviour were changing[6, 2].

As wolves expanded their territorial range within Yellowstone, a change was observed in the foraging behaviour of the resident elk population[1]. Elk decreased their foraging time on plants such as willow, aspen (*Populus tremuloides*), and cottonwood (*Populus angustifolia*) as they became more vigilant of wolf predation[2, 8]. With this increase in awareness of their surroundings and decreased foraging pressure, a recovery of plants began in the region.

Elk were wolves' primary prey within Yellowstone as they had a high abundance and pack cohesion made them easily obtainable[2, 1]. The increased predation on elk has slowly resulted in the suppression of elk as well as their foraging habitats. This has had a compounding effect on plants, allowing regrowth and reshaping of the environment[2, 1, 8].

As an apex predator, wolves interact with all trophic levels directly or indirectly[9]. Indirectly, they interact with the grizzly bear that is also considered an apex predator, but in the sense that it is an omnivore[8]. Prior to wolf reintroduction, grizzly bear populations were affected by the overgrazing of plant biomass by elk populations[2, 8]. In spring after hibernation, grizzly bears need easily obtainable calories usually in the form of insects, carrion, and vegetative material, including berries and grasses[8]. The overgrazing by elk reduces plant biomass available to the bears and also reduces cover required later in the season for safe foraging[8].

In fall, grizzly bears enter a period of hyperphagia where they need to store enough energy from carbohydrate rich foods for hibernation[8]. This time is especially critical for female bears as they gestate, give birth, and lactate while hibernating. Wolves have an indirect relationship with the grizzly bears by directly reducing the elk population that overgraze on essential plants needed by the bears[2, 8, 10]. The wolves also provide carrion left behind at their kill sites that grizzly bears scavenge off of with low energetic costs, providing some of the essential calories for winter survival[2, 8].

The removal of wolves from their ecosystem negatively affected the conservation of the fauna and flora in the region. The riparian systems; ungulate, beaver, and grizzly bear populations; and mesopredators (coyotes) were all impacted, causing a trophic cascade[2, 1, 8]. The removal of apex predators can also result in a mesopredator release, where a smaller predator such as the coyote is suppressed by a larger predator no longer has that inhibiting effect[11].

Wolves have the ability to change the behaviour of the animals they interact with based on their predatory status. In the presence of wolves[1], coyotes changed their behaviour and foraging habits to avoid areas populated with wolves and their dens. These dens were previously used as hunting grounds for coyotes after the wolves were extirpated from Yellowstone in the early 20th century[1]. After wolves were reintroduced, coyotes were deterred from foraging near the wolf dens as the new wolf packs reclaimed these denning areas for themselves[1]. An inverse relationship was also observed between coyotes and small mammals inhabiting areas near wolf dens: as coyote populations decreased, small mammal populations increased. This correlation appears to happen because coyotes would prey upon small mammals, and as wolves gradually reclaimed their old territory pushing the coyotes further out, the small mammals moved back toward the dens because of less predation pressure[1].

Through trophic interactions with other trophic levels and their ability to alter animal behaviour, wolves play an integral part in their habitat. Their profound effects they have on the ecosystem of Yellowstone have been shown through studies and numerous ecosystem changes. With these changes that are already apparent, it warrants further investigation down the line to continue observations of Yellowstone and how this ecosystem continues to recover from a time of absence with wolves.

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Review

Evolutionary Perspectives on Male Homosexuality: A Review

Yasmina Mashmoushi[1], Mitan Mzouri[1]

[1]Dept. of Biological Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2

Corresponding Author: Y. Mashmoushi (mashmouy@myumanitoba.ca)

Abstract

This review provides a comprehensive coverage of the leading evolutionary hypotheses to date on male homosexuality: the sexual antagonism model, the tipping-point model, and the kin selection hypothesis. It does so by first, surveying the most prominent findings on the biological causes of male homosexuality; second, discussing the effects of male homosexuality on individual fitness; and third, outlining the currently contending evolutionary theories on male homosexuality and critically evaluating each against current, pertinent empirical evidence. This review reveals that male homosexuality is a complex, multifaceted phenomenon influenced by an interplay of genomic and environmental factors that may have had unique evolutionary trajectories. Thus, there is likely more than one evolutionary mechanism at play responsible for the maintenance of gay alleles in the human population. Current research largely supports the notion that the alleles responsible for male homosexuality bestow fitness benefits in heterosexual carriers. The tipping-point model and sexual antagonism model, but not the kin selection hypothesis, are in line with such evidence. Future research into the genomic underpinnings of sexual orientation in homosexual males and its genetic equivalents in heterosexual males and females may allow for further evaluation of these hypotheses.

Keywords: Human Evolution, Evolutionary Psychology, Mating Preferences, Sexual Orientation, Male Homosexuality

1 Introduction & Background

Homosexuality refers to sexual attraction, romantic attraction, or sexual behaviour toward members of the same sex[1]. As a sexual orientation, it is the enduring pattern of sexual or romantic attraction and behaviour toward members of the same sex[1]. According to research, human sexual orientation exists along a continuum that ranges from exclusive homosexuality to exclusive heterosexuality and includes many forms of bisexuality; it does not exist as a mere heterosexual–homosexual dichotomy[2, 3, 4].

Homosexual behaviour has been documented in more than 500 different species of animals: in various primate species and in every major animal group (mammals, birds, reptiles, amphibians, fish, insects, and invertebrates)[5, 6, 7, 8]. This includes courtship, affection, sexual activity, pair bonding, and parenting all observed in multiple settings: in the wild, in captivity, and in the laboratory[5, 6, 7, 8]. Homosexuality has also been documented in human societies over several millennia and archaeological evidence (petroglyphs, ancient paintings, tomb figurations, etc.) has confirmed its occurrence prehistorically[9, 10, 11]. Since human sexuality varies along a continuum, reliably measuring the prevalence of homosexuality in the human population is challenging for researchers. Moreover, due to the widespread heterosexist discrimination found in many societies, many homosexual individuals do not openly identify as such[1]. The documented prevalence of homosexuality has been found to vary largely over time and geographic region[12]. According to some surveys, 2–11% of people in the West have had some form of same-sex sexual contact in their life[13]. This percentage rises to 16–20% when both same-sex behaviours and same-sex attractions are considered.In a 2006 Australian study, 20% of respondents anonymously reported some homosexual feelings, although only 2–3% identified themselves as exclusively homosexual[14]. In the scientific community, the consensus is that approximately 2–9% of females and 1–10% of males in the West are exclusively homosexual[12, 15]. Thus, homosexuality represents a small but significant sexual minority phenotype in humans.

The persistence of homosexuality throughout the evolutionary history of primates and other animals has been coined an "evolutionary paradox"[16]. Several competing evolutionary hypotheses have attempted to shed light on this Darwinian puzzle, however, no consensus has yet been reached on a single, prevailing evolutionary account of the matter[17]. Because most research in the scientific literature pertains to male homosexuality and the empirical studies investigating lesbianism are sparse, the evolution of lesbianism remains understudied. Consequently, only male homosexuality will be examined in this review.

This review is divided into three main sections: "Biological Links", "Measures of Maladaptiveness", and "Evolutionary Perspectives". The first section surveys the most notable research on the nature and causes of male homosexuality from a psychobiological perspective. This provides us with a backdrop of knowledge against which we can appropriately assess evolutionary explanations later on. The second section discusses the evolutionary maladaptiveness of male homosexuality and its effects on individual fitness. The third section surveys the most prominent evolutionary theories to date on male homosexuality and critically evaluates each against recent empirical evidence.

1.1 Biological Links

Scientists believe that male homosexuality is the result of an interplay of biological and intrauterine environmental factors, and that it is shaped very early in life[12, 18, 19, 20]. Scientists generally do not believe that one's sexual orientation is a matter of choice[12, 18, 19, 20].

1.1.1 Genetics

Studies have shown that male homosexuality is not evenly distributed within the population but rather runs in families, generally on the maternal line[14, 21, 22]. Despite numerous attempts, no single "gay gene" has been identified; however, there is evidence for the presence of multiple contributing genetic factors for homosexuality throughout the human genome[12, 23, 18, 22, 24, 25, 26, 27, 28, 29]. Some association has been found with the Xq28 region on the X-chromosome of homosexual males[25, 28, 29]. In addition, a recent study has found a higher proportion of homosexual males with type A blood and with Rh-negative blood than heterosexual males[24]. Since these traits are controlled for by genes located on autosomal chromosomes, this indicates a possible autosomal genetic contribution to the development of a homosexual orientation[24]. However, this may also be the result of a confounding association between blood group and the X-linked androgen receptor gene[30].

Male homosexuality has also been found to be more common in male relatives on the same maternal line[14, 21, 22]. Moreover, identical twins are more likely to share a sexual orientation than fraternal twins[12, 18, 26, 31]. While some research on identical twins has revealed a 50% concordance rate for homosexuality among the siblings, other studies have found a 20% concordance rate[12, 18, 26, 31]. This research indicates that approximately a third of variation in sexual orientation is attributable to genetic differences among the siblings[18, 31]. Given the differences in sexual orientation in many sets of identical twins, researchers conclude that sexual orientation cannot be attributed to genetic factors alone[12, 18, 26, 31]. Hypotheses addressing these differences consider epigenetic, developmental, and environmental modifiers, such as differences in intrauterine blood transfusion and hormone exposure among the siblings[12, 18, 26, 31].

1.1.2 Environmental Factors (Nurture)

There is no scientific evidence that the social environment after birth has an effect on an individual's sexual orientation[12, 19, 20, 32]. Likewise, there are no empirical studies that support attributing a homosexual orientation to early abuse, trauma, family dysfunction, abnormal parenting, or any other adverse life events[12, 32, 33]. Moreover, there is no evidence that the use of psychological interventions (i.e. conversion therapies) can change one's sexual orientation[34, 35].

1.1.3 The Uterine Environment

Research suggests that sexual differentiation of the human brain occurs during fetal development, programming our gender identities and sexual orientations while we are in the womb[20, 36]. Circulating testosterone from the developing testes is said to act as an organizing factor for the developing nerve cells during a brief critical period, promoting the development of permanent male-typical neuronal patterns[20, 36, 37]. Female-typical neuronal development is said to occur in the relative absence of this hormone surge[36, 37]. Research suggests that male sexual orientation is influenced by intrauterine factors that affect fetal testosterone production, thereby influencing the masculinization of the male fetal brain[19, 20, 36, 37]. This is consistent with documented observable differences in the brains of homosexual and heterosexual males[19, 20, 36, 37]. A number of variations in brain structure have been reported between homosexual and heterosexual men, including the size of the suprachiasmatic nucleus and INAH3 neuronal group of the hypothalamus, with homosexual men typically exhibiting sex-atypical dimorphisms[19, 20, 36, 37, 38]. These findings are consistent with the role of the hypothalamus as a regulator in reproductive function[36]. Furthermore, concentrations of intrauterine testosterone may be influenced by maternal consumption of certain drugs, direct injection of the hormone, maternal immune system reactions, and maternal stress[39]. While some scientists speculate that homosexual males may have been exposed to lower androgen levels in the womb, others maintain that genes still undiscovered may play a role in reduced androgen sensitivity responses in male fetuses that grow up to be homosexual[32, 37, 40].

1.1.4 Maternal Stress

Research suggests that if a woman experiences severe emotional stress during her pregnancy, the likelihood of her giving birth to a homosexual son may increase[39, 41, 42, 43]. This

is said to be because circulating maternal stress hormones (e.g. cortisol) cross the placenta and disrupt fetal testosterone levels and their synchronization with neurodevelopmental epochs[41, 42, 43].

1.1.5 Fraternal Birth Order

Boys with older brothers are significantly more likely to be homosexual, with the chance of homosexuality increasing by about 33% with every older brother[18, 19, 37, 42, 44, 45, 46]. In fact, fraternal birth order is now considered to be one of the most reliable, cross-culturally robust epidemiological variables identified in the study of homosexuality[18, 19, 45, 46]. Consistent with this finding is that the finger length ratio between the index and ring fingers (the 2D:4D ratio), a crude measure of prenatal exposure to testosterone, decreases as fraternal birth order increases[18, 40]. To explain the fraternal birth order finding, it has been proposed that male fetuses provoke a maternal immune system reaction that becomes stronger with each successive male fetus[44, 45]. Maternal antibodies, part of this immune reaction, cross the blood–brain barrier and attack the proteins that play a role in the masculinization of the male fetal brain[45].

1.2 Measures of Maladaptiveness

1.2.1 History as a Psychological Disorder

In 1952, when the American Psychiatric Association (APA) published its first Diagnostic and Statistical Manual of Mental Disorders (DSM), homosexuality was classified as a psychological disorder[47]. This classification was later scrutinized when research failed to provide an empirical basis for its support[48, 49, 50]. The APA concluded that this classification reflected untested assumptions based on once-prevalent social norms and removed homosexuality from the DSM, stating that it implies no impairment to general, social, or vocational abilities[47, 48, 49, 50, 51]. Thereafter, the APA urged mental health professionals to act as leaders in helping combat the stigma of mental illness that has long been attributed to homosexual orientations[48]. Today, scientists and mental health professionals agree that homosexuality poses no intrinsic obstacle to leading a healthy, happy, or productive life in the full array of social institutions[18, 32, 48, 49].

1.2.2 Mental Health

Male homosexual youth continue to be at an increased risk of compromised mental health than their heterosexual peers[52, 53, 54]. A recent US study interviewing a community sample of gay youth between the ages of 16 to 20 found that approximately 18% of gay participants met the diagnostic criteria for major depression, 11.3% for PTSD in the past 12 months, and 31% for suicidal ideation[53]. When comparing these findings to national mental health diagnosis rates for the general population, the difference is stark: The rates for these diagnoses and behaviours among youth are 8.2%, 3.9%, and 4.1%, respectively[55, 56]. Gay youth have also been found to be at an increased risk of substance abuse, bullying, and psychiatric comorbidity[54]. In all, researchers agree that the compromised mental health of gay youth is the cause of social ostracism, isolation from family and peers, internalization of negative societal stereotypes, and/or limited support structures in place for them in society[52, 53, 54].

1.2.3 Reproduction

From an evolutionary standpoint, the fundamental maladaptiveness of homosexuality is evident. Homosexual individuals, most typically, do not have children of their own. Although modern methods such as *in vitro* fertilization and artificial insemination are now being used by same-sex couples to produce biological children, these methods have only been developed in the past century and could not have been responsible for the passing on of gay alleles throughout human history[12].

1.3 Evolutionary Perspectives

1.3.1 Homosexual Individuals as "Helpers-in-the-Nest"

In the *Origin of Species*, Darwin described how entire family groups or bloodlines (not just individuals) can compete for selection[57]. The *kin selection hypothesis* of male homosexuality, popularized by Wilson in 1975, posits that homosexual individuals can compensate for their lack of biological children by maximizing the reproductive success of their family members. Thus, rather than reproducing themselves (i.e., *direct fitness*), homosexual individuals enhance the reproductive success of those who share their genetic code (i.e., *indirect* or *inclusive fitness*)[58, 59]. According to this model, although the alleles that predispose individuals to a homosexual orientation do not get passed on through the reproduction of homosexual individuals themselves, they may still get passed on to the next generation by their relatives[58, 59].

Theoretically, there are many ways that homosexual individuals can be said to increase the reproductive success of their family members, such as by contributing resources (eg., food, shelter, etc.), performing "uncle-like" activities (eg., taking care of offspring), and helping family members in times of stress (eg., providing defense, supervision, resources, or care)[59, 60, 61]. The kin selection hypothesis views homosexual individuals as essentially "helpers-in-the-nest"[58]. This hypothesis also argues that homosexual individuals may contribute substantially to the emotional wellbeing and overall cohesion of their family[60, 61]. This idea is consistent with studies showing lower levels of hostility and higher levels of emotional intelligence, cooperation, and empathy in homo-

sexual men[15]. Evolutionarily speaking, the ability of family members to bond with and cooperate cohesively within their familial group may have determined in many cases whether the group survived or perished[60].

The kin selection hypothesis was tested by Vasey, Pocock, and VanderLaan on the Pacific island of Samoa in 2007. In this island, Samoans live in a highly primitive and traditional society reminiscent of the human ancestral past, and Samoan homosexual and transgendered males are socially accepted by the majority of Samoans[61], see also[62, 63]. Vasey et al.'s 2007 study found that gay Samoan men were significantly more willing to help their kin than were straight, childless men, providing the first ever evidence in support of the kin selection hypothesis. However, a later study by Vasey and VanderLaan[64] found that homosexual men in Japan were no more generous or attentive towards their nephews and nieces than were childless, heterosexual men and women. More evidence against the kin selection hypothesis later surfaced in several studies across the United Kingdom, United States, and the West, with homosexual individuals not found to provide more care or resources to family members than their heterosexual counterparts[16, 64, 65]. This remained true regardless of the types of measures used, whether these measures were subjective (e.g., feeling of closeness to the family) or objective (e.g., frequency of contact with the family, distance residing from relatives, etc.)[16, 64, 65]. However, researchers currently disagree on whether these results implicating a lack of support for the kin selection hypothesis could be the cause of data being gathered in modern, industrialized societies (e.g. the UK, USA, and Japan), which are less remnant of the human ancestral environment and are characterized by fervent social intolerance towards homosexuals[62, 63, 66].

1.3.2 Additional Functions of "Gay Alleles"

This explanation posits that the group of alleles that code for a homosexual orientation in gay males also confer strong reproductive advantages in heterosexual individuals, resulting in the persistence of gay alleles in the gene pool as their successful heterosexual carriers pass them down[67, 68, 69].

1.3.3 Coding for Femininity in Males

The *tipping-point model of male homosexuality*, popularized by Edward Miller, posits that the group of alleles that code for a homosexual orientation in gay men confer strong fitness benefits in heterosexual men by coding in them a certain level of psychological femininity[68]. According to Miller, if only a few of these alleles are inherited by males, their reproductive success is enhanced via the expression of attractive, albeit feminine traits such as kindness, empathy, and sensitivity[68]. However, if too many of these alleles are inherited by males, a tipping-point is reached, at which even their mate preferences become feminized[68].

Miller came up with a simplified version of his theory to better illustrate it. He asks the reader to imagine that there are five different genes that each help code for an individual's place along a masculine–feminine continuum. Each of these five genes have two respective alleles: one that pulls the individual to the masculine side of the continuum and one that pulls the individual to the feminine side of the continuum. According to his simplified model, if a man inherits all five of the "feminine-pulling alleles", he will be homosexual and if he inherits less than five, he will not. Homosexuality would continue to persist in the human population if a strong reproductive advantage is conferred on individuals possessing some copies of these feminine-pulling alleles. According to Miller, a low dose of these feminine-pulling alleles significantly enhances a heterosexual male carrier's reproductive success. But in the less common, spontaneous occasion that a significantly large dose of these feminine-pulling alleles is inherited, the male carrier's sexual orientation is altered and his fitness adversely affected. Nonetheless, these alleles would continue to persist in the population if they confer an overall reproductive advantage on their male carriers[68].

Consistent with the tipping-point hypothesis, homosexual men are reported to be more sensitive, kind, and empathetic than heterosexual men, which have been characteristically deemed to be feminine attributes[70]. Furthermore, studies have found that a higher level of psychological femininity in straight men is associated with a greater number of female partners, suggesting that psychological femininity is attractive to women[71, 72]. This could be because psychological femininity indicates a nurturing disposition which could help rear offspring. In another study, researchers predicted that if the tipping-point model of male homosexuality were correct, then heterosexual men with a homosexual male twin should have more attractive feminine-pulling alleles and thus more opposite-sex partners than members of heterosexual twin pairs[15]. The findings of this large community-based twin study (N = 4904) supported this prediction; heterosexual males with a homosexual male twin had significantly more children, significantly more opposite-sex partners, and were significantly younger at their first age of intercourse than members of heterosexual male twin pairs ($p<0.001$)[15]. The results of these and similar studies have made the tipping-point model one of the leading evolutionary theories on male homosexuality to date[67].

1.3.4 Coding for Femininity in Males & Females

Another possibility is that the alleles responsible for male homosexuality code for psychologically or physically feminizing traits in both men and women[21, 67]. The *sexual antago-*

nism model suggests that an allele that is detrimental to the fitness of one sex could be maintained in the population so long as it is beneficial to the fitness of the other sex[21]. An allele that makes its bearer attracted to men and more feminine provides an obvious reproductive advantage to women, but an obvious reproductive disadvantage to men[21]. This allele would code for same-sex attraction if it appears in a male's genome but would maintain a net evolutionary benefit if this occurs rarely[21].

There is a significant amount of evidence for this theory. Numerous studies have found significantly greater fecundity in the female matrilineal relatives of homosexual men (i.e. their mothers, aunts and grandmothers) as compared to heterosexual men[21, 73, 74, 75]. Some other studies have also found that the female relatives of homosexual males have significantly fewer abortions and gestational complications than the female relatives of heterosexual males[12, 74]. Moreover, homosexual men have been found to have an excess of matrilineal but not patrilineal male homosexual relatives as compared to heterosexual men[21, 73]. According to researchers, even a modest increase in the reproductive capacity of females carrying these gay alleles could easily account for their maintenance at high levels in the population[21, 76].

2 Review & Discussion

As previously mentioned, significant maternal stress during pregnancy can disrupt fetal testosterone production and increase the likelihood of giving birth to a homosexual son[39]. As such, in the case of highly stressful environments, a family would benefit from having help in providing resources, shelter, and protection to its members. Additionally, because homosexual individuals do not have offspring of their own, this would prevent the family from becoming overburdened with more children in the future and would allow for the sole allocation of resources towards existing family members. Thus, the kin selection hypothesis is consistent with the maternal stress finding and may argue that homosexuality is activated epigenetically by environmental triggers linked to resource feedback, environmental stress, and the general need for help-in-the-nest.

The kin selection hypothesis is also logically consistent with the fraternal birth order finding[19]. If a family is already flush with children, epigenetic switches that alter the sexual orientations of subsequent fetuses and prevent them from adding more offspring to the family would be evolutionarily favorable[19]. Thus, homosexuality may be nature's way of ensuring that families do not have an unmanageable number of mouths to feed. A family flush with children would also benefit from added help-in-the-nest. Moreover, avoiding familial problems arising from competition for mates or for the allocation of resources towards one's own offspring could improve a family's overall health, cohesion, and success. This could be of vital importance in times of stress, when resources are scarce and mates not ample. Nonetheless, if homosexuality is indeed the result of an epigenetic switch that codes for a needed helper-in-the-nest who does not have offspring of his or her own, why does fraternal birth order, in particular, act to trigger such a switch but not birth order more generally? Moreover, no such correlation between birth order and homosexuality has been found for females[19]. The kin selection hypothesis does not address this fundamental disparity.

Ultimately, the kin selection hypothesis suggests that homosexuality is a switch in reproductive strategy: a trade-off between mating effort and alloparenting effort (i.e., parenting offspring other than one's own). However, why would individuals intended to be alloparents be anything but asexual? In insects of the order Hymenoptera (e.g., bees, wasps, and ants), individuals that alloparent are asexual[77]. These individuals do not expend any time, effort, or resources on courtship or on pair bonding with members of the same or opposite sex[77]. The kin selection hypothesis could explain the asexuality in these bugs destined to be alloparents but does not seem to account for homosexuality in humans.

In all, little empirical evidence has been found in support of the kin selection hypothesis. Researchers have concluded that homosexual individuals generally do not provide more care or resources to family members than heterosexual individuals[16, 65, 64]. Moreover, the kin selection hypothesis' feasibility can be questioned from an evolutionary standpoint. Because individuals share at most 25 percent of their genes with their nephews and nieces, they must compensate for every child they do not have themselves with the birth and success of at least two nephews or nieces. This is an inefficient method of passing on one's genetic material from one generation to the next.

As previously mentioned, the tipping-point model of male homosexuality is supported by a variety of evidence. One purported finding that supports this hypothesis is that heterosexual individuals in homosexual–heterosexual twin pairs tend to be younger at their first age of intercourse and tend to have a greater number of opposite-sex partners than members of heterosexual twin pairs[15]. However, there may be an alternate explanation for this large-scale finding. Throughout their life course, twins try to assert their individuality and unlikeness from each another[78]. Thus, heterosexual individuals in heterosexual–homosexual twin pairs may experience an added pressure to act in a more heterosexual way as compared to their twin because it is a distinguish-

ing factor between the pair. Therefore, twin studies that support the tipping-point hypothesis should be regarded with scrutiny. Alternative possible study procedures may involve evaluating the psychological femininity and reproductive success of the fathers and male relatives of homosexual and heterosexual men, not just their twins. Men carrying a greater number of feminine-pulling alleles should be more likely to produce homosexual sons, have a higher level of psychological femininity, and have had a greater number of sexual partners in their lifetime. Another potential area for future research involves investigating the applicability of the tipping-point model to female homosexuality, i.e., whether lesbianism is caused by way of "masculine-pulling alleles." Future studies can investigate whether men find masculine traits attractive in women—analogous to how females find feminine traits attractive in men—to determine whether the tipping-point model is relevant to lesbianism.

The tipping-point model of male homosexuality supports a polymorphic view of male homosexuality (and male sexuality more generally), since it suggests that multiple, "feminizing" alleles are at play and have an additive influence on male sexuality[69]. Therefore, the tipping-point model is consistent with the fluid sexual orientation continuum that scientists agree on today. However, the tipping-point model does not account for the fraternal birth order finding or maternal stress finding of male homosexuality. If male homosexuality is caused by way of feminine-pulling alleles, do intrauterine factors related to fraternal birth order and maternal stress epigenetically activate these alleles? On the one hand, the link between fraternal birth order, maternal stress, and male homosexuality could be viewed as the result of meaningful epigenetic action on these feminine-pulling alleles; making a male fetus more "feminine" after several brothers are born or in times of stress may be evolutionarily favorable. On the other hand, the connection between fraternal birth order, maternal stress, and male homosexuality could be considered arbitrary, a cause of nothing more than chance variations that influence fetal testosterone production and coincidentally affect the development of the male fetal brain. Future research into the early uterine environment's influence on fetal gene expression and its relation to sexual orientation may elucidate the nature of this relationship.

As previously noted, the sexual antagonism model of male homosexuality is supported by a variety of evidence. However, this model cannot yet account for the relatively low frequency of homosexuality in males[21]. According to the principles of sexually antagonistic competition, the alleles that mutually code for homosexuality in men and increased fecundity in women should steadily increase in prevalence in the human population over time, since females that inherit them are met with greater reproductive success[21]. Thus, genotypic ratios within the sexes would become altered[21]. This would result in the steady increase of male homosexuality over time and could hypothetically lead to the eventual "sterilization" of the male sex[21].

However, the maintenance of male homosexuality at a generally fixed ratio and relatively low frequency in the human population for millennia contradicts this assertion[12]. To address this discrepancy, it has been proposed that these sexually antagonistic alleles are commonly expressed in females, but only sporadically expressed in males[21]. However, why there would be such an asymmetry in the expression of these alleles among the sexes remains unclear[21]. In order to move forward with this hypothesis, future genomic research must locate these genetic associations and confirm or disconfirm their asymmetry in expression among the sexes[21].

It is also important to note that, like the tipping-point model of male homosexuality, the sexual antagonism model does not account for the fraternal birth order and maternal stress connection to male homosexuality. Likewise, it may view this connection as either arbitrary or epigenetically meaningful. Future research into the early uterine environment's influence on fetal gene expression in relation to sexual orientation is needed to unravel the nature of this connection.

As previously mentioned, fraternal birth order increases the likelihood of homosexuality[19]. This is believed to be the result of an immune system reaction in the mother that develops after several males are born and interferes with the proteins that have a role in the masculinization of the male fetal brain[44]. It is possible that this immune system reaction has not been sufficiently selected against and hence eliminated by evolution because it can only come into play after several siblings are born, most of whom are heterosexual and go on to have children. Clearly this cannot solely account for the persistence of male homosexuality throughout our evolutionary history, since some individuals are born gay without having any older brothers; however, it may be part of the mechanisms at play.

3 Conclusion

Male homosexuality's persistence in the human population for millennia has been termed an "evolutionary paradox"[16]. There are several competing evolutionary hypotheses that attempt to shed light on this matter, some more supported by evidence than others. Male homosexuality has proven to be a complex, multifaceted phenomenon for both researchers and evolutionary theorists alike[12, 18, 63, 67, 79]. Several coexisting factors may influence the development of homosexuality

in males, whether independently or in conjunction with one another, and each of them may have had unique evolutionary trajectories[12, 18]. Thus, there is likely more than one evolutionary mechanism at play responsible for the persistence of gay alleles in the human population[12, 18].

Current research on male homosexuality primarily supports evolutionary perspectives arguing that "gay alleles" confer strong fitness benefits on heterosexual individuals. The tipping-point model and sexual antagonism model are the two most empirically supported evolutionary theories on male homosexuality to date[18, 21, 67, 69]. Future research into the genomic underpinnings of sexual orientation in homosexual males and its genetic equivalents in heterosexual males and females may allow for further evaluation of these hypotheses. If further research supports both the tipping-point and sexual antagonism models of male homosexuality, it may be that both models account for a unique piece of the evolutionary puzzle and must be merged into a single coherent account of the matter. In all, male homosexuality has proven to be a convoluted evolutionary phenomenon that a substantial amount of additional research is needed to elucidate.

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Review

The Genomic History of Elephants Supports the Existence of a Third Modern Species and has Important Implications for Conservation

Kateryna Kratzer[1]

[1]Dept. of Biological Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2
Corresponding Author: K. Kratzer (kratzerk@myumanitoba.ca)

Abstract

*African elephants have historically been classified as a single species. New research into the genomic history of proboscideans has confirmed that the African savanna elephant (*Loxodonta africana*) and the African forest elephant (*Loxodonta cyclotis*) are in fact distinct species. The depauperate genetic variability of* L. africana *which may be due to a bottleneck in their evolutionary history increases their species vulnerability.* Loxodonta cyclotis *is currently vulnerable and its numbers are in sharp decline. Reclassifying these elephants as distinct species would prove useful to future studies and promote unique conservation approaches for each species, which are likely necessary to prevent the further decline and potential extinction of elephant populations.*

Keywords: Proboscidean, Phylogeny, Species, Genetic Bottleneck, Conservation

1 Introduction

The predecessors of modern elephants, such as giant mammoths and mastodons, originated in Africa and roamed throughout much of North America and Eurasia. Comprising 164 recognized species and subspecies[1], these ancient proboscideans were much more diverse than the modern species they gave rise to: the African elephant (*Loxodonta africana*) and its smaller cousin the Asian elephant (*Elephas maximus*). From Carl D. Illiger's designation of elephants as Proboscidea in 1811[1] up until very recently, these two were thought to be the only living proboscideans. However, according to genetic evidence, African elephants actually comprise two separate species, bringing the total number of extant proboscidean species up to three[2]. The African savanna elephant (*Loxodonta africana*) and the African forest elephant (*Loxodonta cyclotis*) should be classified as distinct species by conservation organizations so that both unique groups may be preserved. The number of elephants in Africa was estimated to have decreased by between 104,000 and 114,000 from 2007 to 2016 due to poaching and loss of habitat[3]. Despite the World Wildlife Fund listing African forest elephants (designated as a subspecies of African elephants) as vulnerable and "in sharp decline", there is no official estimate of their population size. Updating the modern elephant family tree to include a third species is an important scientific advancement with implications for both elephant conservation and future study. By recognizing forest and savanna elephants as distinct species, conservation efforts can be designed to address the specific needs of each species to ensure their ongoing preservation. Furthermore, updating the elephant family tree to reflect accurate data will be useful for researchers studying both ancient and modern proboscideans.

The earliest proboscideans first appeared on the African continent between five and ten million years ago[2,1]. These creatures were relatively small and had short trunks[1], although they would eventually give rise to the larger and more recognizable modern elephants. The extant Asian elephant is clearly morphologically distinct from its African relatives with its shorter stature, smaller ears, elongated skull shape, and straighter, more downward-facing tusks[4]. There are also notable differences between the two African species, although these have previously been insufficient to officially recognize them as distinct. *Loxodonta cyclotis* is smaller, has rounded ears, straighter and thinner tusks, and different skull morphology than its savanna-dwelling cousin[5]. In addition, the habitat of forest elephants is much smaller than that of savanna elephants[6].

Distinguishing species based solely on morphology has incorrectly dictated ideas of elephant evolutionary history. It was previously[1] suggested that the Asian elephant was most closely related to an extinct species called the straight-tusked elephant (*Palaeoloxodon antiquus*) based on their morphology, but recent work[2] has shown that this is unlikely. This more precise classification of species, based not only on morphology but also on phylogeny and biology, led to two conclusions[2]: first that the most recent common ancestors

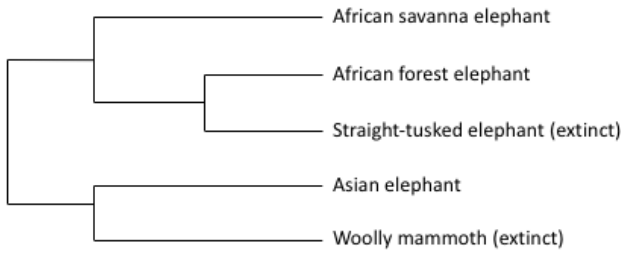

Figure 1: *Revised tree of phylogenetic relationships among elephant species based on straight-tusked elephant genome analysis. Branch lengths are not proportional to time. Data from Meyer et al.*[7]

(MRCAs) are different than previously determined, and second that there are three extant elephant species. African elephants should therefore be officially recognized as two distinct species based on their morphology, phylogeny, and biology.

2 The Elephant Family Tree

Of the 164 recognized proboscidean species[1], only two were believed to be extant. However, Shoshani[1] acknowledged that there may be three living species of elephants, and later studies outlined by Grubb et al.[8] suggested that African forest and savanna elephants should be classified separately based on morphology. This evidence was insufficient to justify the separate classification of African species. A recent phylogenetic tree (Fig. 1) based on genomic analysis of *P. antiquus*[7] recognized separate African species. This was confirmed[2] by performing genetic analysis on samples from a variety of living and extinct proboscideans.

The MRCA of the extinct straight-tusked elephant and the African forest elephant lived closer to modern times than did the MRCA of both modern African species (Fig. 1). The forest elephant is therefore more closely related to *P. antiquus* than it is to the concurrent savanna elephant. In fact, *L. africana* and *L. cyclotis* have been genetically isolated, meaning there has been no genetic exchange between their ancestors, for approximately 500,000 years[2]. Since the two African species diverged earlier in history than the forest elephant and the straight-tusked elephant, they have been genetically distinct species for a long period of time.

3 Modern Elephant Genetics

Palkopoulou et al.[2] sequenced the genomes of 14 proboscideans and aligned them to a reference genome from an extant African savanna elephant. Different molecular features of the data such as divergence between nucleotides and mitochondrial diversity were used to construct phylogenetic trees, which gave overviews of the relationships between the genomes. This and other genetic studies of modern and extinct elephants provide two points of compelling evidence for the existence of two distinct species in Africa.

First, there is no significant gene flow between modern African forest and savanna elephants[2, 6, 9], although there was considerable interbreeding between ancient elephant species[2]. The two types of elephants currently living in Africa are therefore arguably more distinct than their ancient relatives, which are classified as separate species.

Second, despite having a smaller geographic range[6] and therefore less opportunity to accumulate genetic diversity, forest elephants are more genetically diverse than their savanna counterparts[5]. Genetic samples from savanna elephants were nearly identical[5], indicating a genetic bottleneck event in their past. Bottlenecks decrease genetic variability in a species and can be extremely detrimental to species survival. Lack of genetic variation in other African animals such as the cheetah (*Acinonyx jubatus jubatus*) can lead to difficulty breeding in captivity, high rates of juvenile mortality, spermatozoal abnormalities, and increased vulnerability to zoonotic diseases[10]. These factors are important considerations for the field of conservation biology as they can complicate efforts to conserve threatened species.

4 Conclusion

Proving the existence of two African elephant species has important implications for immediate conservation efforts as well as future scientific research. The classification of forest and savanna elephants as separate species will directly impact conservation and management approaches by promoting individualized efforts to protect each species as a distinct group. Widely accepted data that accurately depicts the relationships between modern and extinct elephant species will allow for more consistent data collection in the future, furthering our biological understanding of the past and present.

The loss of a subspecies is not as devastating as the extinction of an entire species. Organizations such as IUCN and the World Wildlife Fund can focus their efforts on saving both African elephant species from extinction if they recognize them as such, rather than classifying *L. cyclotis* as a subspecies of African elephant. Unique conservation efforts directed towards each species are important[2, 7, 6, 9]. Despite the vulnerability of forest elephants in particular, the official census[3] paints a grim picture for all remaining elephants in Africa, making them a broad priority for conservation efforts. Separate studies of forest and savanna elephants are required in order to better understand these unique animals

and shape conservation approaches tailored to each. A wider approach, based on the old single African species idea, could result in the loss of one or both African elephant species.

Using our current knowledge of each species ecology, we can begin to identify better conservation approaches. African forest elephants live in dense vegetation and are therefore difficult to identify and study without specialized techniques such as thermal imaging[11]. More comprehensive study of these elusive animals requires the use of techniques and technologies that are customized to their behaviour. Savanna elephants are easier to observe in their open habitat but likely require different conservation approaches due to their limited genetic variation. Careful breeding programs to prevent any further decrease in savanna elephant genetic diversity would not be applicable to the more genetically diverse forest elephant populations. Interbreeding between the two species, which may occur if they are housed together in captivity under the assumption of belonging to a single species, would destroy any genetic differences between the species. If these species are not conserved separately, we may end up losing both.

In addition to the importance this has in regard to elephant conservation, accurate genetic data is critical to general scientific inquiry and knowledge. An updated elephant family tree will help guide future molecular studies on genetics and genomic histories, as well as more traditional biological studies on the behaviour, distribution, and population changes of African forest and savanna elephants. In addition, the techniques used[2] to determine historical relationships between proboscidean genomes can be applied to broader studies on the genomic history of other species.

The history of elephants is more interesting and complicated than once thought. Two species of elephants reside in Africa; *L. africana*, the savanna elephant, and *L. cyclotis*, the forest elephant. They differ in their morphology, phylogeny, and biology. The two types have been genetically distinct for half a million years and show different levels of genetic diversity. They should therefore be recognized as separate species. Additional genomic analysis of ancient specimens can help further unravel the past while the recognition of distinct African elephant species will shape current studies and conservation efforts, thereby helping save more elephant species from extinction. Genetic study is vital to the field of biology in terms of the past, present, and future.

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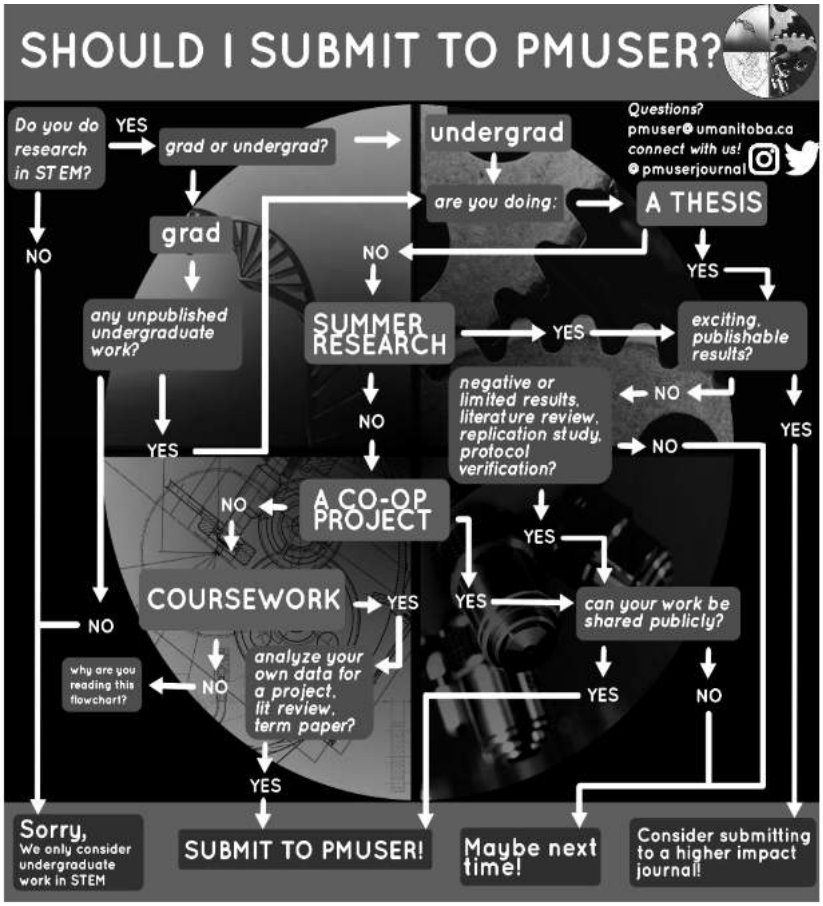

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Puzzle

The Hapless Editors

Puzzle 1: Easy

As the editors were carrying the stack of review papers accepted for publication in Volume 4, Issue 1 their skates (we're in Canada, eh) flew from underneath them and the papers were scattered.

Before the editors could gather the pages, they were shredded by a Zamboni (it was a terrible day so let's not dwell on the details, alright?). Each author submitted a single article, and all articles were on different topics.

After debating a while, the following information is all that the editors' faulty and fallible collective memory could recall.

Can you help the hapless editors piece together the surname of each author with the topic of the paper and the order they think the six reviews were to have been published in?

1. The second review was written by Dueck.
2. Alsip's review was not published before Mashmoushi's.
3. The homosexuality review was written by Mashmoushi.
4. The review about wolves was published immediately after the one about homosexuality.
5. Kratzer did not write the fourth review.
6. The Antarctic fish review was published three ahead of Mashmoushi's.
7. The review about Antarctic fish was published immediately in front of the one about elephants.
8. The editors could only be completely certain about the author and topic of the first of the six reviews.

Stuck? Already? Fine. Here's a hint. http://tiny.cc/v4i1p1h1

Still stuck?? http://tiny.cc/v4i1p1h2

Want a grid for *Puzzle 2*? http://tiny.cc/v4i1p2h1

What!
You're expecting a second hint for the *hard* puzzle?
There is no second hint for *Puzzle 2*...
Okay, fine. You can try to beg us for it,
But we'll tell you to read Volume 4!

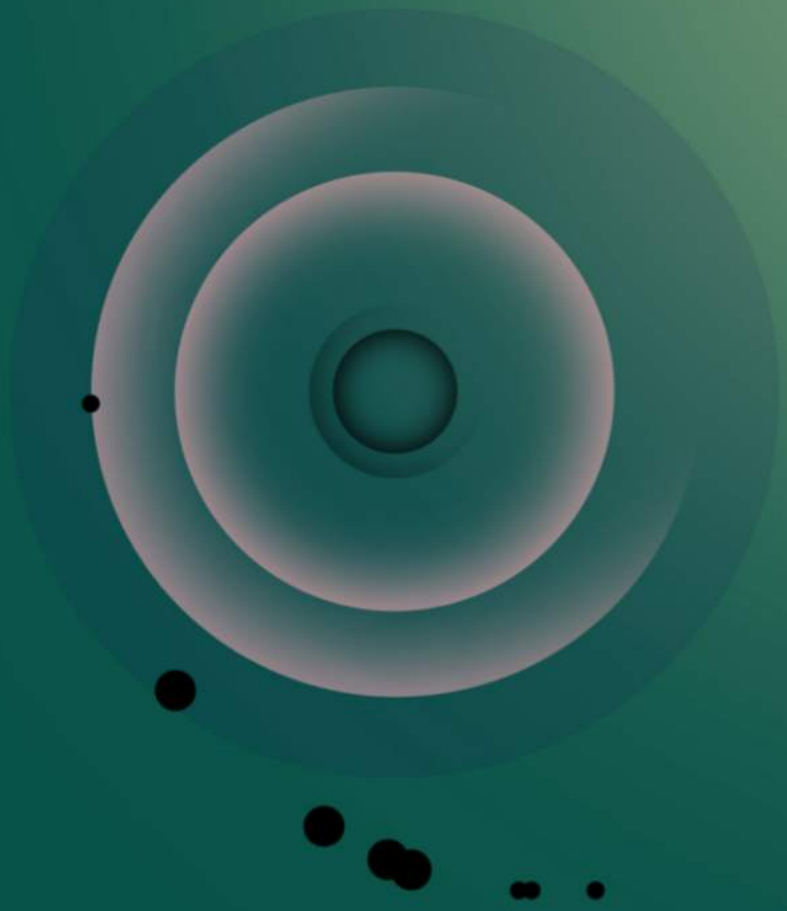
Puzzle 2: Hard

Now, can you reassemble the unique first and surnames of each first author of each research article with their unique digital object identifiers, abbreviated topic areas, and publication order? Because after the Zamboni, the editors then faced the cataclysmic catastrophe as their publishing platform crashed and obfuscated the digital bits of the eight research articles just prior to publishing...

All that remains is the following corrupted cryptobabble...

1. The article by Edwards was published one earlier than the article focused on STEM factors.
2. The article by Saj either discussed AGGR or was written by Danielle.
3. The article by Guo was published six after the one with ID 613.
4. The last article published was three after Danielle's.
5. The eight research articles are the one with ID 620, the fifth published (with ID 660), the eighth published, the one mentioning CPR, the one discussing RNN, and the ones by Guo, by Wuzinski, and by Carter.
6. The paper written by Lindsay, neither the third published nor the one discussing A, has an ID less than 630.
7. The paper drawing attention to CSPMA was published immediately after ID 604 and before the sixth article published.
8. First author of Carter Ives either made use of the FME or had the sixth article published.
9. The article by Edwards was published earlier than the one with ID 616.
10. The paper published sixth, by Steven, was published four after the one by Wuzinski, and has a lower ID than the one by Lindsay.
11. Beom-Jin Park's research was published two papers after Carter's.
12. Xiang Zheng's research was published immediately after that discussing SNPs and more than two after Saj's.
13. Article 613 was published before 637 or 604.
14. The paper with the largest ID was published last.
15. Zheng's research was published five articles after the one with ID 604.
16. AGGR, not the topic of the final publication but written by Danielle or Michelle, has ID 637 or 670.

Done? Now, how does your attempt to recreate the publication differ from the correct Volume 4?
Got the answer? Email us at pmuser@umanitoba.ca for fame, recognition, and a hearty handshake.



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pmuser@umanitoba.ca
ojs.lib.umanitoba.ca/pmuser