PMUSER

Proceedings of Manitoba's Undergraduate Science and Engineering Research

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Frontiers of Undergraduate Research

VOLUME 5

PREFACE

2019
December

The climate is in crisis.
Evidence is absurdly overwhelming
with blizzards, droughts, fires, floods, hurricanes,
and volcanoes; natural processes of Mother Earth are
metastasizing like a tumour at peak angiogenesis. The catalyst is humanity.
Neo-colonialism fuelled by the climate crisis is the largest threat to all of humanity.
Should humanity dismiss the calls to action, billions of people will suffer and die.

The University of Manitoba spent seven million dollars on fossil fuel products, according to 2017 documents on campus sustainability. Solutions include solar panel integration on the Fort Garry campus infrastructure. Although realistic, these solutions are not enough to reverse carbonic emissions. Humanity must protect, restore, and fund solutions to the climate crises. Solutions must protect trees, food, and natural water sources. Solutions must restore or maximize areas to capture carbon and self-sustain diverse ecosystems. Funding climate crises solutions is necessary in every human capacity worldwide, through any means possible.

Proceedings of Manitoba's Undergraduate Science and Engineering Research Journal is a platform that reflects a critical moment in undergraduate endeavors. Volume 5 of PMUSER Journal follows a three-fold value system; provide open access to undergraduate research, assist in recognition of researchers for their disciplined work, and further overall undergraduate participation and development of research publications. Despite adversity, Volume 5 presents Manitoba's thriving innovation on the frontiers of undergraduate research.

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invent the future

About PMUSER

Proceedings of Manitoba's Undergraduate Science and Engineering Research (*PMUSER*) is an open-access, peer-reviewed 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 any topic concerning 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 students to explore the frontiers of undergraduate research and add additional value and learning opportunities to students' degree programs through publishing their work.

Aim & 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.

Image Credits

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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.

Instructions for Authors

Undergraduate authors are encouraged to submit their perspective, research, review, or rapid communication articles! 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.

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About the Cover

The cover features the 2019 Cover Art Contest winner, William Steele. "As a piece that involves the theme of science and its continued progress, I thought about including imagery that evokes mythological connotations of trees that provide greater wisdom and knowledge; the world tree of Yggdrasil as well as the Tree of Knowledge of Good and Evil are both iconic in their own ways, and even referring to the myth of Isaac Newton's discovery of gravity through the simple falling of an apple from a tree. Science as a field is interconnected in many different ways amdist its branching studies, and only by working together can any of its greater fruits be grasped."

Letter from the Editor-in-Chief

In Pursuit of Life's Purpose

The proceedings are a critical reflection of my time leading PMUSER Journal. Financial support, professional development, community reputation, and conflicts of interest are discussed. After a year at the helm of a research publication platform, I consider what defined 2019 for me. Professional and personal lives are kept separate mostly but the inherent political burden of being a full-status Indian under the Indian Act is an additional layer of complexity in leading interprofessional teams. This complexity offers integrity but also a limitation on what is achievable for those who come next.

Not-for-profit and student led, PMUSER Journal offers a glimpse into the forefront of research across the province of Manitoba. This platform is desperately needed in our University community, yet it is funded by very few. Special thanks to Dr. Jay Doering and to the Faculty of Science, their financial support of Volume 5 is most appreciated. Others are encouraged to reach out with resources as Volume 6 will be coming soon.

So many were unable to make this Volume's deadline. The work done just to translate their research into English, execute their work, and translate it back to their original language is a burden I must acknowledge. There is a heavy and unnecessary sacrifice of language, culture, storytelling, and journalism that comes with research publication, but the University of Manitoba offers a culturally safe place to explore ways to transfer this knowledge.

In 2015, I was laid off from my career as a heavy machinery operator in the oilfield. My love of physics led me to a lot of injuries, so I chose a medical degree in Nursing. By 2017, I found proficiency in analyzing policy and legislation. Summer of 2017 and 2018 when I wasn't studying Nursing, I worked at Health Canada. A decade after middle school, my passion in journalism returned when I read Medicine Unbundled by Garry Geddes. I was reminded to go beyond just "doing my job"; the most important values within my family cannot be bought.

Practicing as a Nurse, working at the federal government, reading empirical data on my culture and bloodline; I began to ponder what my descendants will know of me and how I will make the world a better place. For the first time in my adult life, I was in a psychological space safe enough to choose where my energy is spent. The opportunity to act as Editor-in-Chief of PMUSER Journal is one most-privileged. It doesn't pay the bills at all but the experience to lead a team and contribute to Manitoba-based research I'm proud of.

I must acknowledge my own potential conflicts of interest. Earlier I spoke to my Indian status as jury selection, among other Canadian practices, screen us from participation for "political bias". I can assure you my contributions to a higher knowledge are not propaganda to benefit corrupt or otherwise political entities in Canadian society. Policy in Canada, however, refers to me as permanently disabled due to my expressions of autistic and AD/HD traits. Health Canada provided summer employment and an Indspire bursary at the start of my Nursing education. My Nursing tuition in 2018 and 2019 is sponsored by the Indian Act chief and council to whom I am a member with.

I most recently won UMSU's 2018 Award for Indigenous Community Leader. I am also part of the inaugural cohort for the President's Student Leadership Program. Most grateful, but my yearly income averages $7,900 with personal care-taking, clinical rotations, Nursing studies, Two-Spirit research, journal management, community events, volunteerism, etc. I participated in the five-year strategic planning of the Aboriginal Nursing Cohort Initiative, and even sat on the Student Advisory Council for the Dean of Nursing in 2018/2019. I want to help save the world, but my biggest conflict is impoverishment and financing. I've spread myself too thin helping everybody else and forgot about me.

To conclude, my involvement with PMUSER Journal was life changing and I see much potential going forward. I hope my work, and that of PMUSER Journal, has challenged you to pursue a higher truth. I also hope openly sharing some of the more intricate parts of who I am serves both the community and my own self-growth. Certain visions haven't reached fruition yet, but I never imagined a reality like today. I hope my legacy with PMUSER Journal lasts longer than my tenure.

Thanks for reading,

River Steele, UMSNIV
PMUSER Editor-in-Chief

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University of Manitoba Faculty Profiles

Jose Luis Rodriguez-Gil, PhD
Research Associate, IISD-ELA / University of Manitoba

Photo by:
Riley Brandt, University Relations, University of Calgary

Why is experience valuable for undergraduate students?

There are the obvious professional development aspects of spending some time in a lab, which always looks good in a CV. In my opinion, however, there are also some really valuable soft skills that can be learned from partaking in a research project. During an undergrad program, a lot of focus is placed in the acquisition of knowledge, but not so much in the application of this knowledge. Solving problems when they come. Oh! They do come! It requires making connections between all those pieces of knowledge that the student gathered before and using them to come up with creative solutions. That is something that can only be developed when conducting research hands-on.

Anyone that has published anything in the peer-reviewed literature knows the hard work that goes into it.
— Jose Luis Rodriguez-Gil, PhD

What opportunities are available for undergraduate students?

I am a new recruit to the department, so I am learning that myself, however, within the coming months, myself and Dr. Hanson will be looking for undergraduate students to work with us in a number of projects that we have coming down the pipe. No pun intended, a large portion of my current research centers around pipeline spills in freshwater systems. This research is being carried out at the world-renown IISD — Experimental Lakes Area (IISD-ELA). The IISD-ELA has an excellent program of internships for students that allows you to spend a whole summer working at the World's Freshwater Laboratory. This program includes some Manitoba-specific scholarships, so if you like lakes and forests, get in touch with us.

What value do undergraduates get from publishing?

Again, there is a double value here. Of course, it looks great in your CV. Not only as another line on it, but as a very impressive one. Anyone that has published anything in the peer-reviewed literature knows the hard work that goes into it. Seeing a peer-reviewed publication in an undergraduate student's CV speaks to a great number of skills and personal characteristics from that student that no other section of a CV would. At the same time, there is the part of being able to say that you have contributed to create new knowledge in your area. If the research doesn't get published, independently of how hard you worked on it, in the view of the rest of the world, it was not done. That is sad, because of the waste of your own work and effort, but also because it could lead to waste of other people's resources and effort if they end up doing it again because they didn't know it already had been done.

Of course, these decisions need to be based in the scientific knowledge acquired previously, but how that knowledge gets used when things don't go according to plan is where a great researcher shines.
— Jose Luis Rodriguez-Gil, PhD

What does it take to be successful in research?

As I mentioned earlier, one of the most common occurrences in research is things not going as planned. Problem solving capabilities and a bit of creativity to come up with new ways around the problem are great skills to have in a lab or in the field. Of course, these decisions need to be based in the scientific knowledge acquired previously, but how that knowledge gets used when things don't go according to

plan is where a great researcher shines. Many of these times could very well be opportunities for new ideas and future research waiting to happen. On a more work-life balance side of things, that constant problem-solving can be tough, and as such, to be able to do it for an extended period of time (your whole career!) you do need to learn to take a step back, have a break and come back to it with fresh eyes... and if it does not work, learn to let go!

Who has influenced you the most?

I am going to have to go with Dr. Mark Hanson. Mark was my PhD co-advisor, and we have been working together for over a decade now! More than in my science, where he has obviously had an influence, I believe his influence is more noticeable in the kind of researcher/advisor/mentor I want and try to be. He is approachable, he cares, and somehow, he manages to stay on top of things, even when he is incredibly busy! You never fall off his radar. He also taught me, early on, about the importance of being involved in the greater scientific community by joining and partaking in activities and organizations. That has payed off countless times over my career. Fortunately for me, I am now at the University of Manitoba, so we will get to work together for many years. Come join our labs!

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— Jose Luis Rodriguez-Gil, PhD

How will your current job help achieve long-term career plans?

I am currently a Research Fellow splitting my time between the IISD-ELA and U of M. The IISD-ELA is known as "The world's freshwater laboratory" and it has been compared to the CERN, or the International Space Station of aquatic research, for it is the only place in the world where the kind of work that we do (large ecosystem-scale freshwater research) can be done. Obviously, working at the IISD-ELA is a dream shared by most people working on fresh-water related issues, so I feel incredibly fortunate to be able to say that I work there. As a research associate, my job is to support research currently being carried out (like the oil-spill program mentioned earlier) but also to come up with my own research agenda of research that can take advantage of what this unique facility has to offer. At the same time, my work at U of M allows me to remain in touch with the university community and the students, who after all, carry out most of the research. While it is an early-career position, I believe there is a long future and great room for growth within IISD-ELA and U of M, and I am looking forward seeing where it takes me.

Describe a problem you faced. What did you learn?

I am not going to talk about one single problem, but a time in my life when I had to deal with many problems. During the past 2 and a bit years I have been project coordinator for a large project (3 PIs, 14 grad students) which involved simulating a series of oil spills in lake enclosures. This involved very hard work with a 6-month field season at the IISD-ELA. Every day we had to face "problems", from equipment malfunctioning, to fish not wanting to work with us and of course, all the cascading effects that those things would have in the general project and the work of the different students. I think my main learnings from that time are summarized in my previous answer to "what makes a successful researcher". Problems are not rare, but the norm in research, one has to learn how to get used to them, both in the professional sense (you do get better at coming up with a "plan B") and in the personal sense. You need to understand that it is just part of the job, and while you do want to do your best, sometimes, you need to leave the problem for a bit, go home, enjoy your family and friends and come back to it with a fresh set of eyes. Even if at the time it looks like the most important thing in the whole world... It likely can wait.

Solutions are easier to see with a fresh pair of eyes.
— Jose Luis Rodriguez-Gil, PhD

How do you motivate a researcher going through a low point?

I know it seems like your whole life right now, but it is just work... Go home and take a break.

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Perspectives

Innovation and Expanding Horizons within Research at the University of Manitoba

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1 Introduction

The University of Manitoba is at its peak in novel research discoveries eliminating any shrouds of ambiguity that permeate its walls.

2 Discussion

Diversity and inclusivity are the driving forces behind the cutting-edge research at the University of Manitoba. This innovation extends to all levels of academia, from undergraduate through to post-graduate studies. Dr. Jason Kindrachuk from the Department of Medical Microbiology & Infectious Diseases and Dr. Mojgan Rastegar from the Department of Biochemistry and Medical Genetics are two of the many research supervisors at University of Manitoba's Bannatyne Campus currently addressing issues associated with human health. Dr. Kindrachuk's research centres on the mechanisms of Ebola virus transmission, pathogenesis and persistence while Dr. Rastegar's research centres on the neurological disorder, Rett syndrome.

2.1 Dr. J Kindrachuk's Research Lab

Dr. Kindrachuk places a large emphasis on understanding the intersection of basic and clinical research in emerging viruses not only in terms of the physiological repercussions that result from infection but the role they play at the level of individual tissues and cells. He stresses the importance of "understanding how these viruses usurp and undermine our own defense systems," as well as the lasting effects these diseases may have at the molecular level. To accomplish these tasks, Kindrachuk and his students employ various complex lab techniques. An area of interest to Kindrachuk are methods for evaluating the maintenance of blood-tissue barriers, including the blood-testis barrier. This interest stemmed from Kindrachuk's experience in West Africa when he and his fellow colleagues learned that male Ebola virus disease survivors can still carry the virus within their testicular tissue after recovery.

Furthermore, testing these patients with current diagnostic methods lead to a negative result. This is of great concern according to Kindrachuk as "patients that have been released to the general public have been shown to be able to spread the disease sexually and carry high amounts of virus, in the absence of disease.

[Thus] we can start to use systems like blood-testis barrier or our kinome platform within our labs to better understand how these viruses are able to do this." The persistence of this virus within the human body raises many more questions than we currently have answers for. Kindrachuk recalls another patient who relapsed twice due to viral penetration into and out of the central nervous system. Kindrachuk believes the virus gained entry, laid dormant, and then resurfaced in the blood.

To further elucidate, what really allowed the virus to transverse the barrier in the first place? Furthermore, what caused the essential breakdown of the barriers and allowed for virus transmission across these internal physiological barriers within the human body? Interestingly, research within Kindrachuk's lab has shown that bilateral navigation of Ebola virus is not seen across the blood-testis barrier. This was reflected in one of their subjects that had 10,000 times higher concentrations of virus in semen than was found during peak Ebola virus infection in the patient. Kindrachuk hopes to dissipate the enigma of Ebola virus's mechanisms within the body and hopes to answer many unanswered questions including why the virus can only enter the blood-testis barrier in one direction but can easily traverse across the blood-brain barrier in both directions.

"Patients that have been released to the general public have been shown to be able to spread the disease sexually and carry high amounts of [Ebola] virus"
— Dr. Jason Kindrachuk, Department of Medical Microbiology & Infectious Diseases

2.2 Brayden Schindell & Ebola survivors

PhD student Brayden Schindell from the Department of Medical Microbiology & Infectious Diseases has worked in the emerging and re-emerging virus's laboratory since January 2018. He was intrigued with the research conducted on Ebola virus and began pursuing his PhD degree in Dr. Kindrachuk's lab in January of 2018. He studies how Ebola virus persists in the reproductive

tracts of male and female survivors and how these patients maintain the virus despite remaining largely asymptomatic. Currently the lab is focused on utilizing polymerase chain reaction based diagnostics similar to those employed during Ebola virus disease outbreak. Kindrachuk states that it is "much more sensitive than an ELISA (enzyme linked immunosorbent assay) and is capable of sensing very remote traces of the virus." The issue with this technique arises when the virus is housed in a more precarious location such as the brain or the testes where the immune system is more limited in its ability to initiate a response. Schindell and Kindrachuk believe responses to current outbreaks is a top priority.

Dr. Kindrachuk hopes to dissipate the enigma of Ebola virus's mechanisms within the body and hopes to answer many unanswered questions including why the virus can only enter the blood-testis barrier in one direction but can easily traverse across the blood-brain barrier in both directions

The goal of Schindell's project involves studying the mechanisms of establishment of persistent infections in the reproductive tracts of males and females. He is also collaborating with researchers in West and Central Africa to study the long-term reproductive health effects on survivors of Ebola virus disease. He will be studying the reproductive health effects by working with cohorts of survivors in Sierra Leone conducting survey-based analysis and sample analysis. Currently there are two vaccines for Ebola in clinical trials.

2.3 Research Opportunities in Winnipeg

Part of Winnipeg's flourishing research opportunities can be attributed to the National Microbiology Laboratory located in Winnipeg that provides students with direct lines to many lead researchers in many viral and bacterial fields. The facility houses several classes of containment laboratories as well as the only containment level four laboratory in Canada. Having this facility in such close proximity to the University provides students with the opportunity to conduct novel experiments and analysis on high containment viruses such as Ebola virus. "The work environment is very supportive and extensively collaborative," Schindell states. He also believes the University houses a world-class facility in conjunction with the heavy focus on community engagement locally and abroad. "It allowed me to venture beyond what I believed I was capable of accomplishing and has allowed me to gain new perspectives on my research with Ebola virus."

2.4 How Integrating Labs can Facilitate Change and Innovation

The collaborative nature of the Bannatyne campus allows for students with a myriad of different strengths and backgrounds within the field of science to share their ideas and innovations to formulate new discoveries. The Regenerative Medicine unit and the Department of Medical Microbiology and Infectious Diseases are indicative of this breadth of cooperation as well as the collaborations between community groups that directly translate research to the community. A great example of this collaboration is reflected in the work of Dr. Keith Fowke's laboratory (Medical Microbiology and Infectious Diseases) with the community organization Sunshine House. Sunshine House supports the largely underserved groups including those affected by HIV/AIDS, those who use substances, those exploring gender and sexual identity and those experiencing homelessness. They provide a community drop-in and resource centre that promotes harm reduction and social inclusion by providing programs, which fulfils social, health, and recreation needs of the community.

In 2005, Sunshine House reached out to the Fowke lab because the community wanted to understand the effects of solvent use on the body. In collaboration with Sunshine House, the Fowke lab has uncovered novel findings about the impact of solvent use on some aspects of the immune system. The study results have been discussed with community members to arrive at a collective understanding and interpretation of the findings. This work has resulted in not only answering the communities initial question, but has sparked new discussions and new questions leading to new projects that involve more researchers at the University of Manitoba and from other institutions.

"It allowed me to venture beyond what I believed I was capable of accomplishing and has allowed me to gain new perspectives on my research with Ebola virus."

— Brayden Schindell PhD, Department of Medical Microbiology & Infectious Diseases

Dr. Mojgan Rastegar joined the University of Manitoba in 2009 as the primary member of the Regenerative Medicine program, at the Rady Faculty of Health Sciences. Since then, she has built a strong research program on epigenetics, stem cells, and neurodevelopmental disorders. Apart from running her own research laboratory and training of graduate and undergraduate students, she is the director of multiple courses at the graduate level, while teaching undergraduate courses. At the University of Manitoba, she is a member of the Senate, and Senate Committee on Admis-

sion, Senate Committee on Planning and Priorities, as well as Faculty and College Executive member.

2.5 Novel Techniques

Dr. Rastegar's research program is focused on the role of epigenetics in neural stem cell self-renewal and differentiation and their abnormalities in X-linked neurodevelopmental disorders such as Rett Syndrome. In these regards, her lab uses a combination of *in vitro* and *in vivo* approaches, using different types of stem cells, primary brain cells, human cell lines, and transgenic mice. Her lab pioneered the research on developing tools and reagents to study different protein variants of the MeCP2E1 and E2, in the brain. Dr. Rastegar has now taken a leadership role in Canadian research on Human Rett Syndrome Brain Tissues. Earlier this year, she received an award from the Ontario Rett Syndrome Association (ORSA) to establish the "Human Rett Syndrome Brain Bio-Repository Laboratory" in Manitoba that is currently located at the Children's Hospital Research Institute of Manitoba (CHRIM).

The grand opening of this laboratory was September 2019, which was well attended by Rett Syndrome parents (from Ontario, British Columbia, Manitoba) and the president of ORSA. This new laboratory is a unique resource in Canada. Dr. Rastegar is now studying the molecular basis of intellectual disability in neurodevelopmental disorders, aiming to use cutting edge techniques in stem cell biology, epigenetics, genomics, and drug repurposing to find potential novel avenues for devastating diseases that currently have no cure, such as Rett Syndrome.

Dr. Rastegar is now studying the molecular basis of intellectual disability in neurodevelopmental disorders, aiming to use cutting edge techniques in stem cell biology, epigenetics, genomics, and drug repurposing

3 Conclusion

Compared to other research laboratories across the province, Dr. Rastegar believes the University of Manitoba has an extremely strong and diverse research team with scientists from all across Canada with her lab specifically bringing expertise from Europe, United States, and Canada. Despite the various challenges encountered working with neural stem cells for Dr. Rastegar and the strenuous experimental procedures required for Ebola research for Dr. Kindrachuk, both research labs are continually inspired and face these inevitable road blocks with an open approach and an equally open mind.

4 Acknowledgements

This piece would not have been possible without the tremendous contributions and effort put forth by Dr. Jason Kindrachuk, Dr. Mojgan Rastegar, Dr. Keith Fowke, Brayden Schindell, Monika Kowatsch and the community at Sunshine House. Your patience, time, and commitment put forward in assisting with the publication of this piece has not gone unnoticed. It was a privilege to interview and engage with all of you and furthermore, share with all our readers the research taking place within the University of Manitoba which certainly has the potential to change the world.

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Research Article

Estimation of Ectoparasites in an African Ground Squirrel

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Abstract

Studying the parasites of wildlife necessitates the accurate estimate of ectoparasites of free-ranging animals, often in a field setting. The objective of this study was to test the relative accuracy of ectoparasite estimate in a rodent species, the Southern African ground squirrel (Xerus inauris). *Estimates of ectoparasites using combing were compared to total counts of ectoparasites on sacrificed animals. Results suggest that our combing method and visual inspection was a reliable method to estimate flea and lice intensity and abundance for* Xerus inauris *species. However, differences were found in prevalence of these parasites between estimated and total collected, as the total was 1.5 times that of the estimates. These results demonstrate successful estimation of parasites in a live small mammal species without requiring anaesthesia.*

Keywords: Ectoparasites, Field Techniques, Fleas, Lice, Squirrels

1 Introduction

Parasites can have high impacts on hosts in terms of fitness (survival and reproduction) and more directly on their behaviour and physiology[1]. Host parasite infestations are usually extremely variable, with a high proportion of parasites concentrated in a few host individuals[2, 3]. Infestation includes a minimum of one parasite per host. To understand the factors that can influence infestation and ultimately variances in the fitness of hosts, it is important to have accurate parasite estimates[4]. Absolute and mean values can be of limited use in most parasitology studies due to the over-dispersion of parasite distributions[2]. The most common measures to assess parasites are estimates of prevalence (percentage or proportion of hosts with the parasitic infestation) and intensity (quantity of parasites on infested hosts[3]). However, these measures rely on accurate estimates of the parasites, which are often difficult to assess under field conditions where sacrificing the host is not an option[4].

In mammals, a common technique to quantify parasites include live-animal combing to estimate the relative infestation of ectoparasites[5, 6]. The accuracy of the combing method is controversial due to parasite specific differences such as duration of attachment, parasite size and mobility, as well as variances in host size, age, skin type, immune status, ectoparasite density, and even differences in the time spent examining hosts by the researcher[7]. This controversy requires methodological studies to assess the accuracy of the combing technique in difference species to assess parasitic infestation.

Ectoparasite estimation can be influenced by the species, morphology, and behaviour of both the host and the parasite, as well as magnitude of infestation, time available for inspection, and experience of the investigator[7]. Due to these variations, studies have addressed potential parasite sampling errors by using subsampling. Subsampling techniques aim to standardize the estimation process by either decreasing the time spent examining individuals, using a predefined sampling time, or to decrease the surface area that is examined. Thus, by examining certain parts of the individual, subsampling can be used as a procedure to evaluate relative quantities of parasites with less chance of sampling errors[7]. Previous studies have found samples of ectoparasites by paying specific attention to the ears, face and genital areas of small mammals after combing for ectoparasites[8]. Another study in gerbils (*Gerbillus andersoni*) demonstrated aggregation patterns of mites and ticks to the mouth, ears, nose, and hind legs[9].

The objective of this study was to assess the relative accuracy of parasite estimates in a small mammal, the Cape ground squirrel *Xerus inauris*, in a field setting. We compared these field estimates of parasites to the number of parasites collected after subsequent sacrificing of the animal. Hitherto, we will refer the number of ectoparasites found on the sacrificed animal as the "total collected" ectoparasites. If combing is a good relative estimate of ectoparasite infection, then the total ectoparasites collected will positively correlate

with combing estimates.

2 Materials and Methods

2.1 Study Species, Trapping and Handling

Cape ground squirrels are a highly social, semi-fossorial species of squirrel living throughout the arid regions of southern Africa [10]. The ectoparasites recorded on Cape ground squirrels include fleas (*Ctenocephalides connatus, Echidnophaga bradyta, Echidnophaga gallinacea, Synosternus caffer, Chiastopsylla rossi, Demeillonia granti, Pulex irritans*), lice *(Neohaematopinus faurei)*, and ticks *(Rhipicephalus theieri)*[11, 12]. Only ectoparasites large enough for visual observation without the need of a microscope were collected during this procedure. The study was conducted on two farms near Bultfontein, Free State Province, South Africa (28.28°S, 26.15°E) in July 2013, where squirrels are routinely removed as control measures to reduce crop damage. We handled all squirrels that had been captured in live traps (Tomahawk 15x15x50cm) using techniques described in Hillegass et al.[6], where squirrels were immediately placed in a cloth handling bag.

We immediately estimated ectoparasite numbers by combing three strokes on the left, middle and right plane of the animals back, from the shoulders to the base of the tail with a metal flea comb. Any collected ectoparasites (fleas, ticks, and lice) were placed into a petri dish with 70% ethanol and counted. We modified Hillegass et al.'s[6] procedure by also including a careful examination of the groin and inner thighs of the squirrel and collecting any visible ectoparasites from these areas using forceps. Upon completion of ectoparasite combing, squirrels were given to local farmers and we received carcasses from the farmers following euthanization by chloroform placed on cotton pads, the best method to collect ectoparasites[13, 14, 15]. The carcasses were then held by the tail or foot over a white paper in an enamel tray and the entire body was rubbed to remove the remaining ectoparasites[16].

We also searched all parts of the body, handling bag and tray and removed any remaining visible parasites. All collected fleas, lice and ticks were counted and stored in 70% ethanol. As ticks are rarely found on *Xerus inauris*[6], we found only four ticks in total. These ticks were excluded in this analysis. Mites were also excluded from this study as they are not collectable by combing methods. All trapping and handling was in accordance with the American Society of Mammalogists' guidelines[15], and the University of Manitoba Animal Care Committee (Protocol #F10-030).

The abundance (mean number of parasites found on all individuals), prevalence and intensity of the parasites, were calculated using Quantitative Parasitology 3.0 web version[17, 3]. We used exact unconditional 95% confidence intervals to estimate prevalence of infestation[18] and sign tests to compare our estimates and total counts. A 2000 replication bootstrap with replacement two-sample t-test was used to compare abundance and mean intensity and for all analyses we reported 95% confidence intervals (CI) using Clopper-Pearson estimates, unless otherwise specified[3]. We used JMP© 10.0 (SAS Institute Inc., Cary, North Carolina, USA) for our sign, Spearman's correlation, and Wilcoxon signed ranked tests.

3 Results

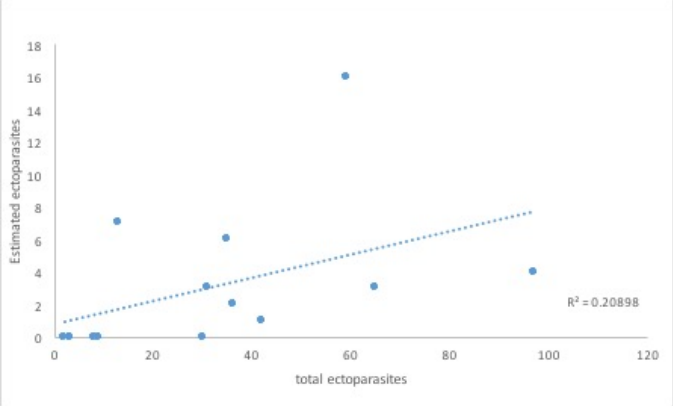

Figure 1: *Estimated ectoparasites from 15* Xerus inauris *hosts using a combing method were positively correlated with total number of ectoparasites (Spearman's correlation, P < 0.05).*

A total of 15 ground squirrels were collected, including eight females and seven males. The confidence intervals of overall ectoparasite prevalence from before and after euthanasia overlapped (Table 1), but prevalence of all species was higher in the euthanized estimates (sign test: M = 3, P = 0.03). Differences in the prevalence of lice were also significant (sign test: M = 6, P = 0.0005) while fleas were close to significantly different (sign test: M = 2.5, P = 0.063). As expected, median intensities of live estimated ectoparasites were lower than the median intensities of total ectoparasites (mean intensity was only 6.5±2.6% of total intensity; Table 1) and we found similar lower intensities in estimates of fleas (21.7±0.07%) and lice (0.64±0.46%).

However, the relative abundances of ectoparasites were reflected in our estimates. We found a significant relationship between the total estimated mean abundance of ectoparasites and the total ectoparasite abundance of sacrificed squirrels (Spearman's correlation; $\rho = 0.666$; P = 0.007; n = 15; Figure 1). The estimated mean abundance of fleas (2.5; CI = 1.13-4.87) was correlated with total flea abundance (Spearman's correlation: $\rho = 0.702$; P = 0.0035; n = 15) and the estimated lice abundance was also correlated with total lice abundance ($\rho = 0.504$; P = 0.055; n = 15).

Table 1: *Estimated and total prevalence, intensity, and abundance results of ectoparasites on Cape ground squirrels (n=15).*

n	Prevalence total (%)	Prevalence estimated	Mean Intensity total	Mean Intensity estimated	Median Intensity total	Median Intensity estimated	Mean Abundance total	Mean Abundance estimated
Total	100.0	60.0	39.8	4.8	34.0	3.0	39.8	2.9
15	(78.2-100.0)	(32.3-83.7)	(24.6-61.0)	(2.7-8.9)	(8.0-68.0)*	(1.0-7.0)**	(23.5-60.9)	(1.4-5.9)
Fleas	93.3	60.0	12.6	4.1	6.5	3.0	11.7	2.5
15	(68.1-99.8)	(32.3-83.7)	(7.4-21.2)	(2.2-7.2)	(2.0-16.0)	(1.0-7.0)	(6.5-19.4)	(1.1-4.9)
Lice	100.0	20.0	27.4	1.3	25.0	1.00	27.4	0.3
15	(78.2-100.0)	(4.3-48.1)	(14.5-47.0)	(1.0-1.7)	(1.0-31.0)***		(14.7-48.2)	(0.0-0.6)

Parentheses indicate 95% confidence intervals unless otherwise specified by an asterisk (*96.5-96.9% CI; **97.0-97.1%CI; ***98.2% CI).

4 Discussion

Our estimates via the combing method correlated positively with the overall parasite amounts collected post-mortem and may have accurately predicted the total counts up to a point, but with an associated error rate. As well, the abundance of fleas and lice between estimated and total measurements were significantly correlated. In this study, we were only concerned with parasite species visible to the naked eye as in field estimation of parasites, equipment is not always available. We do acknowledge the limitations of the combing method, as it does not accurately detect very small ectoparasites, and data collection requiring finer ectoparasite counts or a smaller error rate should avoid the combing method.

As well, previous ectoparasite collection from *Xerus inauris* was performed by combing the individuals from the shoulders to the base of the tail in the two lateral and one medial planes of the back[19]. Thus, we used the modified Hillegass et al.[6] combing technique as previously stated with special attention to the groin and inner thigh of the individuals in attempts to produce the most accurate estimation of the parasites. Heckenberg et al.[20], using a similar combing technique (without our direct observation of the groin and thigh) on dogs (*Canis lupus familiaris*), recovered between 67 and 75% of the total flea burden.

In comparison, Eads et al.[21] used a combing technique on anesthetized prairie dogs (*Cynomys ludovicianus*). They combed for three fifteen second intervals including combing the dorsal, lateral and ventral surfaces without using direct observation and detected a 5.4% error in prevalence if only one 15 second comb was used. There are other studies that also suggest an accurate estimation method of lice may be one that involves anesthetizing the animals and using combing with visual estimation or the use of a fumigant insecticide powder[22, 13, 16]. However, while these methods may allow for more thorough checking of the animal, they also require more handling and stress to the subject as well as the risks and prolonged recovery times of anesthetic use (including numerous undesirable side effects such as regurgitation, aspiration and hypothermia[23]). Our results suggest that combing and visual inspection result in good estimates of relative parasite prevalence, intensity and abundance for both fleas and lice without a need to use any anesthetic.

One of the reasons why tick abundance and prevalence is low in this study could be the seasonal pattern of ticks occurring on this mammal, as the study was done only in winter. Also most of the tick's lifecycle is not on the host, thus those mammals with a larger body mass (more surface area) and those that travel long distances (more chances to become infected with ticks), are more likely to be parasitized by ticks. Ticks are also most often found on vegetation and the larger the host species, the more likely it is to come into contact with the surrounding vegetation and become infested by ticks[24]. *Xerus inauris* is a small species, which may relate to the low density of ticks found on their bodies.

We hope future studies will focus on estimation of different species of ectoparasites to see if there is a pattern amongst or between species, and if there is a correlation amongst parasites that are too small to be estimated without using specialized equipment. In addition, it would be interesting to repeat this study in different seasons to account for varying parasite patterns throughout the year. To conclude, our data suggest that the combing method was a reliable method to estimate fleas and lice in Cape ground squirrels.

5 Acknowledgements

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Research Article

Crushed Glass as a Constructed Wetland Substrate: Invertebrate Community Responses to Simulated Wastewater Inputs

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Abstract

Constructed wetlands (CWs) are an increasingly common polishing step prior to the release of municipal wastewater treatment facility effluents, especially in smaller and more isolated communities. It is hypothesized that recycled crushed glass could be a suitable alternative matrix for CW construction. In comparison to commonly used substrates, recycled crushed glass has several advantages: it is less expensive, more environmentally friendly, and it can be transformed into various sizes to meet specific design requirements. The material is inert, transparent, has large pore spaces, and significant surface area. Components that impair receiving water quality (e.g., nutrients, pharmaceuticals, and pathogenic bacteria) could be reduced by enhancing light penetration, macrophytes for uptake and assimilation, surface area for microbes, and overall retention time. To explore the ability of crushed glass to support relevant biological communities, twelve outdoor mesocosms were established with and without emergent plants, and crushed glass was contrasted with a typical gravel base in triplicate. Specifically, we examined the response of the zooplankton community. After these systems were acclimated, they were treated with a single pulse of synthetic wastewater (e.g., nutrients, pharmaceuticals, and salts). Mesocosms exposed to the synthetic effluent developed a significantly ($p<0.05$) different invertebrate community response in total abundance when compared to the unexposed control treatment. There were no significant ($p>0.05$) differences among the mesocosms with crushed glass as a substrate (including controls) for all diversity indices, indicating that the addition of synthetic effluent and macrophytes had no significant impacts on the invertebrate community structure. Overall, recycled crushed glass was determined to be suitable matrix for zooplankton communities, with water quality and effective treatments being maintained relative to gravel systems. Though the treatments with a gravel substrate had greater total invertebrate abundance, it was found that the gravel treatments were significantly ($p<0.05$) less diverse (Shannon's index) and had less evenness than all other treatments with glass substrates. We recommend that future studies should explore the effectiveness of recycled crushed glass in CWs on a larger scale, as these results suggest that recycled crushed glass could be a viable surrogate for gravel in subsurface filtration processes.

Keywords: Constructed Wetlands, Pharmaceuticals, Alternative Substrate, Zooplankton, Substrate

1 Introduction

Constructed wetlands (CWs) are an increasingly common polishing step prior to the release of municipal effluents, especially in small communities. Constructed wetlands can be a cost-effective treatment option for the removal of contaminants and excess nutrients from both treated and untreated wastewater effluents[1,2]. The systems make use of aerobic conditions, aquatic plants, and extended hydraulic residence times to promote degradation of pharmaceuticals and related contaminants[3]. Organic contaminants, such as pharmaceuticals and personal care products (PPCPs), are often not fully degraded or absorbed in the bodies of human users[4]. Consequently, these compounds are present in municipal wastewater facilities and surrounding surface waters[5]. PPCPs incorporate a wide variety of different groups, such as hormones, antibiotics, disinfectants, synthetic fragrances, and preservatives. Discharge from wastewater facilities is the primary source of PPCPs in surface waters, as the removal of PPCPs and their metabolites by these systems is often incomplete, resulting in a continuous discharge that can make these contaminants "pseudo-persistent"[6,7,8]. These low concentrations of PPCPs are unlikely to pose an acute risk to aquatic organisms; however, there is potential risk for chronic toxicity in non-target organisms downstream of effluent releases[9,10,11].

Constructed wetlands have been used as secondary or

tertiary steps for wastewater treatment. In these systems, macrophytes have been utilized in several designs for the attenuation of PPCPs to improve overall removal efficiency[12]. Multiple plant species have been incorporated into these systems, including emergent, submergent, and free-floating plants. The most common species are *Phragmites australis, Typha spp., Typha angustifolia,* and *Typha latifolia*[13]. The role of macrophytes in constructed wetlands is typically to stabilize the substrate surface, uptake nutrients and contaminants, prevent channeled flow, insulate against freezing via litter production, and to shield algae from solar radiation[14]. Still, the value of their role in PPCP removal is unclear[15].

Constructed wetlands provide habitats for micro- and meso-fauna, and promote zooplankton grazing of remaining algal solids[16]. Zooplankton play a significant role in aquatic ecosystems, as they drive nutrient cycles via the consumption of primary producers (e.g., phytoplankton), and function as prey for planktivorous fish. Additionally, their community dynamics of growth, mortality, diversity, and distribution, structure the ecosystem through trophic interactions. Their intermediate trophic position in aquatic food webs makes them susceptible to bottom-up as well as top-down trophic cascades, as their biomass has been shown to increase with nutrient enrichment[17]. The most common zooplankton species in freshwater ecosystems are copepods, cladocerans, and rotifers[18]. They are highly sensitive to abiotic factors, such as temperature, dissolved oxygen, pH, salinity, turbidity, heavy metals, and contaminants (e.g., pesticides[18]).

Zooplankton exhibit a diverse range of life-history patterns, rates of reproduction, and life cycles. Furthermore, studies, such as the one conducted by Shurin et al.[19], have used the species richness of zooplankton to test their association with environmental variability. Therefore, zooplankton are effective indicators of aquatic ecosystem health. As a result, similar studies, such as the one conducted by Lobson et al.[20], have utilized the response of zooplankton communities in mesocosms to address indirect effects of a contaminant. Mesocosms have been commonly used to address concerns of non-target effects, as key variables can be manipulated to better observe both direct and indirect effects of a stressor on a lentic system[21].

Various substrate materials have been used in CWs, with the most popular being gravel, sand, and light-expanded clay aggregate[13, 22]. Dordio and Carvalho[22] found that light-expanded clay aggregate is a suitable substrate for agricultural wastewater treatment, as ionic contaminants are primarily adsorbed to substrates via electrostatic interactions. In gravel-bed CWs, wastewaters are treated via subsurface-flow through shallow channels, where the gravel provides surfaces for sorption and biofilm growth, physical support for macrophyte growth, and promotes the settling and filtration of suspended solids[23]. Recycled crushed glass represents a potential alternative substrate, but there is currently a lack of knowledge surrounding the performance of recycled crushed glass as a surrogate substrate material in CWs. There is an abundance of recycled crushed glass available in Canada, due to the immense quantities produced annually.

In comparison to the common silica sand substrate, crushed glass has several advantages: it is less expensive, more environmentally friendly (since it is a recycled material), and it can be transformed into various sizes to meet specific design requirements[24]. The material is inert, with significant surface area, and has large pore spaces. This means no risk of chemical contamination, a suitable matrix for microbial growth, and a large aerobic zone and support for root development of aquatic plants (e.g., cattail). This research is part of a larger study exploring the effectiveness of crushed glass as a substrate in CWs. Recycling of glass is at an impasse.

Few options exist at the moment for how this material can be made economically viable. It was hypothesized that recycled crushed glass could be a suitable matrix for constructed wetlands. The ability of recycled crushed glass to provide the same, if not better, nutrient and contamination removal as gravel will be examined. The objective of this study was to characterize the mesocosm zooplankton communities to determine the suitability of a glass substrate to support natural populations. These data can serve as the basis for further development of cost-effective and environmentally friendly wastewater treatment facilities based on recycled glass.

2 Methods

2.1 Experimental Design

This study occurred at the Prairie Wetland Research Facility (PWRF) at the University of Manitoba, Winnipeg, MB (49°48'35.9"N, 97°07'33.0"W). An array of twelve individual wetland mesocosms were installed at the PWRF. Each mesocosm consisted of a flat-bottomed, circular, low-density polyethylene tank (2.7m in diameter × 0.72m in height; 3.49m^3 in volume), with no outflows or inflows. The two substrate materials assessed were recycled crushed glass and gravel. One of four treatments were randomly assigned, in triplicate, to each mesocosm (described in detail in Figure 1). The addition of synthetic wastewater and selected pharmaceuticals occurred to all treatments (excluding control treatments) in a single pulse application on August 14th, 2018.

2.2 Preparation of Mesocosms

Each mesocosm received approximately a 30-cm layer of recycled crushed glass or gravel. Gravel with a diameter ranging from 1.5-2.5cm was added to match the average approximate size of the crushed glass material. Tap water from the City of Winnipeg was used to fill the tanks to a volume of approximately 2400L. Floating debris from the recycled crushed glass (e.g., plastic caps, labels, corks, etc.) was removed from the tanks upon filling. Macrophytes (*Typha spp.*) were collected from Oak Hammock Marsh, MB (50°11′15″N, 97°7′30″W) on July 19th, 2018. Macrophytes were planted in the Gravel and Plant-Glass treatments, at a density of 5-10 plants per square metre, for a total of 25 plants per tank. The macrophytes were acclimated in the system for 26 days prior to the start of the experiment.

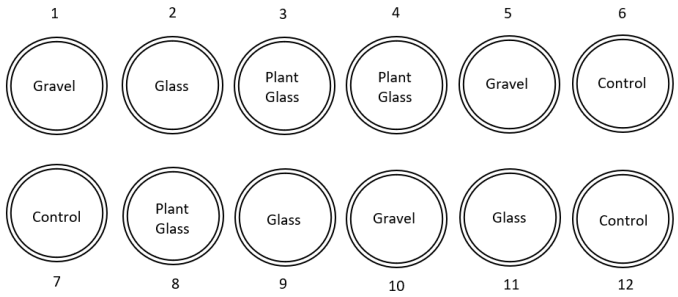

Figure 1: *Layout of randomly assigned treatments in twelve mesocosms. The treatments consist of: Control (crushed glass as substrate, unplanted), Gravel (gravel as substrate, planted with* Typha spp.*, addition of pharmaceuticals and synthetic wastewater), Plant Glass (crushed glass as a substrate, planted with* Typha spp.*, addition of pharmaceuticals and synthetic wastewater), and Glass (crushed glass as substrate, unplanted, addition of pharmaceuticals and synthetic wastewater).*

Zooplankton and benthic invertebrates were also collected from Oak Hammock Marsh using drag nets and kick nets near shore (35-μm and 73-μm mesh). Invertebrates were collected and introduced into the system at 26 and 18 days prior to the start of the experiment (July 19th and July 27th, 2018, respectively). Water containing collected organisms was added to each tank in equal volumes, and the mesocosms remained uncovered to allow for natural aerial colonization by insects throughout the duration of the experiment. Amphibians and fish were not included in the mesocosms, as a result of potential confounding effects on invertebrate-specific assessments.

2.3 Water Quality Monitoring

General water quality parameters – including dissolved oxygen (DO), chlorophyll-a, pH, temperature, oxidation-reduction potential (ORP), and conductivity – were measured every weekday morning, and once a week in the afternoon, using a YSI 6600 V2 Sonde. The concentration of DO provides an indication of the consumption and production rates of organic matter in the systems. Primary production by phytoplankton can be represented by chlorophyll-a concentrations, as chlorophyll-a is an indicator for phytoplankton biomass[25]. The YSI measurements began 29 days prior to the start of the experiment (July 16th, 2018), and continued for 57 days thereafter (October 10th, 2018). Depths were measured bi-weekly in five different locations for each tank, and then averaged to account for evaporation and to monitor the water volume. Photosynthetically active radiation (PAR) was measured at mid-day once a week. A total of two litres of synthetic wastewater and the mixture of pharmaceuticals (concentrations ranging from 5-10μg/L) were added to the corresponding treatments after the acclimation period, marking the start of the experiment (August 14th, 2018).

2.4 Mesocosm Treatment

Synthetic wastewater was added to the treated mesocosms (excluding controls). The synthetic wastewater contains (per litre): 32g peptone, 19g Lab Lemco powder meat extract, 6.7g $(NH_4)_2SO_4$, 3g urea, 3g yeast extract, 2.9g K_2HPO_4, 2.3g KH_2PO_4, 0.27g $CaCl_2{\cdot}2H_2O$, and 0.2g $MgSO_4{\cdot}2H_2O$. One litre of secondary wastewater from Dunnottar, MB (50°27'16.9"N, 96°57'06.5"W), was added to the mesocosms to provide established microorganism colonies. Pharmaceuticals were selected based on their frequency of detection in common wastewater treatment facilities, the low likelihood of acutely affecting aquatic organisms, and their persistence in the environment. The selected pharmaceuticals included atenolol ({β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).

2.5 Zooplankton Sampling Protocol

Zooplankton samples were collected from each mesocosm using passive traps as to not disturb the system; traps were deployed for 24 hour periods. The passive trap consisted of a clear 1-L Mason glass jar with a 243-mL Nalgene polypropylene powder funnel attached via rubber bands and s-hooks (adapted from Murkin et al.[26], Sibley et al.[27]). Each trap was filled with control mesocosm water prior to deployment into the treated mesocosms. Three passive traps were deployed on the substrate surface in an array for each tank. Replicate traps were integrated into a single sample from each mesocosm, and stored in 120-mL French square bottles,

with 5% sugar formalin and distilled water to preserve the samples for further analysis. Samples were taken on Days -7, -1, 0, 1, 7, 14, 21, 28, 42, and 56, for a total of 120 samples.

2.6 Zooplankton Enumeration and Identification

Samples were selected randomly (using a random number generator) for enumeration to avoid a potential counting bias. Prior to enumeration, zooplankton samples were adjusted by concentrating the sample volume of 120mL to a consistent volume of 50mL. To ensure an even distribution of organisms, samples were inverted several times and mixed thoroughly prior to subsampling. Subsamples (5mL) were transferred into a Bogorov zooplankton counting chamber via an air displacement pipette. Subsamples were analyzed using a dissecting microscope at four to five times magnification. A minimum of one 5mL subsample (10% of the total sample volume) was enumerated entirely for each mesocosm sample. For taxa that did not have an abundance of at least 40 individuals in the first subsample, an additional 5mL subsample was enumerated (adapted from USEPA (2016)). The key of Balcer et al.[28] was used to identify cladocerans to genus, copepods to order, ostracods to class, and rotifers to phylum.

2.7 Statistical Analysis

Diversity metrics, such as number of taxa, evenness, Shannon's diversity, and Simpson's diversity, were calculated along with total and individual taxon abundance for each mesocosm. A single factor ANOVA ($\alpha = 0.05$) was used to identify significant differences in Shannon's diversity index, Simpson's diversity index, number of taxa, and evenness among sampling days and treatments. If significant differences were present, a two-sample t-Test assuming unequal variances was used to identify significant differences between treatments. Principal response curve (PRC) analysis was conducted to compare the response of the zooplankton community between different treatments over the course of the study compared to the control mesocosms[29, 30]. PRC analysis was performed using the *vegan* package (Version 2.5-5) and *ggplot2* package in RStudio (Version 1.1.463)[31, 32, 33].

3 Results

3.1 Water Quality

Temporal trends of the measured water quality parameters can be found in the SI (Figures S1 to S4). Mean temperatures for the mesocosms displayed a general decline over time (Figure S1). Mean pH values remained relatively consistent throughout the duration of the study, except for a brief decline and rebound in all exposed treatments (excluding controls) following the addition of synthetic wastewater and pharmaceuticals on August 14th, 2018 (Figure S2). Similarly, mean DO concentration displayed a significant ($p<0.05$) decline and rebound in concentration in all exposed treatments (excluding controls) following the addition of synthetic wastewater and pharmaceuticals on August 14th, 2018 (Figure S3). The decline and rebound in DO was most pronounced in the Glass treatments (glass + effluent) (Figure S3). All treated mesocosms exhibited a brief increase in mean chlorophyll-a concentration following the addition of synthetic wastewater and pharmaceuticals relative to the controls (Figure S4). As with DO, the Glass treatments experienced the greatest increase in chlorophyll-a following the pulse exposure of synthetic effluent (Figure S4), and the Gravel treatments (gravel + effluent + plants) experienced the least increase in chlorophyll-a.

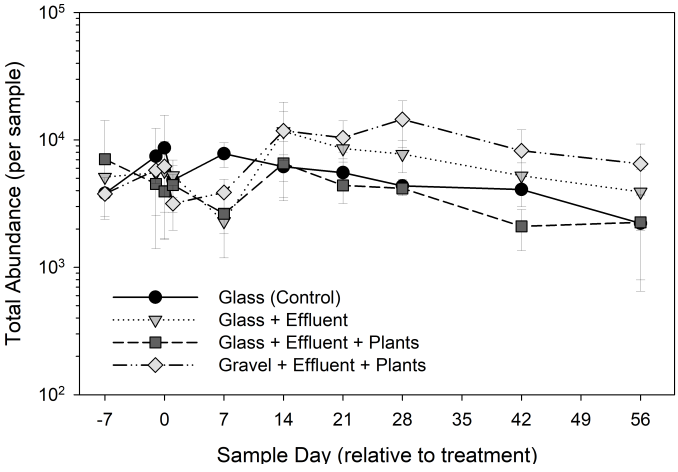

Figure 2: *Mean total invertebrate abundance following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). Error bars represent standard deviation.*

3.2 Invertebrate Abundance

Eleven zooplankton taxa were identified in the mesocosms (calanoid copepods, *Ceriodaphnia sp.*, chydorids, cyclopoid copepods, *Diaphanosoma sp.*, *Macrothrix sp.*, copepod nauplii, ostracods, rotifers, *Scapholeberis sp.* and *Simocephalus sp.* A total of two aquatic insect taxa were identified in mesocosms (*Chaoborus sp.* and Ephemeroptera larvae). The treatments with the greatest total abundance averaged across the three replicates for each treatment are in decreasing order as follows: Gravel (gravel substrate + effluent +

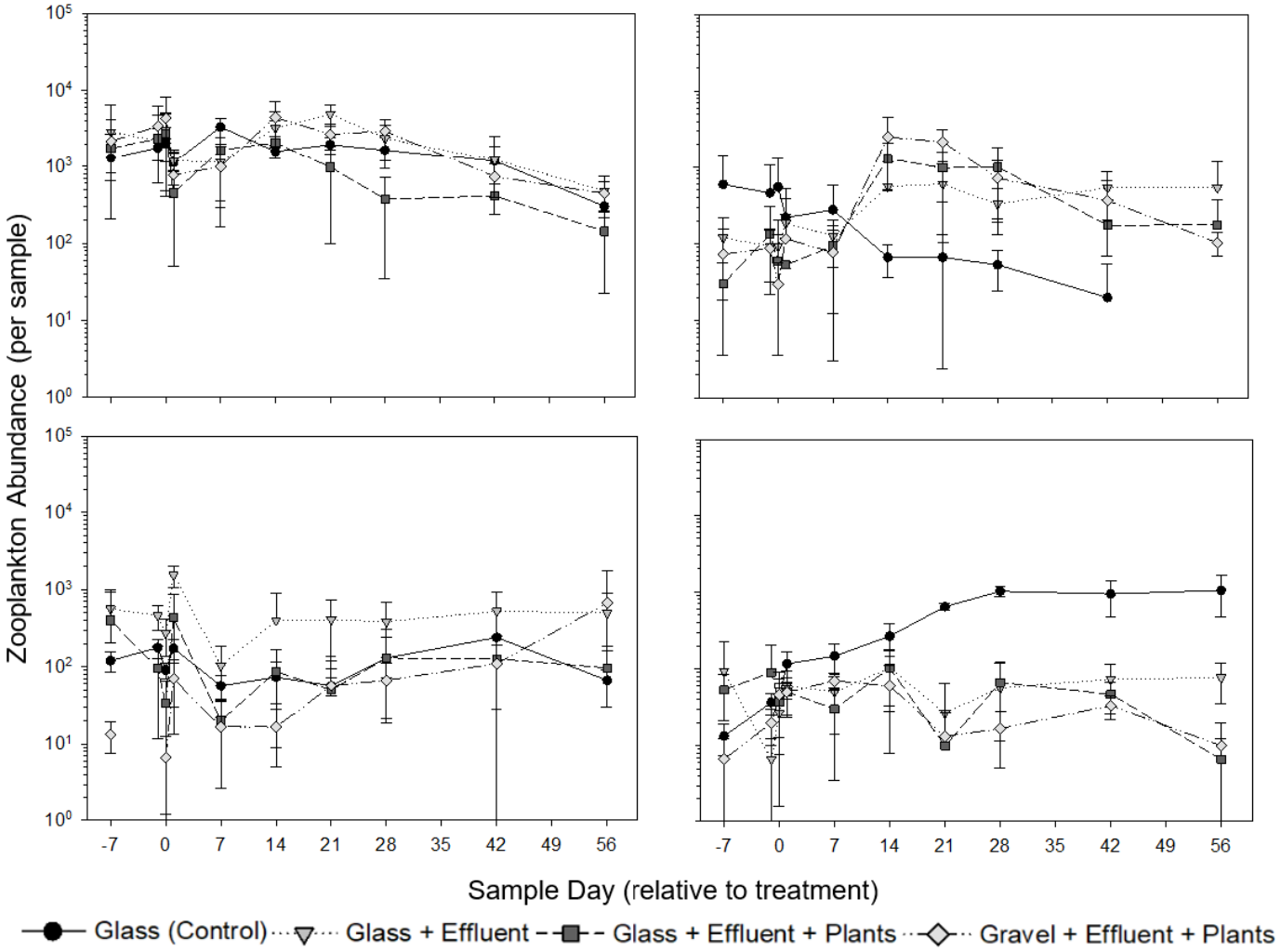

Figure 3: Ceriodaphnia sp. *(A),* Simocephalus sp. *(B), Chydorid (C), and Calanoid Copepod (D) abundances per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). Error bars represent standard deviation.*

plants), Glass (glass substrate + effluent), Control (glass substrate), and Plant Glass (glass substrate + effluent + plants) (Figure 2). The total invertebrate abundance declined for all treatments (including controls) on Day 1 relative to the pre-treatment abundance on Day -1, with only the exposed treatments (excluding controls) continuing to decline one week after the pulse exposure to synthetic effluent (Figure 2). The abundance in Control treatments declined from Day 7 through the remaining seven weeks of the study duration (Figure 2). All the exposed treatments experienced a rapid increase in total abundance from Day 7 to Day 14 (Figure 2) followed by a decline from Day 28 to Day 56 (Figure 2).

Of the thirteen invertebrate taxa that were identified, four distinct trends in abundance were observed. Abundant taxa, such as *Ceriodaphnia sp.*, experienced a decline in abundance for all treatments (including controls) on Day 1 (Figure 3A). The exposed treatments displayed a relatively rapid increase in *Ceriodaphnia sp.* abundance from Day 1 to Day 7 (Figure 3A). From Day 21 to Day 56, all exposed treatments exhibited a decline in *Ceriodaphnia sp.* abundance (Figure 3A). The abundance of *Ceriodaphnia sp.* in the Control treatments declined steadily from Day 7 through Day 56 (Figure 3A).

Less abundant taxa, such as chydorids, experienced an increase in abundance for all exposed treatments on Day 1, which then was followed by a steep decline on Day 7 (Figure 3C). The Glass treatments had the greatest abundance of chydorids and remained relatively consistent from Day 14 to Day 56 (Figure 3C). The Gravel treatments displayed an increase in chydorid abundance from Day 14 to Day 56

(Figure 3C). The chydorid abundance in the Control mesocosms remained relatively consistent throughout the duration of the study (Figure 3C). Taxa that experienced significantly ($p<0.05$) different abundances in the exposed treatments when compared to the unexposed control treatments include *Simocephalus sp.* and *calanoid copepods*.

The abundance of *Simocephalus sp.* increased rapidly on Day 14 for all exposed treatments and declined steadily until Day 56 (Figure 3B). The Control mesocosms experienced a steady decline in *Simocephalus sp.* abundance throughout the duration of the study (Figure 3B). The abundance of calanoid copepods increased steadily throughout the entire study duration in the Control treatments (Figure 3D). The exposed treatments experienced a steep decline on Day 21, and rebound on Day 28, with less of a rebound in the Gravel treatments (Figure 3D). Additionally, *Diaphanosoma sp.* experienced a unique response in abundance when compared to the other taxa (Figure 4).

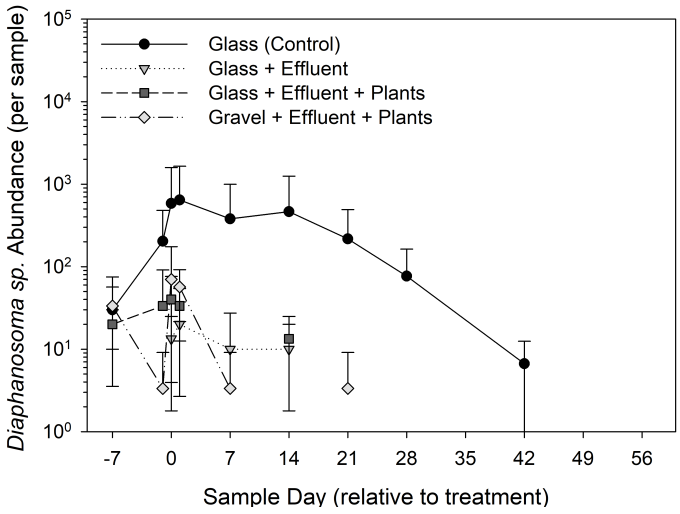

Figure 4: Diaphanosoma sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). Error bars represent standard deviation.*

The abundance of *Diaphanosoma sp.* was rare for all exposed treatments, with none being found after Day 21 (Figure 4). The exposed treatments experienced a decline in abundance from Day 0 to Day 7 (Figure 4). *Diaphanosoma sp.* was present in all Control treatments until Day 42, with a steady decline in abundance occurring from Day 1 through Day 42 (Figure 4).

The principal response curve (PRC) analysis revealed that 30.3% of the constrained variance is explained by the first PRC axis in Figure 5 relative to the control treatment. Conditional variance (i.e. time) accounted for 21.0% of the total variance, with the total constrained variance (i.e. treatment, interacting with time) accounting for an additional 34.8% of the total variance. Species scores were used in development of the PRC's community response (C_{dt}) from the study start date (Day -7, August 7th, 2018) to Day 56 (October 9th, 2018). Scores associated with the first axis explained 30.3% of the constrained variance in Figure 5. The permutation test for first constrained eigenvalue (first axis) resulted in an F value of 19.076 and a p value of 0.004.

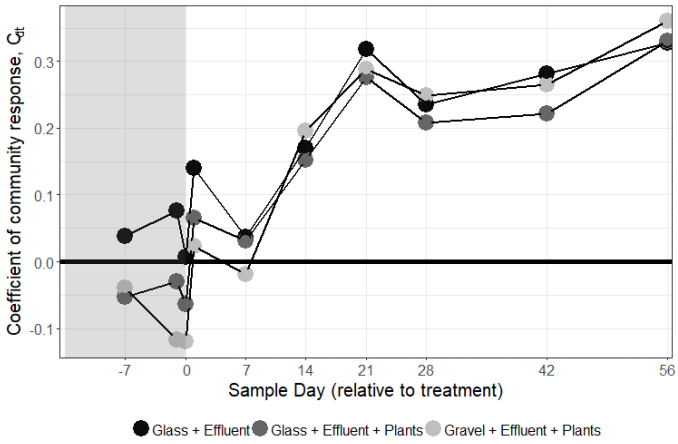

Figure 5: *Principal response curve (PRC) with species scores (b_k) showing the invertebrate community response (C_{dt}) following a single pulse exposure to synthetic effluent in outdoor mesocosms relative to the control treatment from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The black horizontal line (C_{dt} = 0) represents the control treatment and is used as the basis in determining response coefficients for each treatment relative to the control. The shaded region represents the pre-treatment response prior to the single pulse exposure on Day 0 (August 14th, 2018). 30.3% of the constrained variance is explained by the first axis, with conditional variance (i.e. time) accounting for 21.0% of the total variance, and the total constrained variance (i.e. treatment, interacting with time) accounts for an additional 34.8% of the total variance. Permutation test for first constrained eigenvalue (first axis) resulted in an F value of 19.076 and a p value of 0.004.*

A sustained difference in total zooplankton abundance in all treatments relative to the control is observed in the PRC. The PRC demonstrates that there is a significantly different ($p<0.05$) community response in the exposed treatments when compared to the controls (Figure 5), which can be shown in the contrasting abundance trends in Figures 3B and 3D. The main taxa driving the change observed include *Simocephalus sp.* and calanoid copepods; the former taxa decreased in abundance in the Control relative to the treatments, whereas calanoid copepod abundance increased in the Control only, as represented by opposite species scores (b_k) determined following redundancy analysis and PRC development. Additionally, *Diaphanosoma sp.* also increases

in the Control, distinguished by its high negative species score (Figures 4 and 5).

There was a degree of within-treatment variability among the triplicate mesocosms, as invertebrate abundances were observed to change with time among the same treatments. External factors such as weather, fauna grazing from preferred mesocosms, or unevenly distributed invertebrates, may be attributed to the within-treatment variability among the triplicate mesocosms. The relative abundance and diversity of zooplankton present in the mesocosms are comparable to a similar study conducted at the Prairie Wetland Research Facility[20]. While the diversity of the taxa found in the mesocosms was lower than what has been observed in the field, the abundances of zooplankton present are representative of communities that have been observed in prairie wetlands[34]. Similar zooplankton densities have been found in samples from two separate mesocosm studies, both of which used activity traps similar to the passive traps used in this study[35, 36].

3.3 Invertebrate Diversity

The sample day and treatment with the greatest zooplankton diversity was the Glass treatment on Day 56, with evenness, Shannon's index, and Simpson's index values of 0.840, 2.014, and 6.646, respectively (Table 1). The sample day and treatment with the lowest diversity was the Gravel treatment on Day 42, with evenness, Shannon's index, and Simpson's index values of 0.382, 0.916, and 1.623, respectively (Table 1). It was determined by a single factor ANOVA and two-sample *t*-test (assuming unequal variances for Shannon's index), that the Gravel treatment was significantly ($p<0.05$) less diverse than all other treatments, including controls. The mean values for Shannon's diversity index of the Control, Gravel, Plant Glass, and Glass treatments were 1.67, 1.26, 1.68, and 1.66, respectively. The mean values for Simpson's diversity index of the Control, Gravel, Plant Glass, and Glass treatments were 3.99, 2.79, 4.16, and 3.98, respectively.

In terms of Shannon's and Simpson's diversity, none of the treatments were significantly ($p>0.05$) different from each other. The mean number of taxa of the Control, Gravel, Plant Glass, and Glass treatments were 12.5, 12.1, 12.2, and 12.1, respectively. The mean values for evenness of the Control, Gravel, Plant Glass, and Glass treatments were 0.662, 0.505, 0.673, and 0.668, respectively, with the Gravel treatment having significantly ($p<0.05$) lesser evenness than all other treatments, including controls. Though the Plant Glass treatment is not significantly ($p>0.05$) different from the Glass treatment, the greater mean values for the diversity metrics of the Plant Glass treatment is an indication that the presence of macrophytes may increase the diversity in the system.

4 Discussion

To explore the ability of crushed glass to support relevant biological communities in CWs, twelve outdoor mesocosms were established with and without emergent plants, and crushed glass was contrasted with a typical gravel base in triplicate. After these systems were acclimated, they were treated with a single pulse of synthetic wastewater. Mesocosms exposed to the synthetic effluent developed a significantly ($p<0.05$) different zooplankton community response in total abundance when compared to the unexposed control treatment. Four distinct trends in abundance were observed, with these trends being expressed most prevalently in taxa such as *Ceriodaphnia sp.*, chydorids, *Simocephalus sp.*, *Diaphanosoma sp.* and calanoid copepods. There were no significant ($p>0.05$) differences among the mesocosms with crushed glass as a substrate (including controls) for all diversity indices, indicating that the addition of synthetic effluent and macrophytes had no significant impacts on the invertebrate community structure. Though the Plant Glass treatment is not significantly different from the Glass treatment, the greater mean values for the diversity metrics of the Plant Glass treatment provides an indication that the presence of macrophytes may increase the diversity in the system. Overall, recycled crushed glass was determined to be suitable matrix for zooplankton communities in the context of CWs, with water quality and effective treatments being maintained relative to gravel systems.

Zooplankton abundance was predicted to decline in all treatments over time, as zooplankton are sensitive to temperature changes[37], and a declining trend in temperature was found throughout the duration of the study. This temperature-sensitive decline in abundance was reflected most prevalently in the Control treatments. Mean pH values remained relatively consistent throughout the experiment in each mesocosm, so pH was not likely a factor in differences observed among mesocosms and over time. Trophic interactions likely lead to a temporary increase in zooplankton abundance following the increase of primary production (phytoplankton) one week after the pulse exposure to synthetic wastewater and selected pharmaceuticals[38]. This response can be found in abundant taxa, such as *Ceriodaphnia sp.*, which experienced declines and rebounded following the single pulse exposure.

Table 1: *Summary of diversity indices across four treatments on sample Days -7, 0, 7, 14, 42, and 56. The four treatments are Control (glass), Glass (glass and effluent), Plant Glass (glass, effluent, and plants), and Gravel (gravel, effluent, and plants).*

Day	Treatment	Number of Taxa	Evenness	Shannon's Index	Simpson's Index
-7	Control	13	0.703	1.804	4.651
-7	Glass	11	0.641	1.537*	2.878*
-7	Plant Glass	13	0.530	1.359*	2.670*
-7	Gravel	13	0.530	1.265*	2.507*
0	Control	13	0.572	1.468	2.929
0	Glass	13	0.553	1.419	2.810
0	Plant Glass	13	0.472	1.211*	1.980*
0	Gravel	12	0.410	1.019*	1.909*
7	Control	13	0.622	1.594	3.467
7	Glass	13	0.635	1.629	3.306
7	Plant Glass	11	0.551	1.322*	2.324*
7	Gravel	13	0.602	1.545	3.545
14	Control	13	0.730	1.872	5.060
14	Glass	13	0.642	1.647	3.556*
14	Plant Glass	13	0.765	1.961	5.481
14	Gravel	12	0.623	1.547*	3.938*
42	Control	12	0.671	1.667	4.588
42	Glass	12	0.744	1.849	5.312
42	Plant Glass	12	0.810	2.013*	6.237*
42	Gravel	11	0.382*	0.916*	1.623*
56	Control	11	0.595	1.426	3.109
56	Glass	11	0.840*	2.014*	6.646*
56	Plant Glass	12	0.666	1.656	3.500
56	Gravel	11	0.487	1.168*	2.206*

*Asterisks indicate significant difference in zooplankton diversity of treatment relative to control treatment at specific sampling day ($p<0.05$).

Inversely, less abundant taxa, such as chydorids, experienced an increase and then decline following the single pulse exposure. This inverse relationship is likely due to competitive release[39], where the less abundant taxa can fill the niches of the more abundant taxa, possibly as a result of reduced pressure for resources. The cause for these relationships is likely a bottom-up trophic cascade, with nutrient addition being the primary driver, as the concentrations of pharmaceuticals used are not likely to pose an acute risk of toxicity to the organisms present[9, 10, 11]. In a similar study examining the effects of pharmaceutical mixtures on aquatic communities in outdoor microcosms, the authors indicate that organic enrichment may be contributing to the found effects of increased abundance and decreased diversity of zooplankton in the highest concentration treatment, resulting in trophic interactions with phytoplankton[40]. *Simocephalus sp.* had the most positive species score, as the taxon decreased in abundance in the Control relative to the exposed treatments.

Inversely, calanoid copepods and *Diaphanosoma sp.* had the most negative species scores, as both taxa increased in abundance in the Control relative to the exposed treatments. These four trends may be attributed to certain taxa being more resilient than others under the observed conditions[41], which may additionally confound our ability to observe treatment-specific impacts.

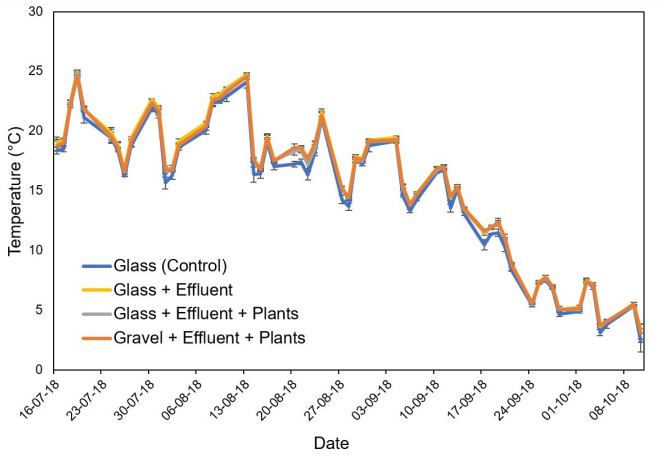

Figure S1. *Mean temperature (°C) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Temperature values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. Error bars represent standard deviation.*

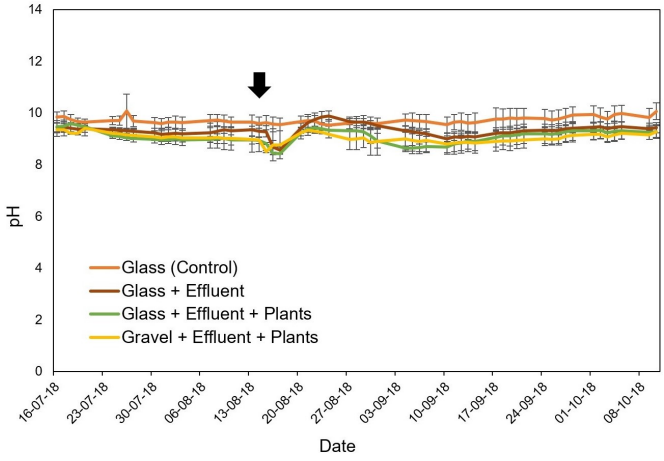

Figure S2. *Mean pH of treated mesocosms at the Prairie Wetland Research Facility (PWRF). pH values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls). Error bars represent standard deviation.*

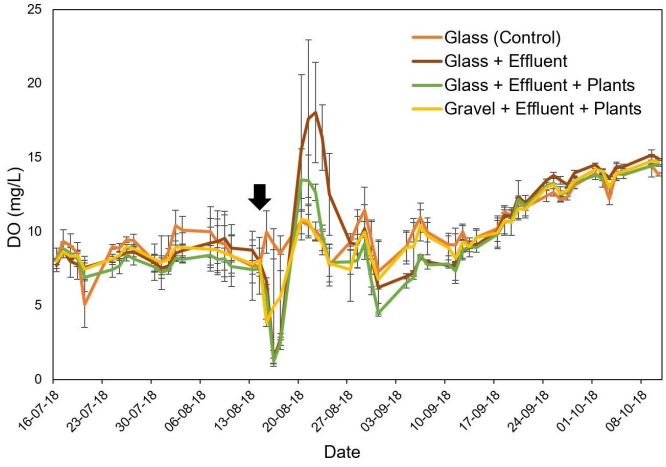

Figure S3. *Mean dissolved oxygen concentration (mg/L) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Dissolved oxygen values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. The arrow indicates when the synthetic wastwater was added to the exposed treatments (excluding controls). Error bars represent standard deviation.*

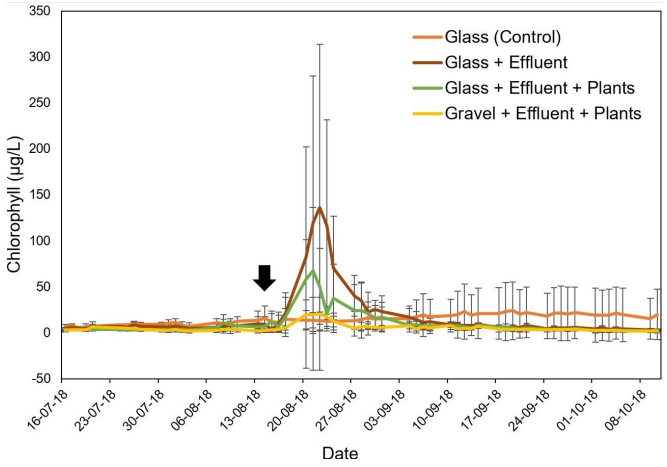

Figure S4. *Mean chlorophyll-a concentration (µg/L) of treated mesocosms at the Prairie Wetland Research Facility (PWRF). Chlorophyll values were averaged across replicates (n=3) for each of the four treatments (control, gravel, plant glass, and glass). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16th, 2018 to October 10th, 2018. The arrow indicates when the synthetic wastwater was added to the exposed treatments (excluding controls). Error bars represent standard deviation.*

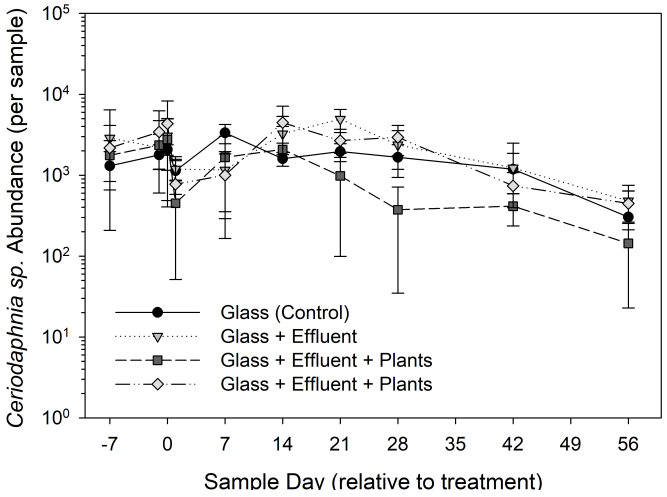

Figure S5. Ceriodaphnia sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

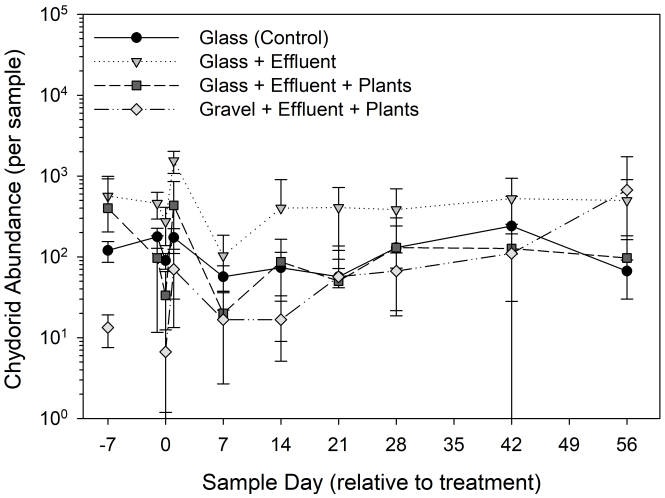

Figure S6. *Chydorid abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

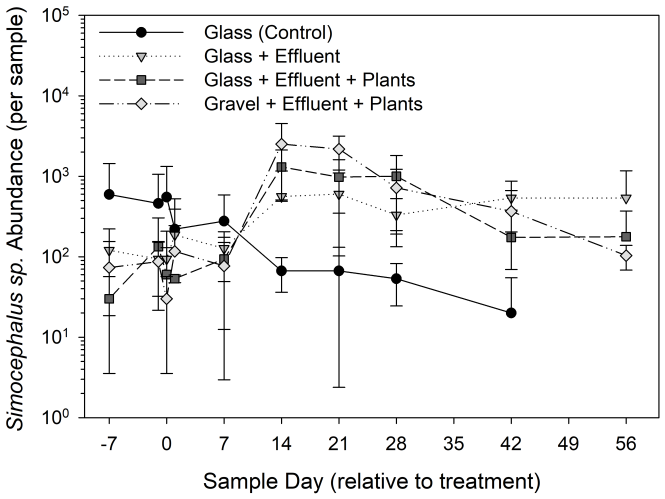

Figure S7. Simocephalus sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

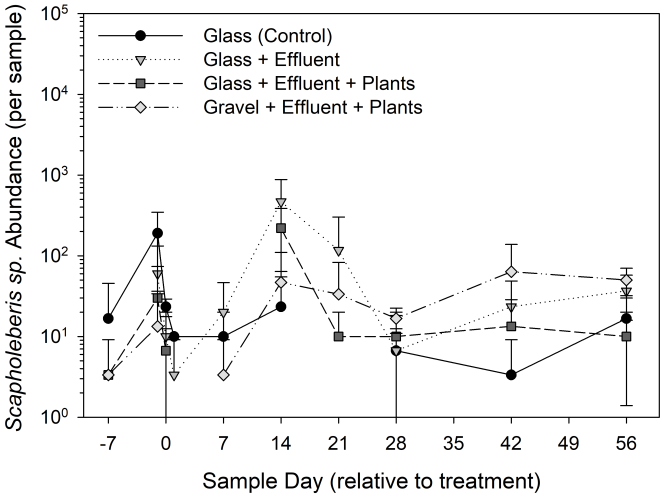

Figure S8. Scapholeberis sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

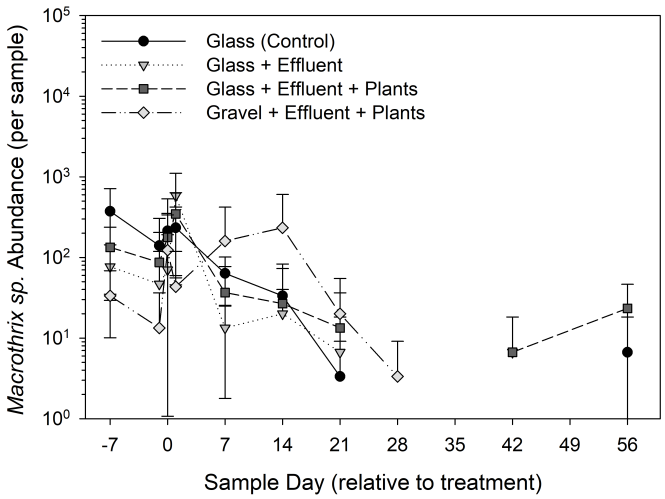

Figure S9. Macrothrix sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

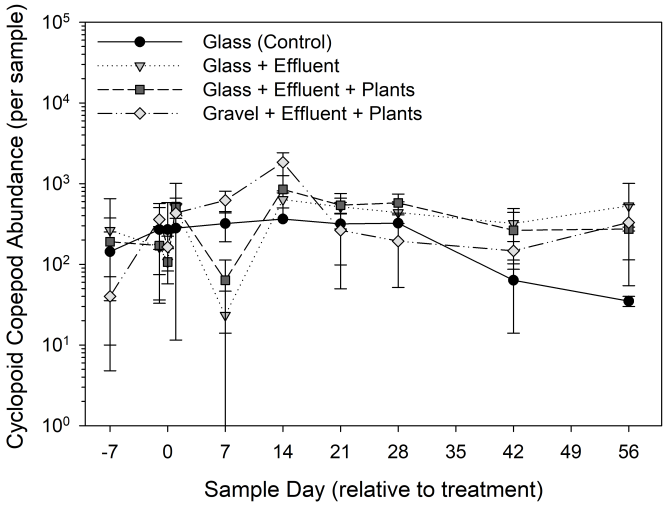

Figure S10. *Cyclopoid copepod abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

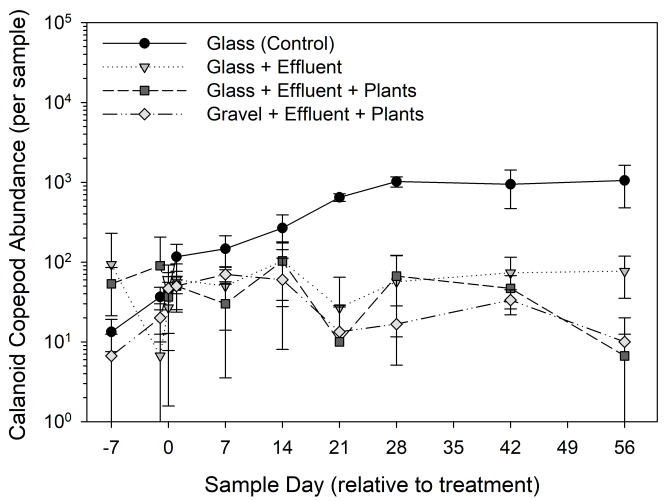

Figure S11. *Calanoid copepod abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

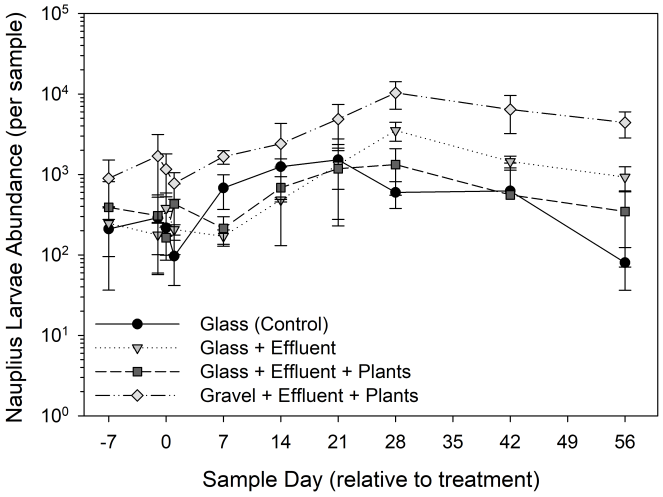

Figure S12. *Nauplius larvae abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

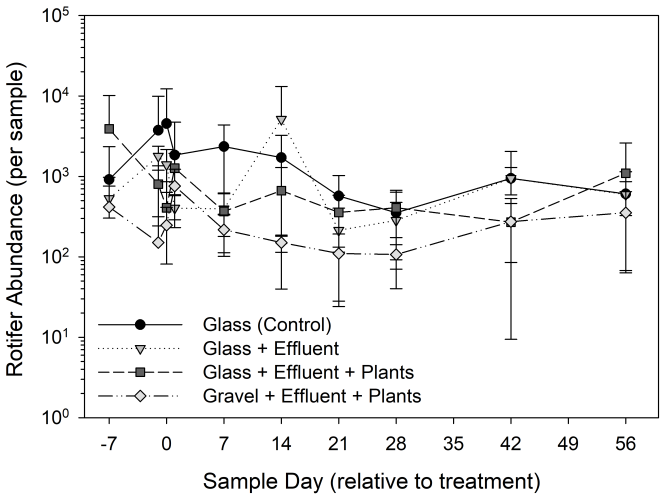

Figure S13. *Rotifer abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

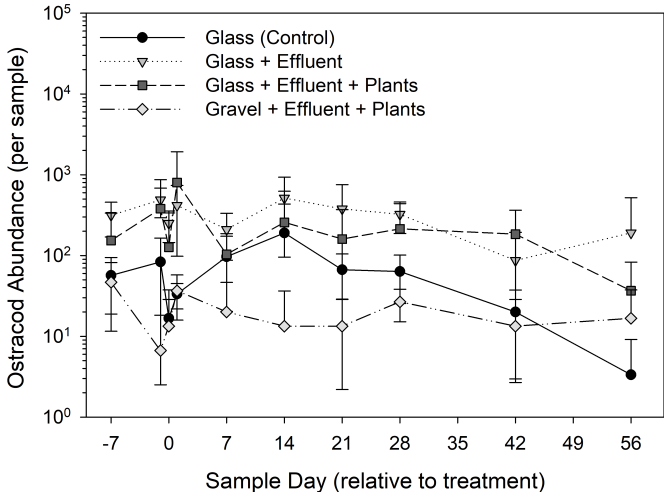

Figure S14. *Ostracod abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

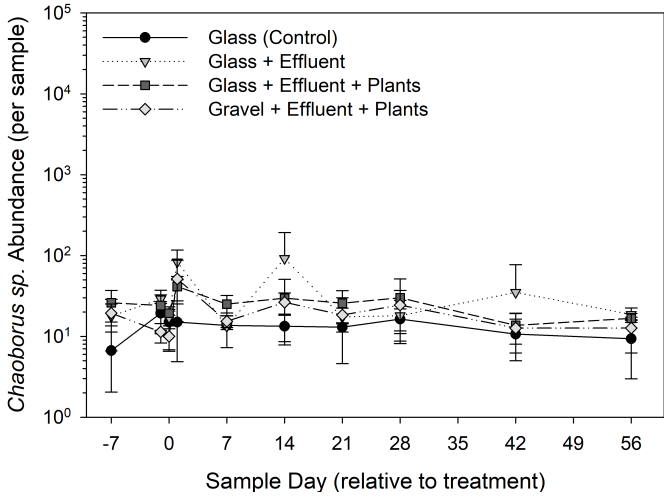

Figure S15. Chaoborus sp. *abundance per 50mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

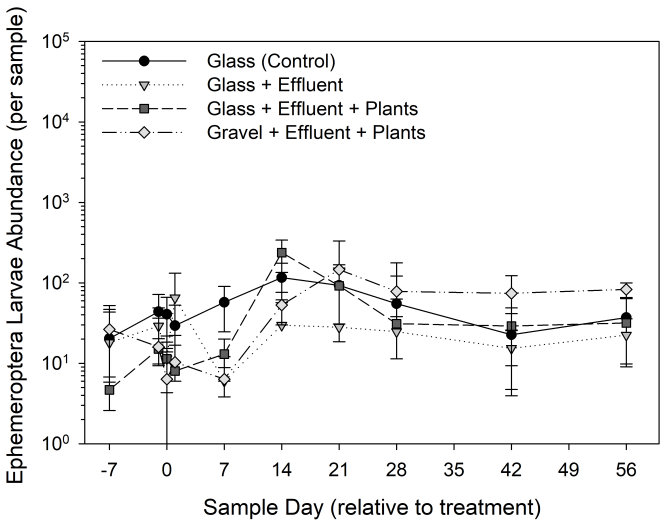

Figure S16. *Ephemeroptera larvae abundance per 50 mL sample following a single pulse exposure to synthetic effluent in outdoor mesocosms from Day -7 (August 7th, 2018) to Day 56 (October 9th, 2018). The synthetic effluent contains nitrogen, phosphorus, salts, proteinaceous material and selected pharmaceuticals. The selected pharmaceuticals include atenolol (β-blocker), carbamazepine (anticonvulsant), ketoprofen (anti-inflammatory), and sulfamethoxazole (sulfonamide antibiotic).* Typha spp. *was used in treatments with plants. Error bars represent standard deviation.*

Systems with high levels of diversity are more likely to be resilient to structural changes in the community, with functional responses such as energy flow, biomass production, decay processes, and nutrient cycling being maintained through redundant roles in the ecosystem[42]. Using Shannon's diversity index, it was determined that the Gravel treatment is significantly less diverse than the other treatments. Additionally, the Gravel treatment was determined to have significantly less evenness than all other treatments. However, the Gravel treatment was not significantly different from other treatments when using Simpson's diversity index. In terms of diversity indices, Simpson's index is more sensitive to abundant species when compared to Shannon's index, which is likely resulting in the found differences in statistical significance[43]. In contrast, the Plant Glass treatment had the greatest mean values for Shannon's diversity index, Simpson's diversity index, and evenness, thereby supporting the hypothesis that recycled crushed glass can provide a matrix for natural populations comparable to, or an improvement, relative to gravel substrate.

There was a degree of within-treatment variability among the triplicate mesocosms. This variability may be confounding our ability to observe actual impacts from the treatments, as zooplankton abundance was observed to change with time among the same treatments. This within-treatment variability may be attributed to external factors such as weather, fauna grazing from preferred mesocosms, or unevenly distributed invertebrates. The natural variability among these systems could be reduced by allowing for the macrophytes and invertebrate communities to establish themselves for a longer duration prior to the exposure period. Biomass was not estimated in this study; however, it is worth investigating in similar future studies, as biomass estimates would help to provide more information on toxicity and grazing patterns, allowing for a clearer and more concise conclusion. Zooplankton are reported as number of organisms per cubic metre[44], which is a challenge for passive traps, since flow rate and volume of water filtered are required for the calculation. As a result, the abundance results in this study were reported as abundance per sample for each treatment. Future similar studies should try to incorporate a flow-rate monitor into the experimental design.

5 Conclusion

In conclusion, recycled crushed glass is a suitable matrix for natural populations. There was no significant difference between the Control, Glass, and Plant Glass treatments for all diversity indices, indicating that the addition of synthetic effluent and macrophytes had no significant impacts on the invertebrate community structure. Though the effect of repeated pulsed exposures was not examined, it is unlikely that several repeated pulses, at much lower concentrations and environmentally relevant intervals between events would cause significant impacts on the zooplankton community dynamics when using recycled crushed glass as a substrate[20, 45]. Therefore, these results suggest that recycled crushed glass could be a viable surrogate for gravel in subsurface filtration processes, and further exploration is warranted.

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Review

Role of RNA-interference in Crop Pests and Disease Vector Control

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Abstract

Insect pests are a threat to meeting food demands of the ever-increasing human population. They are also the cause of many vector borne diseases in humans leading to countless deaths. Present insect pest control strategies including chemical pesticides, developing transgenic plants and organic certified chemical pesticides have numerous limitations in terms of their effectiveness and target specificity. However, genetic method that makes use of the sequence specificity of RNA interference (RNAi) has great potential in controlling pest insect populations. RNAi is a naturally occurring conserved process responsible for protection against viral pathogens. Efficiency of RNAi is variable among different pest insects. It is dependent on method of double stranded RNA (dsRNA) delivery, gene selection techniques, dsRNA expression and presence of off-target effects. Moreover, environmental risks involved in use of RNAi based insecticides in natural crop field scenario is debatable. Despite the challenges faced, RNAi mediated gene knockout of different pest insect genes has potential usefulness in controlling pest insect growth and survival.

1 Introduction & Background

Climate change is a universal phenomenon that is impacting all the organisms on this planet. Insect population growth is linked to increases in temperature due to global warming[1], leading to increases in the frequency and intensity of periodic insect outbreaks[2]. The most worrisome increase in insect population outbreaks is of the disease vectors and pest insect populations as they cause human suffering and destruction of crops. Every year more than 1.5 million human lives are lost to vector borne diseases[3], when the easiest method of prevention of such diseases is elimination or population control of vectors and pest insects.

Insect population control is key to preventing the spread of vector borne diseases like malaria, dengue, yellow fever, chikungunya and lymphatic filariasis that are targeted to be eradicated globally in the near future[4, 5]. To control insect populations, more efficient, target specific and cost-effective insecticides and delivery methods must be explored[6]. Presently, narrow and broad-spectrum insecticides both are heavily reliant on the use of chemical insecticides.

Two basic types of insecticides, narrow and broad-spectrum include wide varieties of chemical insecticides that act through inhibition of enzyme activities in pests[7]. Chemical insecticides are delivered mostly by aerial spraying, however very small percentage (0.003-0.0000001%) of insecticide actually reaches the target crop pests. Spraying, although instantly effective, has many side effects including high dosage administration and off-target effects like death of pollinators[8]. To deal with these side effects, genetic methods are being incorporated into insecticide development and delivery[9]. Genetic methods incorporate wide variety of techniques including chromosomal replacement, translocation formation, sterile insect technique and gene knockout using RNA-interference (RNAi)[10, 9, 11]. RNAi has been used for gene silencing to produce sterile insect males[10]. Gene silencing via RNAi is achieved by administering dsRNA (Table 1) using various introduction techniques to specific cells or the whole organism[10]. It would be pertinent to discuss various issues related to the conventional methods of pest insects and disease vector eradication vis-à-vis genetic method of RNAi. The objective of this literature review is to compare and contrast the effectiveness of last generation chemical insecticides with future generation insecticides using modern genetic technologies such as RNAi, to control crops and disease vector insect populations.

2 Review & Discussion

2.1 Techniques in crop pest control

Some of the earliest attempts at population control of crop pests included biological control by prey species such as use of bats as predator of moths in pecan orchards[12]. Biological control method was introduced as an alternative to chemical pesticides since chemical pesticides had negative impact in terms of biomagnification and off-target effects as was observed in honey bees with use of imidacloprid, a broad spectrum insecticide[13, 6]. Even though the biological method was potentially non-harmful with no off-target effects, researchers faced the difficulty of controlling prey-predator interactions as sometimes the presence of large herds of bats over the pecan orchard was enough to deter the prey moths[6]. The strong interaction between the populations of predator and prey limited this method, as when prey population declined so did the predator population because of resource separation[14, 15]. Additionally, the interspecific competition between introduced predator species and the native predator species for the same prey lead to

population imbalance between the two[16]. It appears that the biological method is limiting in delivering the desired results by not being appropriately controllable and is also not very cost effective.

In addition to biological methods, efforts were also made in development of organic certified pesticides to minimize harm to other off-target species and humans as consumers of crops. Although these organically certified pesticides were safer and much preferred over traditional synthetic pesticides because of absence of effects on non-targeted species, these new pesticides also had issues. The residual persistence of organic certified pesticides was higher than synthetic pesticides despite having low toxicity under laboratory conditions[17, 18, 19]. Thus, organic certified pesticides need further complementary bioassay treatment before being used in integrated pest management (IPM) programs dealing with wide variety of crops[18]. Therefore, despite seeming promising, organic certified pesticides were not found to be of significant advantage over the synthetic pesticides[18, 19].

Emergence of transgenic plant technology led to development of bio-insecticides using Bt-toxin (*Bacillus thurigiensis*) (Table 1), which showed promise in controlling plant as well as disease vector insect populations[20]. The development of transgenic crops that produce insecticidal Bt-toxin against specific insect pests lead to death of target pests upon ingestion of the plant[21]. This technique has been proposed to be useful for mosquito control as it can be administered via feeding through water to the targeted mosquito larvae species[22]. However, Bt-toxin administration in water bodies may impact other aquatic organisms that share the same water body with the target mosquito species[22].

Hence further investigation of off-target effects is required in administration of Bt-toxin via feeding through water. Another common method of administration of Bt-toxin is through the development of transgenic crops that selectively express Bt-toxin in their non-edible parts. The selective expression prevents pest ingestion as observed in crops like soybean, corn and cotton that are affected by defoliating pests including cotton bollworm[23, 24, 21]. Transgenic soybean plants specifically express Bt-toxin in their leaves and the pests are killed by gut perforation[23]. Transgenic plants produced by this method show Bt-toxicity trait retention and generational transfer from the parent plant to offspring plant making bio-insecticides commercially viable[13]. However, evolution of Bt-resistance in pest insects is an issue that has been observed by several different studies[13, 20, 24, 21].

2.2 RNAi in Agriculture Pest Control

Existing pest control strategies have limitations as discussed above. The techniques are not very successful in limiting pest insect populations. Sequence specific gene silencing via RNAi holds great promise for effective agricultural pest management[25]. RNAi mediated gene silencing of genes involved in various key physiological processes of pest insect was found to be detrimental to the growth, development into fertile adult and overall survival of the plant[26]. RNAi has an edge over bio-insecticide technology because of its highly conservative nature. For its activation and function, sequence specific nucleotide base pairing complementarity is required[27]. This implies that there will be minimum off-target effects. Hence, plant mediated RNAi is a powerful weapon in the fight against agriculture insect pests.

One example of efficiency of dsRNA in controlling plant pests is the use of RNAi in development of bollworm resistant cotton plants. When RNAi specific to critical bollworm genes was introduced, cotton yields increased significantly[28, 29]. Cotton plants expressing dsRNA of a reductase gene HMGR (Table 1) showed regression in the growth and development of cotton bollworms, however, it was unclear if expression of dsHMGR affected the number and time of bollworm pupation since most of the tested larvae died before pupation[27]. Therefore, further analysis of role of dsHMGR expression in cotton bollworm is required to explore the pest insect developmental stage that is targeted by RNAi of HMGR gene in transgenic cotton plants.

Efficiency of RNAi is variable among different pest insects. It is dependent on many factors, including the method of double stranded RNA (dsRNA) delivery, the dose that the insect acquires, the choice of the gene target (as not all gene knockdowns will have lethal effects), and the barriers within the insects, such as gut nucleases or reduced uptake mechanisms[30, 31]. One method that shows some promise in terms of increasing efficiency is the use of microorganisms to not only produce the dsRNA, but to deliver it to the feeding insects. Transgenic bacteria[32] and yeast[33] have been produced that express dsRNAs targeting mosquito gene transcripts, and as larval mosquitoes will readily consume these microorganisms in their diet, effective RNAi has been achieved in these insects. It is not yet clear if the microorganism-mediated delivery system is providing protection of the dsRNA from gut nucleases, or if it is providing higher doses of dsRNA to the gut cells, through different uptake mechanisms[34]. Nevertheless, this delivery system is worth further exploration for mosquito control applications.

Off-target effects observed in pest insecticides are undesirable have major concern. To address this issue, dsRNAs was designed to be delivered orally using sequence specificity of RNAi to selectively kill target species. The study also looked at a wide variety of pests including fruit flies *Drosophila melanogaster*, beetles *Tribolium castaneum*, aphids *Acyrthosiphon pisum* and hornworms *Manduca sexta*. These pest insects were selectively killed when fed with species specific dsRNA targeting vATPase transcripts[35].

Moreover, the study also demonstrated the selection ability of RNAi by use of two closely related fruit flies which were both given dsRNA specific for □-tubulin gene. Only those fruit flies that possessed the gene were affected in terms of their development whereas the others showed normal growth and became normal adults[35]. These results show that RNAi is not only specific in attack but can also be used in wide variety of insects.

2.3 Risk Management of RNAi

Environmental risk assessment (ERA, Table 1) involves producing an analysis plan describing relevant exposure scenarios and their potential consequences[36]. It is important to consider risk assessment before field release of any transgenic organisms. For RNAi, many aspects of ERA are similar to those of other genetically modified crops and pesticides[37]. However, difference in

mode of action of dsRNAs make RNAi and other insecticidal technologies very different.

For wide range of species subjected to RNAi, dsRNAs have off-target binding elsewhere in a nontarget species' genome making prediction of toxic effects and designing maximum-hazard dose assays challenging[37]. There is also an issue of safety concerning open field use of RNAi. ERA of RNAi is a necessary step towards widespread its practical utilization in plant pest control. Crop plants are host and food source of herbivorous insects, have tons of biomass, can accumulate a large amount of dsRNAs to provoke the RNAi response and dsRNAs can be continuously produced under varying environmental conditions[38]. Therefore, looking into the importance of crop plants, the challenge of unpredictable ERA of RNAi should not be discouraging in investing further into different ways of RNAi expression in plants.

2.4 RNAi in medicinal disease vector control

Controlling medicinally important disease vector pests using RNAi has not attracted many proponents due to the unique challenges of delivering dsRNA to these pests. As a result, chemical pesticides are still used extensively to control disease vector pests causing large scale harm to pollinator species through the common use of aerial spraying as the delivery method[18, 39].

The basic components of the RNAi machinery are found in all eukaryotes to protect them from the potentially harmful long viral dsRNA[37]. Therefore, the use of RNAi is widely exploited as a reverse genetics tool to assess gene functions in a broad range of species. Application of RNAi in most species under study is only limited by how easily the dsRNA can be delivered to the target cells[39]. The importance of any delivery method of RNAi is in reducing the loss of model insects during the delivery procedure to create loss of function mutants. Ideal pest control methods have some, if not all, of the following characteristics: species specificity, absence of side effects on crops and no or negligible environmental pollution[38]. There are different types of RNAi related delivery methods that show such potential.

Chitosan is an inexpensive, non-toxic and biodegradable polymer. It was found that chitosan-interfering RNA nanoparticles derived from chitosan, when introduced in Aedes aegypti via feeding, were successful in gene knockout[40]. These findings are of great importance as chitosan has potential for use in natural crop fields.

Microinjections are common mode of delivery of dsRNA to whole organisms like adult mosquito larvae[22]. There are two types of micro injections widely used for delivery of dsRNA, including the more common haemocoel injections and the direct injections. Direct injections are preferred over haemocoel injections as they can be administered during any stage of insect development, whereas the use of haemocoel injections is a laborious delivery technique and many insects die during the procedure[39]. Overall, currently both methods are in use depending upon the chosen model species under study.

Ever since the discovery of resistance of dsRNA to gut exonucleases, feeding has become the most favoured form of delivery as it is non-invasive and causes no physical harm to the model insect. dsRNA containing bacteria fed Caenorhabditis elegans had RNAi interference in broad region of their body. It was due to the spreading effect involving inhibition of several genes in bacterial-mediated delivery of dsRNA via feeding. Spreading effect could be due to differences in the susceptibility of some cells or developmental stages to the consequences of ingested dsRNA. Thus, bacterial-mediated delivery of dsRNA via feeding was less effective as RNAi method than direct microinjection[26, 41]. Although bacterial-mediated delivery of dsRNA via feeding method is an example of RNA-mediated transfer of information between organisms and between species, however, it is not yet known whether such RNA-mediated interference-transfer mechanisms participate in natural ecological interactions, such as antiviral defence or communication during biological symbiosis[26]. Soaking is another delivery method used to introduce dsRNA to create sterile males in various mosquito species. This sterile insect technique uses aerial release of sterile males to reduce wild local populations[42, 5]. In one study, spermatogenesis-specific dsRNAs were administered to mosquito larvae by soaking larvae in dsRNA solutions. At higher concentration, dsRNA sufficiently induced sterility in most (72-92%) of the males[22]. However, lower concentrations were found to be inefficient in inducing sterility in males (only 20-35%). Advantageously, upon mixing of low concentrations of different dsRNAs together, sterility frequencies were near 100%[22]. This experiment is an example of the efficiency of the soaking method. Furthermore, in these dsRNA soaking treatments, the dsRNA entered the insects by ingestion, although entry through other routes (e.g. cuticular penetration) could not be excluded. Nevertheless, RNAi clearly spread beyond the initial entry points to reach the testes. Soaking technique for making sterile insects is relatively simple compared to other approaches of transferring dsRNA[10, 22].

3 Conclusion

Looking at the studies performed to improve insecticide delivery and their pros and cons, it is evident that RNAi has great potential for control of insect pest populations. However, all studies involving RNAi were restricted to laboratory conditions only. No references were found pertaining to RNAi expression under open field conditions because of lack of knowledge about the gene sequence of other off-target and economically viable species. In addition, the coupling of other technologies, like Bt-toxin, with RNAi could expand the possibilities of further improvement in pest control and research in the field of molecular biology. It might even provide a solution to the existing problem of Bt resistant pests. To further improve and establish RNAi technology, large scale field tests need to be conducted along with evaluations of the potential risks of this technology. To confirm no off-target effects and better risk assessment, further studies need to be done in closely related members of the same species by administering RNAi specific for certain desired genes present only in one of the members of the species and comparing it with the control. Further, as information on off-target effects of RNAi is limited, future study will focus on prevalence of off-target effects in two closely related disease vector insect.

Despite all the apprehensions, RNAi is still a promising technology of the future as dsRNAs can be chosen from a vast number of potential target genes. In addition, owing to the advancement in technology, identification of more crucial genes involved in the growth and development of various insects can be used as target genes in RNAi based pest resistance in the future.

Table 1: *Full description of scientific terms and terminology used in the literature.*

Terms	Full Form
RNA	Ribonucleic acid
RNAi	RNA-interference
dsRNA	Double stranded RNA
IPM	Integrated Pest Management
SIT	Sterile Insect Technique
Bt-toxin	Bacillus thuriegenis toxin
WHO	World Health Organization
HMGR	3-Hydroxy-3-methylglutaryl reductase
vATPase	Vacuolar Adenosine Triphophatase
ERA	Environment Risk assessment
PF	Problem Formulation
MCL	Mantle Cell Lymphoma
TLRs	Toll Like Receptors

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Review

Gonadal Development, Social Structure, and Implications of Protandry by Aggressive Dominance in *Amphiprion* Anemonefish

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Abstract

Anemonefish of the genus Amphiprion *have developed a mating system involving protandrous sequential hermaphroditism, wherein male sexual maturation occurs prior to female sexual maturation in an individual[1]. This review will summarize changes in the gonad and individual behaviour through the transition from juvenile to male to female, as well as explore the relationship between body size and fecundity in* Amphiprion. *Anemonefish and their eggs obtain protection from predation by living among the tentacles of sea anemones, which are armed to sting most other fish[2]. A sequentially hermaphroditic mating system is advantageous due to the low abundance of host anemones, as it ensures that a migrating anemonefish can find a potential mate in any group it encounters, and the loss of a mate will always trigger a replacement. This strategy ultimately grants* Amphiprion *the ability to withstand unpredictable host abundance and maximize safety and offspring production, encouraging the proliferation of the genus[3].*

Keywords: Anemonefish, Amphiprion, Protandry, Gonad, Aggressive Dominance

1 Introduction

Anemonefish of the genus *Amphiprion* live in symbioses with host anemones of the order Actiniaria[1]. A large anemone can harbour a maximum of two anemonefish, with up to 10 individuals found in a single anemone colony at one time[3]. *Amphiprion* are sequentially hermaphroditic protandrous fish, meaning that male units of sexual reproduction reach maturity prior to female units[1]. This change is socially controlled by a size hierarchy in anemonefish through aggressive dominance by the sole female, the oldest and largest individual of the group[3,4]. The second-largest individual is the only reproductive male, who acts aggressively towards the smaller subadults, resulting in a monogamous relationship between himself and the female.

The mating pair's domination renders the subadults "juvenile", unable to sexually mature[3]. Upon death or removal of the female from the group, her mate changes sex to become the new female while the alpha-subadult, the most dominant in the group of up to eight subadults, quickly reaches sexual maturity to become the reproductive male[3,4]. Remarkably, a transitioning male anemonefish is able to completely alter its physiology, gonadal morphology, and behaviour in as little as 20 days[5,6]. Only recently have researchers begun to elucidate the complex physiological mechanisms underlying *Amphiprion* sex change, which is largely facilitated by steroid hormones[6,7,8], though it has long been known that social behaviour plays a foundational role in the process[3,5]. This review will describe the gonadal development of anemonefish through each social role (subadult, male, female), investigate the behavioural role in mechanisms governing protandrous sex change, and explore the relationship between body size and fecundity in *Amphiprion*.

2 Discussion

Aggressive dominance within a social group of *Amphiprion* anemonefish determines the gonadal development of each member. Fricke and Fricke[3] described subadult males as "psychophysiologically castrated," as pressure from high-ranking group members suppresses gonad growth. The gonads, located in the caudal region below the swim bladder, are smaller in subadults than they are in functional males or females[9]. Juvenile gonads are ovotestes lacking clear boundaries between immature ovarian and testicular tissue, wherein sparse spermatocytes remain undeveloped until the individual assumes the breeding male position[4,9]. When subadults become males, considerable gonad growth is accompanied by the redirection of gonadal development so that the ovary assumes testicular characteristics; the male ovotestis incorporates both mature testicular tissue and immature ovarian tissue[4,7,10].

Sex change from male to female involves the degradation of testicular tissues and the growth and maturation of ovarian tissues; this female gonad is slightly larger than the functional male ovotestis[7,9,10]. Godwin[4] quantified the proportion of oocytes within *A. melanopus* gametogenic tissue throughout sex change, revealing that nearly 60% of male gametogenic tissue is composed of oocytes preceding sex change and spermatogenic tissue can be completely diminished within a matter of weeks (Table 1). Functional female ovaries harbour oocytes in various maturity stages, with testicular tissue degenerated to a narrow band surrounding the gonads[4,7]. Since the testicular tissue of *Amphiprion* deteriorates in the sexual transition from male to female, this process is thought to be irreversible in nature[9]. However, a recent study on *A. clarkii* has found that the functional ovary does retain high sexual plasticity; treatment of a female with an aromatase inhibitor (AI) has been shown to regenerate active spermatogenic tissue within the ovary, likely due to depletion of estrogen levels caused by the AI[8].

Table 1: *Oocytes as a proportion of total gametogenic tissue at various points in sex change of* Amphiprion melanopus.

Days After Female Removal	Proportion of Oocytes in Total Gametogenic Tissue
0	0.58
10	0.69
20	0.89
30	0.93
45	1.0

A study by Godwin[5] on *A. melanopus* verified that protandrous sex change is under social control; induced sex change by female removal from the anemone has led to the observation of several sex change-associated social cues. One noted cue was the absence of female aggressiveness towards the male upon her removal. Taking the female from the anemone and leaving behind the male and subadults eliminates the aggressive approaches by the female, which normally total about 150 per day towards the male. This may act as a signal to the male that he can assume dominance once the female oppressor is absent. However, it would be beneficial in future to study whether keeping the female in an enclosure within the anemone would yield the same effect, as it would eliminate only the aggressive approaches and disqualify the possibility that sex change may be controlled by some visual or chemical cue instead.

Nevertheless, Godwin's results concur with Fricke & Fricke[3], who suggested that sex change in males is suppressed by aggressive female dominance. *Amphiprion* sex change also involves mating pairs bathing, or placing themselves in close contact with host anemone tentacles, near and parallel to one another in a behaviour referred to as visiting. Visiting increases within a day after female removal, with the sex-changing male often visiting the same juvenile to establish which subadult shall sexually mature; this behaviour decreases as the sex-changer characteristically grows more aggressive in the subsequent days. This aggression prevents other individuals within the group from also changing sex to female, securing them as potential mates rather than competitors for mates. Following female removal and initial visiting behaviours, sex-changers increase aggressive approaches on the alpha-subadult compared to other subadults as they attempt to clearly establish their position as the only female[5].

The drastic contrast between the body size of *Amphiprion* sexes suggests the necessity of larger female body size for reproductive success. Fricke and Fricke[3] observed body size in two *Amphiprion* species to differ substantially between sexes; female body length was measured approximately 20 mm longer, while female body weight was roughly double that of male averages (Table 2). Correlations between female body weight and ovary weight have been observed across multiple *Amphiprion* species, indicating that female fecundity increases with body size[1,3]. A larger female can produce and accommodate more oocytes, but an increase in size would not necessarily affect male reproduction because a small male can still mate successfully with a large female and sperm takes up relatively little space and is continuously produced. These observations led to the hypothesis that sex change direction in *Amphiprion* was decided based on larger female body size being more favourable than larger male body size. An important reason for the anemonefish's tremendous growth when transitioning from male to female may be that male reproductive success depends less on body size, while female reproductive success increases with increasing body size[1].

Table 2: *Mean and standard deviation of body length and wet body weight in samples of* Amphiprion akallopisos *and* Amphiprion bicinctus.

	Amphiprion akallopisos		*Amphiprion bicinctus*	
	Male	Female	Male	Female
Length(mm)	73±3	97±5	113±7	129±6
Weight(g)	7.1±1.1	19.5±2.9	28.0±3.9	46.1±7.6

In groups of *Amphiprion*, females control the reproductive state of every member, restricting the breeding population size and suppressing the sexual maturity of female candidates[3]. The absence of a female results in sex change

in the dominant male by the degeneration of testicular tissue and growth and maturation of ovarian tissue[10], coupled with a newfound aggression in the interest of establishing dominance status amongst their peers. Increases in the body size of sex-changing individuals translates to higher female fecundity, though to what extent remains unexplored. Future studies might venture to ascertain the exact growth-to-fecundity increase ratio, as well as pinpoint the cause of the extreme body size increase in sex-changing anemonefish (the dominant fish might feed more, for example). Nevertheless, the evolutionary advantages of sequential hermaphroditism in *Amphiprion* extend far beyond fecundity.

Considering the low population density of anemonefish and erratic distribution of host anemones, chances of a migrating individual (usually juvenile) encountering an anemone hosting an opposite-sex potential mate would be scant if not for this mating system[3]. A study by Hattori[1] effectively disproved protogyny (females change into males) as a viable option for the anemonefish mating system; one hypothesis suggests that a dominant male would require a harem of smaller females to breed with (polygyny), which would be costly to defend given the low abundance of hosts available. The study was able to mathematically prove the efficacy of protandry over polygyny by determining that reproductive success in various *Amphiprion* species is maximized if the mating system involves monogamous pairing with a dominant female rather than polygyny with a dominant male.

3 Conclusions

Protandry in *Amphiprion* ultimately ensures that there will always be a potential mate available and loss of a mate will always trigger replacement. Once an individual has joined a group, they may never have to leave the safety of their anemone again to find mates[3]. However, it should be noted that larger subadults may pair with each other and move amongst hosts to establish their own territory, securing them a chance to mate if they are outlived by the mating pair in their current anemone[1]. Thus, the evolution of protandrous sequential hermaphroditism has granted Amphiprion the ability to withstand unpredictable host abundance and maximize safety and offspring production in order to promote the proliferation of the genus. The display of aggressive dominance is the foundation of the *Amphiprion* social mating system, suppressing radical changes in the protandrous anemonefish gonad: full transition from juvenile ovotestis to functional male ovotestis to a completely restructured working ovary, contained within a large female capable of producing a plentiful next generation of anemone-inhabiting juveniles.

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Advancing Data Analytics for Decoding Gendered Language in Job Advertisements of STEM Fields

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Abstract

The underrepresentation of women in STEM (Science, Technology, Engineering, Math) is a complex issue that is influenced by several factors. Evidence that gendered wording in job advertisements exists and sustains gender inequality (Gaucher et al., 2011) has shown that word choice in job advertisements affects not only one's perception of their fit for the position, but also how much they feel they belong to that field. Gaucher's study documents the effect of masculine and feminine wording in advertisements.

FEMININE WORDS
'affectionate'
'agree-'
'cheer-'
'child'
'co-operate'
'collab-'
'commit-'
'communal'
'communicate'
'compassion'
'connect'
'considerate'
'cooperati-'
'depend-'
'emotion-'
'empath-'
'enthusias-'
'feel'
'feminine'
'flatter'
'gentle'
'honest'
'inclusive'
'interdependen-'
'interpersonal'
'kind'
'kinship'
'loyal'
'modesty'
'nag'
'nurture-'
'pleasant'
'polite'
'quiet'
'responsible'
'sensitive'
'share'
'sharin-'
'submissive'
'support-'
'sympathy-'
'tender-'
'together'
'trust'
'understand-'
'warm'
'whin-'
'yield'

MASCULINE WORDS
'active-'
'adventurous'
'aggress-'
'ambition-'
'analytical'
'assert-'
'athlete-'
'autonomy-'
'battle'
'boast-'
'challeng'
'champion-'
'compet-'
'competent'
'competit-'
'confident'
'courag-'
'decid-'
'decisi-'
'defend'
'determin-'
'domina-'
'driven'
'fearless'
'fight'
'force'
'forcible'
'greedy'
'headstrong'
'hierarch'
'hostil-'
'impulsive'
'independen'
'individual'
'intellect-'
'lead-'
'logic-'
'masculine'
'methodical'
'objective'
'opinion'
'outspoken'
'persist-'
'principle'
'reckless'
'self-confiden-'
'self-sufficient-'
'selfish-'
'stubborn-'
'superior'
'unreasonab-'

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Background

The underrepresentation of women in STEM (Science, Technology, Engineering, Math) is a complex issue that is influenced by several factors. The 2011 paper, *Evidence that gendered wording in job advertisements exists and sustains gender inequality* (Gaucher et al., 2011), has shown that **word choice** in job advertisements affects not only one's perception of their **fit for the position**, but also how much they feel they **belong** to that field. Gaucher's study documents the effect of **masculine and feminine wording** in advertisements.

Objective

- To analyze job advertisements from the fields of Computer Science, Engineering, and Healthcare using pre-existing **gender decoders** in order to **reveal and compare patterns of language use** in job advertisements
  - ↳ Infer their **effect on gender parity in STEM**.

What is gendered wording?

A word is considered **gendered** if it has an **association with a certain gender**, typically male or female.
- ↳ Usually **implicit** bias; can contradict a conscious opinion

What is a gender decoder?

- A **gender decoder** is tool that **counts and compares** the number of feminine and masculine words that appear in the given text then **returns a result**
  - ↳ Usually "Male", "Female", or "Neutral".
- Every decoder has a curated **word list** that it considers gendered; this list often differs between decoders.
  - ↳ Often, these word lists consist of **word stems** in order to **detect a group of words**
    - eg. The word stem "collab", such as in "collaborate", "collaborative", "collaborating"

Method

I. Collection of Job Advertisements

- Python web-scanning program was developed to **automate the collection** of job advertisements from an online job board.
  - ↳ This yielded **~6000 Engineering** (Mechanical, Electrical, Computer) and **~3500 Computer Science** advertisements.
  - ↳ Additionally, **~2800 Healthcare** listings were collected to compare a traditionally female-dominated field to the STEM fields.

II. Decoding

- **Five online gender decoders** were selected from a web search.
- Python program was created to **simulate the decoders' algorithms** and calculate the **result of each** decoder for each collected advertisement
  - ↳ The program also recorded **which** gendered word stems appeared in the text, and **how often**.

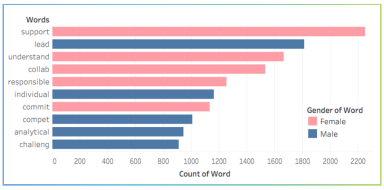

Fig. 1: Most common gendered words stems found in Computer Science advertisements

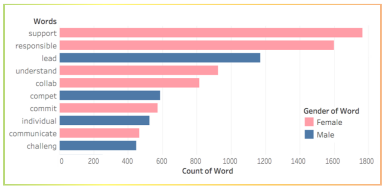

Fig. 2: Most common gendered word stems found in Engineering advertisements

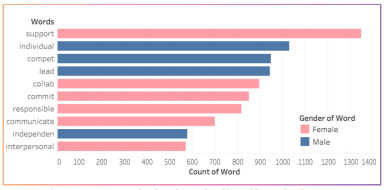

Fig. 2: Most common gendered word stems found in Healthcare advertisements

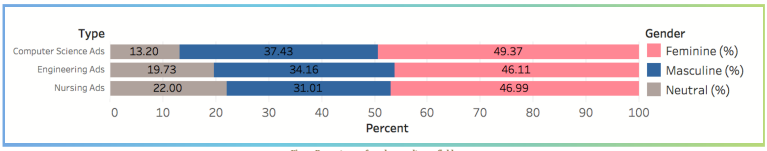

Fig. 4: Percentages of gender results per field

Moving Forward

Next steps include:
- **Dividing** each advertisement **into components** (Company Description, Job Responsibilities, Job Qualifications) and calculating the results of each section.
  - ↳ To obtain a deeper understanding of **how gendered words are used** in advertisements – see if words are being used in a way that reinforces stereotypes of a gender's competencies
- **Creating our own gender decoder** using **machine learning**
  - ↳ Consider the **context** of words to determine if they are being used in a "gendered sense" - prevent advertisements from adding gendered words that aren't used in a gendered sense

eg. The word "kind" as an adjective means "considerate" (feminine), while as a noun it means "type" (neutral)

Results

- Overall, **all three field** samples contained **more feminine-coded advertisements** than masculine- or neutral-coded.
- The **most common word stem** in all fields was "**support**", a feminine word (such as in "supportive", "supporter")
- Four of the five **most commonly used gendered word stems** in the Computer Science and Engineering samples are **feminine** words.

Implications

- More feminine-coded Computer Science and Engineering advertisements could mean that STEM companies are **becoming aware of the effects** of wording on potential applicants
- The **high frequency of feminine words** such as "support" and "collab-" suggest that despite being male-dominated fields, STEM jobs involve **many traditionally feminine qualifications or duties**.

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Female-Specific Larval Lethality in the Yellow Fever Mosquito *Aedes aegypti*

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Abstract

The mosquito, Aedes aegypti*, is the primary vector of dengue, yellow fever, and Zika viruses. Dengue alone threatens over 390 million people worldwide, causing over 300,000 deaths annually. Chemical pesticides are the main method of disease suppression, but new, environmentally friendly methods of mosquito control are needed. The Sterile Insect Technique (SIT) is a pesticide-free method of locally controlling pest insects by releasing large numbers of sterile males, to out-compete wild males for female mates. For this method to work effectively, few or no females should be released with sterile males as sterile females can still spread diseases. Thus, efficient sex-sorting is needed, and to date, no large-scale sex-sorting methods for mosquitoes have been sufficiently effective for use in sterile insect technique.*

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Department of Biological Sciences, Faculty of Science, University of Manitoba

Introduction

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The Sterile Insect Technique (SIT) is a pesticide-free method of locally controlling pest insects by releasing large numbers of sterile males, to out-compete wild males for female mates[2]. For this method to work effectively, few or no females should be released with sterile males as sterile females can still spread diseases. Thus, efficient sex-sorting is needed, and to date, no large-scale sex-sorting methods for mosquitoes have been sufficiently effective for use in sterile insect technique.

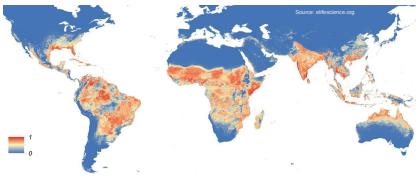


Figure 1: The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*.

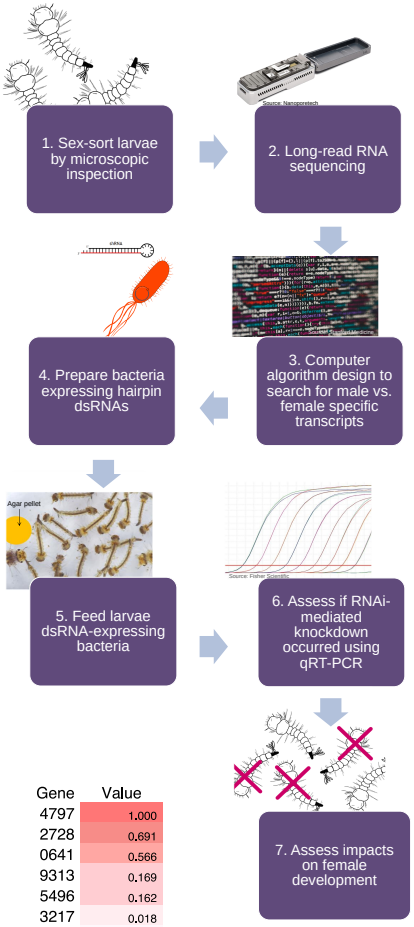
Figure 2: *Aedes aegypti* male (left) and female adults.

Research Objectives

To improve the SIT programs for mosquito control, we will use RNA interference (RNAi) technologies to selectively kill female larvae before they become biting adults (Figure 2). RNAi is a method of silencing a gene's expression by administering double-stranded RNA (dsRNA) to an organism, which results in destruction of the target gene's mRNA. For this project the following objectives must be achieved:

1. Identify genes that are uniquely expressed or processed (spliced) within female larvae.
2. Identify which female genes, when targeted by RNAi, can prevent female development.

Methods



Gene	Value
4797	1.000
2728	0.691
0641	0.566
9313	0.169
5496	0.162
3217	0.018
4814	0.006
2233	0.000

Female-specific
Male-specific

Table 1: A value of 1 signifies a 100% female-specificity, a value of 0 indicates no sex bias, and a value of -1 signifies a 100% male-specificity. This table only illustrates a small subset of the genes analyzed.

Results

- RNA sequencing identified 114 female-specific transcripts (Figure 4).

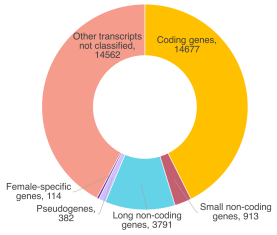


Figure 4: *Aedes aegypti* mosquito has 34 429 gene transcripts. RNA sequencing identified 114 of those to be female-specific in larvae.

- RNA sequencing also identified over 100 new female-specific splice variants (Figure 5).

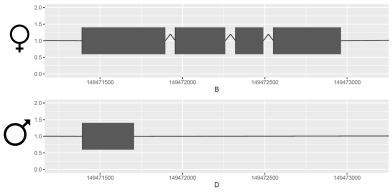


Figure 5. Female- (top) and male-specific (bottom) splice variants.

- qRT-PCR confirms that RNAseq search algorithm is accurate – female specific transcripts are expressed only in females (Table 1).
- Larvae fed on female-specific dsRNAs show delayed growth rates or increased mortality.

Conclusion

- RNAseq search algorithm has identified 114 new female-specific genes expressed in larvae (confirmed by qRT-PCR).
- RNAi-bacterial feeding mosquito larvae is slowing female development, but the search continues for a gene that prevents female development entirely.
- Slowing female development may still work for SIT – if males develop faster, they can be collected from the lagging females more easily.

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Structural Insights into Metal-Organic Connectivity by Paramagnetic NMR

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Abstract

Metal-Organic Frameworks (MOFs) are an emerging class of materials with numerous applications in domains such as gas storage and separation, catalysis and bioimaging [1]. They consist of metallic centres with organic linkers between them, forming a 3D network. These MOFs are difficult to analyze through x-ray diffraction (XRD) methods, thus the hope is that Nuclear Magnetic Resonance (NMR) will provide meaningful insight into their structures. However, many MOFs are paramagnetic, as many positive ions of transition metals contain unpaired electrons. Paramagnetic electrons introduce complications to NMR studies of such compounds. Along with these complications, opportunities to probe chemical structure through paramagnetic interactions arise.

Structural Insights into Metal-Organic Connectivity by Paramagnetic NMR

Lyle Walld[1], Kirill Levin[1], Daniel Padeanu[2], George Shimizu[2], Scott Kroeker[1]

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Introduction

Metal-Organic Frameworks (MOFs) are an emerging class of materials with numerous applications in domains such as gas storage and separation, catalysis and bioimaging [1]. They consist of metallic centres with organic linkers between them, forming a 3D network. These MOFs are difficult to analyze through x-ray diffraction (XRD) methods, thus the hope is that Nuclear Magnetic Resonance (NMR) will provide meaningful insight into their structures.

However, many MOFs are paramagnetic, as many positive ions of transition metals contain unpaired electrons. Paramagnetic electrons introduce complications to NMR studies of such compounds. Along with these complications, opportunities to probe chemical structure through paramagnetic interactions arise.

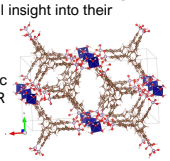


Figure 1: Typical MOF Structure

Purpose

The goal of this project is to make use of the sensitivity of NMR to unpaired electrons to gain insight into the packing structure of ligands in these materials, with the hope that paramagnetic NMR may be extended to a wider variety of MOFs.

Theory of Paramagnetic NMR

Most NMR is performed on diamagnetic compounds. The magnetic moment of paramagnetic electrons results in significant interactions with the nuclei in a material. These interactions drastically alter the NMR spectrum by two mechanisms.

Isotropic Hyperfine (Fermi):

A through-bond effect, which originates in unpaired-electron spin-density ($\rho_{\alpha\beta}$) having a probability to exist at a given nucleus. This interaction results in changes to the observed NMR shifts (δ_{obs}) according to:

$$\delta_{Fermi} = \frac{M(S+1)}{T}\rho_{\alpha\beta}$$

$$\delta_{obs} = \delta_{dia} + \delta_{Fermi}$$

where δ_{dia} is the diamagnetic shift, S is the total electron spin number, T is the temperature of the system, and M has a value of 23.5×10^6 ppm K au^{-1}.

Anisotropic Hyperfine (Dipolar):

The dipolar effect is a through-space interaction between nuclei and unpaired electrons with effects that include dramatic increases in nuclear relaxation rates.

The nuclear relaxation time, T_1, is a significant factor in terms of experimental NMR. Relaxation times vary drastically with interatomic distance r, as $T_1 \propto r^{-6}$. A shorter relaxation period indicates a closer proximity to paramagnetic metal centres, r, the source of unpaired electrons. Nuclear T_1s were measured experimentally and compared with theory to gain structural insight.

$$\frac{1}{T_{1N}} = \frac{2S(S+1)\gamma_I^2 g^2 \beta^2}{15r^6}\omega(T_e)$$

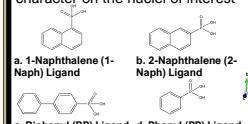
Methods

- **Apparatus Used:**
  - Bruker Avance III 500 Spectrometer
  - 1.3 mm Magic Angle Spinning (MAS) Probe
  - 40-60 kHz MAS Rate
- **Experiments Performed:**
  - ^{31}P, ^{13}C, ^{1}H Hahn-Echo
  - ^{13}C{^{1}H} REDOR used to distinguish carbons bonded to hydrogen from those not bonded to hydrogen.
  - Saturation Recovery and Inversion Recovery to measure T_1
  - CPMG to measure T_2, wherein a $\frac{\pi}{2}$ pulse is followed by a train of π pulses to measure the rate of longitudinal coherence delay, for which T_2 is the time constant.
- **Synthesis:**
  - Samples were synthesized by Daniel Padeanu and Dr. George Shimizu.

Structures

These samples consist of octahedral chromium nodes connected to phosphonate groups via water hydrogen bonds. The 3D framework is formed through phosphonate based organic linkers, see figure 2.

Unpaired electrons from the d^3 Cr^{3+} centres, are delocalized throughout the framework structure, and confer paramagnetic character on the nuclei of interest



a. 1-Naphthalene (1-Naph) Ligand b. 2-Naphthalene (2-Naph) Ligand c. Biphenyl (BP) Ligand d. Phenyl (PP) Ligand

Figure 2: Ligands present in the structures analyzed

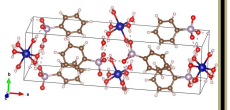


Figure 3: Phenyl based structure (PP), as determined by single-crystal X-ray diffraction

^{31}P MAS NMR

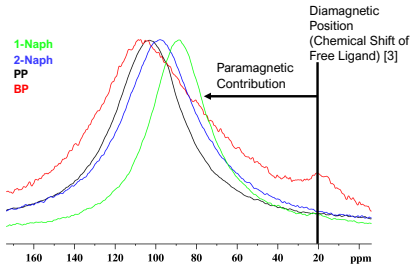


Figure 4: ^{31}P MAS NMR Spectra of four structures, with the approximate diamagnetic position labelled to demonstrate the paramagnetic contribution to shift

- The effects of the Fermi coupling shift the peaks to higher frequency, demonstrating the similar spin delocalization pathways and hence structural connectivity of these samples.

^{13}C MAS NMR

- The differences in aromatic ligand are the driving force behind the observed structural differences between these systems.
- ^{13}C NMR and ^{13}C{^{1}H} REDOR elucidate these differences.
- REDOR experiments were used to determine which carbon signals originate from atoms bonded to hydrogen atoms.

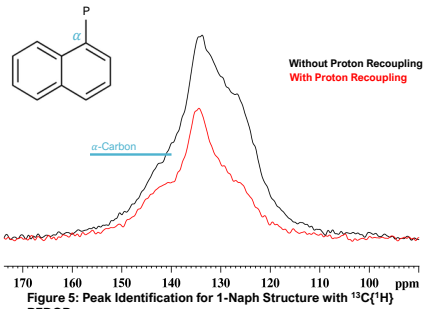


Figure 5: Peak Identification for 1-Naph Structure with ^{13}C{^{1}H} REDOR

- ^{13}C NMR spectra show larger shifts for carbons closer to the metal centre as evidenced by relaxation times and spin density maps.

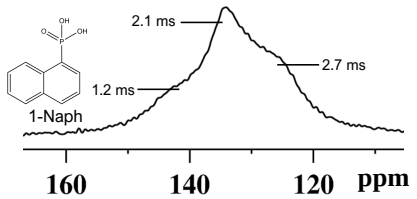


Figure 6: ^{13}C MAS NMR Spectra of 1-Naph, with peaks labelled with corresponding T_1 relaxation times. Uncertainty in T_1 taken as ±20%

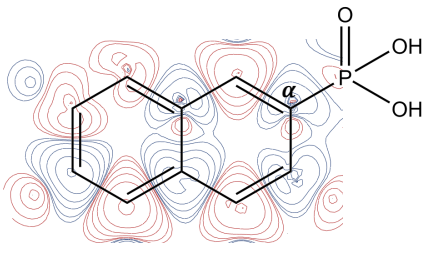


Figure 7: Spin Density Map of 2-Naph, as calculated by HSEH1PBE with 6-31G basis set applied to carbon atoms

- Blue colour of spin density contours at α carbon implies a positive Fermi shift, agreeing with the findings of ^{13}C{^{1}H} REDOR experiments, where the α carbon is shifted to higher frequencies.

Discussion

- ^{13}C assignments are in agreement with the crystal structures of 1-Naph, 2-Naph, and PP.
- Comparing with Figure 2b., this can be rationalized in terms of the measured relaxation times as well.
- The differences in packing arrangement under investigation originate in the degree of interdigitation, or overlap of neighboring sets of ligands, which is detectable via relaxation times.

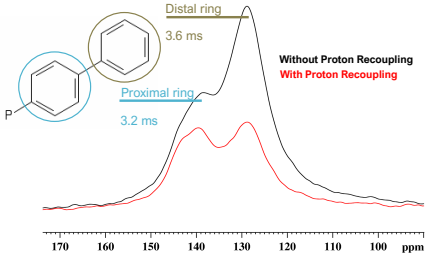


Figure 8: Peak Identification for BP Structure with ^{13}C{^{1}H} REDOR, and T_1 relaxation times

- No crystal structure could be obtained for BP, but structural predictions can be made based on NMR. The relaxation times can be used to gain insight into the packing arrangement.
- The degree of interdigitation of the biphenyl groups can be estimated based on the nuclear relaxation times of the peaks corresponding to each ring.
- Similar relaxation times of signals from neighboring phenyl rings suggests similar distance to a metal from either side of the biphenyl. This implies significant interdigitation in the structure. This is represented in Figure 9.

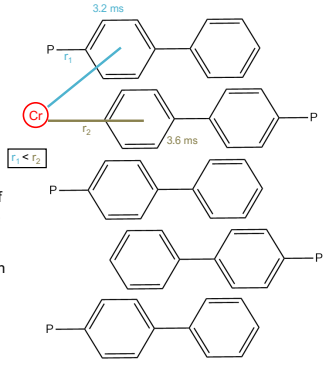


Figure 9: Suggested degree of interdigitation in BP structure

Conclusions

- The sensitivity of NMR peak shifts to paramagnetic electrons has been used to begin developing an understanding of metal-organic connectivity in MOFs.
- By comparison to other structures, insight has been gained on the packing arrangement of the biphenyl structure.
- A more quantitative model for interpreting paramagnetic relaxation times within these, and other periodic systems could potentially triangulate atomic positions.
- This sort of analysis can be extended to similar structures, such as other MOFs, to assist in the development of frameworks with specific and useful properties.

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Effects of Filtration Techniques in Identifying Dissolved Reactive Phosphorus versus Particulates in South Tobacco Creek Watershed

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Abstract

Various research centers, scientists and professionals in analytical chemistry use different types of filter papers to determine the types of phosphorus (P) and particulates responsible for algal blooms and eutrophication in water bodies. However, those filter papers misinterpret results in defining dissolved reactive phosphorus (DRP) versus particulates, by ignoring the fact that particulates which could be enriched in phosphorus (P) or nitrogen (N) present in the water also contribute to eutrophication.

Effects of Filtration Techniques in Identifying Dissolved Reactive Phosphorus versus Particulates in South Tobacco Creek Watershed

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Introduction

Various research centers, scientists and professionals in analytical chemistry use different types of filter papers to determine the types of phosphorus (P) and particulates responsible for algal blooms and eutrophication in water bodies. However, those filter papers misinterpret results in defining dissolved reactive phosphorus (DRP) versus particulates, by ignoring the fact that particulates which could be enriched in phosphorus (P) or nitrogen (N) present in the water also contribute to eutrophication.

Fig 1. The STC watershed

Objectives

The goals of this research are:

- To determine whether particulates pass through six different filter papers and to prove that DRP is not truly dissolved P, but also contains particulates that contribute to eutrophication,
- To quantify and index particulates that pass through the filter papers,
- To demonstrate that sediments move from one source to another through trends in sediment transport in water bodies.

Study Area

South Tobacco Creek (STC) watershed, located near the town of Miami, in the south-western region of Manitoba, Canada, was the selected study area. It is an ideal study area since most of the land in this watershed is used for agricultural production, including: cereal crops, oilseeds, perennial forages and livestock; which drains into the Red River and then, up-north into Lake Winnipeg. The STC contains two sub-watersheds, and more specifically, a reservoir located in the Madill sub-watershed was the selected site for water sample collection.

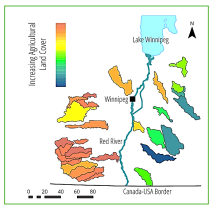

Fig 2. Flow of water from agricultural land.

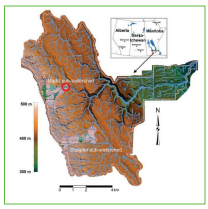

Fig 3. Selected study area circled in red.

Methods

- Fresh homogeneous water samples were collected in the Madill sub-watershed, at 2 locations, namely the "Inlet" and the "Outlet",
- Filtration experiments were conducted in the lab, using six filter papers: 1.6, 1.5, 1.2, 0.8, 0.45 and 0.2 µm, by filtering duplicate 1 L of water through each filter paper, and the samples were labelled: Sample ID 1-12.
- The filtered samples were collected and oven-dried for 18 hours,
- After 18 hours, Gravimetric Analysis was performed to quantify and index Total Suspended Sediments (TSS) present in the water samples,
- The filtrates were refrigerated at T = 4°C; and were then analyzed for the presence of:
  - Particle size distribution via a Master Sizer 2000 Analyzer;
  - P and N by Colorimetry via a Lachat Flow Injection Analyzer.

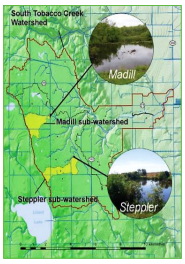

Fig 4. A reservoir located in the Madill sub-watershed was the selected study site.

Results & Discussion

The relationship between Total Suspended Sediments (TSS) and Turbidity in the inlet versus the outlet obtained by Gravimetric Analysis is represented in Fig 5. The trends indicate movement of sediments, where the water sub-samples after filtration, respective to their filter papers, contain more TSS and are more turbid in the outlet than in the inlet.

Table 1. Comparison of TSS in inlet v/s outlet

Filter paper (µm)	Inlet TSS (mg/L)	Outlet TSS (mg/L)
1.6	0.6972	0.9139
1.6	0.6140	0.9747
1.5	0.6803	1.0896
1.5	0.2537	1.0537
1.2	0.6729	0.8488
1.2	0.7045	1.1215
0.8	0.6844	0.9724
0.8	0.7075	1.0457
0.45	0.7701	1.2449
0.45	0.7545	1.3274
0.2	0.7515	1.2616
0.2	0.7800	1.5424

From the largest (1.6 µm) to the smallest (0.2 µm) pore sizes, concentrations of both TSS and turbidity significantly increase as shown in Table 1; from top to bottom.

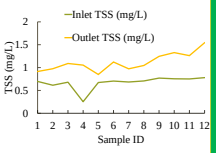

Fig 5. TSS and turbidity are significantly higher in the outlet than in the inlet.

The relationship between DRP and particulates, obtained from the Lachat Analyzer for the inlet and the outlet are represented in Fig 6 and Fig 7 respectively. For both locations, results show that concentrations of NO_3/NO_2 are significantly lower than concentrations of both P and NH_3. However, the inlet contains the highest concentrations of NH_3 whereas the outlet contains the highest concentrations of P.

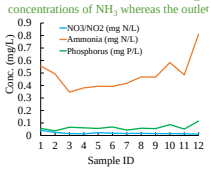

Fig 6. Conc. of P, NO_3/NO_2 and NH_3 for the inlet filtered sub-samples

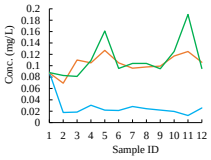

Fig 7. Conc. of P, NO_3/NO_2 and NH_3 for the outlet filtered sub-samples

The relationship between filtered samples and particulates acquired by a Master Sizer 2000 are shown in Fig 8 and Fig 9, for the inlet and outlet respectively. Fig 8. shows that the inlet filtered samples contain sediments of silt and sand, whereas those for the outlet are sediments of silt, since they are all < 50 µm that have been identified to pass through the filter papers. The inlet could be the source of sediments where P also come from.

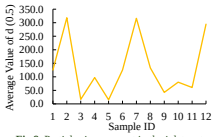

Fig 8. Particle sizes present in the inlet water sub-samples.

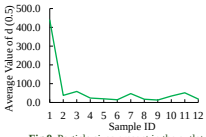

Fig 9. Particle sizes present in the outlet water sub-samples

Conclusions

- Nutrients such as P, NO_3/NO_2 and NH_3 have been identified to be present, that pass through the filter papers and that contribute to DRP fluxes in the outlet.
- More sediments were detected in the outlet water samples, which could be due to increased levels of organic material due to the presence of aquatic vegetation.
- Higher conc. of P were detected in the outlet; hence, particulates that pass through the filter papers are not truly dissolved P, but enriched in P, NO_3/NO_2 and NH_3.

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Phenotypic Analysis of Multi-Drug Resistant Cystic Fibrosis Clinical Isolates of *Pseudomonas aeruginosa* strains

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Abstract

Pseudomonas aeruginosa *is a Gram negative opportunistic pathogen and a leading cause of lung infection in cystic fibrosis (CF) patients. This study was focused on characterizing two multi drug resistant (MDR) cystic fibrosis clinical isolates of P. aeruginosa. These clinical isolates were taken from patients in the Sick Children's Hospital, Ontario. Genomic analysis and phenotypic assays were done to assess the multi-drug resistant and virulence phenotype between these isolates compared to wild type PA01. The strains exhibit very similar resistance profiles apart from meropenem, however a difference is observed in biofilm formation, virulence, and growth in minimal media.*

Phenotypic Analysis of Multi-Drug Resistant Cystic Fibrosis Clinical Isolates of *Pseudomonas aeruginosa* strains

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Abstract

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Introduction

P. aeruginosa is the leading Gram negative infective agent in CF patients (*1).* CF is a genetic disorder characterized by mucous accumulation in the lungs, which facilitates bacterial colonization *(2).* Many strains of this bacterium exhibit MDR phenotypes, limiting the number of antibiotics that can be used for treatment. One of the resistance mechanisms used by *P. aeruginosa* is through chromosomally encoded genes (use of efflux pump or hydrolyzing enzymes). There are four clinically relevant Resistance-Nodulation-Division (RND) efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY) present in *P. aeruginosa* and these pumps have broad antibiotic specificity *(1)*. With antibiotic resistance increasing rapidly in many strains, this study aims to understand mechanisms of resistance in MDR strains in the presence and absence of RND efflux pump overexpression.

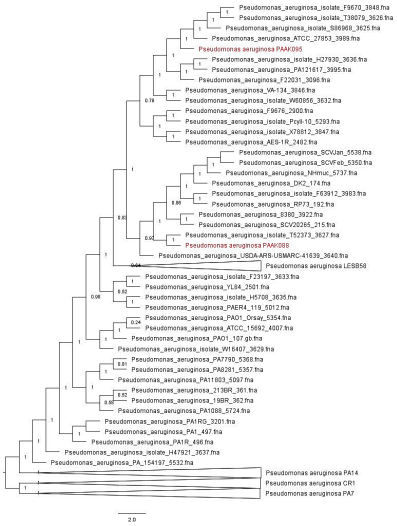

Figure 1. Phylogenetic tree for *P. aeruginosa* genomes *(2)*. Whole genome SNPs based phylogeny was created using Harvest tools, allowing genetic recombination. Both of the isolates formed clusters with nosocomial clinical isolates but in two different clades.

Antibiotic Susceptibility Profiles

Table 1. Minimum Inhibitory Concentrations determined by two fold dilution method in MHB broth or disc diffusion assay. *(1)*

Antibiotic	PAAK088	PAAK095	PA01
Amikacin	≥128	≥128	≤8
Aztreonam	≥64	≥64	≤8
Cefepime	≥64	≥64	≤4
Ceftazidime	≥64	≥64	≤1
Ciprofloxacin	≥8	4	≤0.5
Gentamicin	≥32	≥32	≤2
Meropenem	≤1	≥16	≤0.5
Piperacillin	128	≥256	≤16
Piperacillin/Tazobactam	≥8	≥256/4	≤8/4
Tobramycin	≥16	≥16	≤2
Colistin[#]	S	S	S
Chloramphenicol[#]	I	I	I

*Concentrations are given in µg/mL. S=sensitive, I=intermediate.
[#]Carried out by disc diffusion.

qRT-PCR

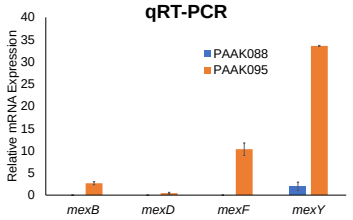

Figure 2.Relative detection of mRNA expression for RND efflux pumps in *P. aeruginosa* PAAK088 and PAAK095 in comparison to PA01 *(1)*. PAAK095 shows overexpression *of mexB, mex F*, and *mexY* while PAAK088 does not overexpress any RND efflux pump genes.

Growth Curve

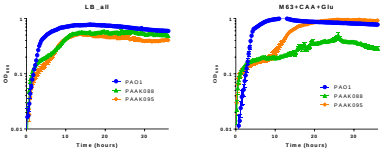

Figure 3. Growth curves in two types of media. LB broth and M63 media with 0.4% Glucose, 1mM MgSO4, and 0.5% CAS amino acid. The experiment was done with 2 biological and 5 technical replicates. PAAK088 and PAAK095 do not exhibit growth in M63+ Arg+ $MgSO_4$ media so we chose to study their growth in a different type of minimal media. Both of these strains showed reduced growth compared to that of PA01, in both minimal and complex media.

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Biofilm Formation

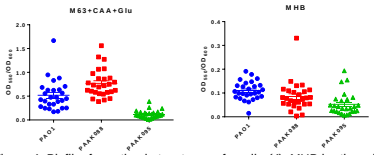

Figure 4. Biofilm formation in two types of media *(4)*. MHB broth and M63 media with 0.4% Glucose, 1mM $MgSO_4$, and 0.5% CAS amino acid. Biofilms were done using crystal violet staining method and normalized with A_{600} after 18 hours of static growth. PAAk088 showed more biofilm formation than PAAK095 in both minimal and complex media. In minimal media, PAAK088 also showed greater biofilm formation than wild type PA01.

Virulence Assays

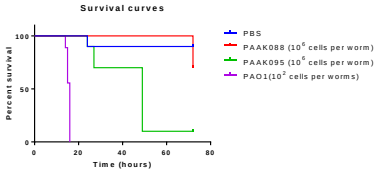

Figure 5. Virulence assay in *Galleria mellonella (*wax worms). Wax worms were infected with a standardized amount of cells in 10 µL volume and number of worms dead were counted every hour. Worms were determined dead when unresponsive and discolored. *(5)* 10 worms were used per experimental condition with PBS as injection control. We found that PAAK095 was more virulent than PAAK088 but both strains were less virulent in comparison to PA01.

Discussion

Both of the CF clinical isolates showed MDR phenotype, but only PAAK095 exhibited overexpression of RND efflux pump genes. This suggests other resistance mechanisms present in PAAK088 that confers resistance to different classes of antibiotic. Both of the isolates show a growth defect in comparison to PA01 which could be an adaptation property of clinical isolates. Interestingly, PAAK088 showed high biofilm formation although this strain shows the slowest growth and least virulence. Numerous surface associated motility assays were carried out but no conclusive data was obtained. It appears that both strains do not exhibit significant motility on the media used..

Conclusion

Since both isolates showed similar antibiotic resistance phenotypes but different virulence and growth phenotypes, further study is required to understand the resistance and virulence mechanisms that differ in these two isolates. Comparative genomics on these isolates will be helpful in explaining the phenotypic differences and resistance profiles observed. Understanding the mechanisms of resistance and virulence in CF isolates will help in developing therapeutic options for treating infections in CF patients.

Acknowledgements

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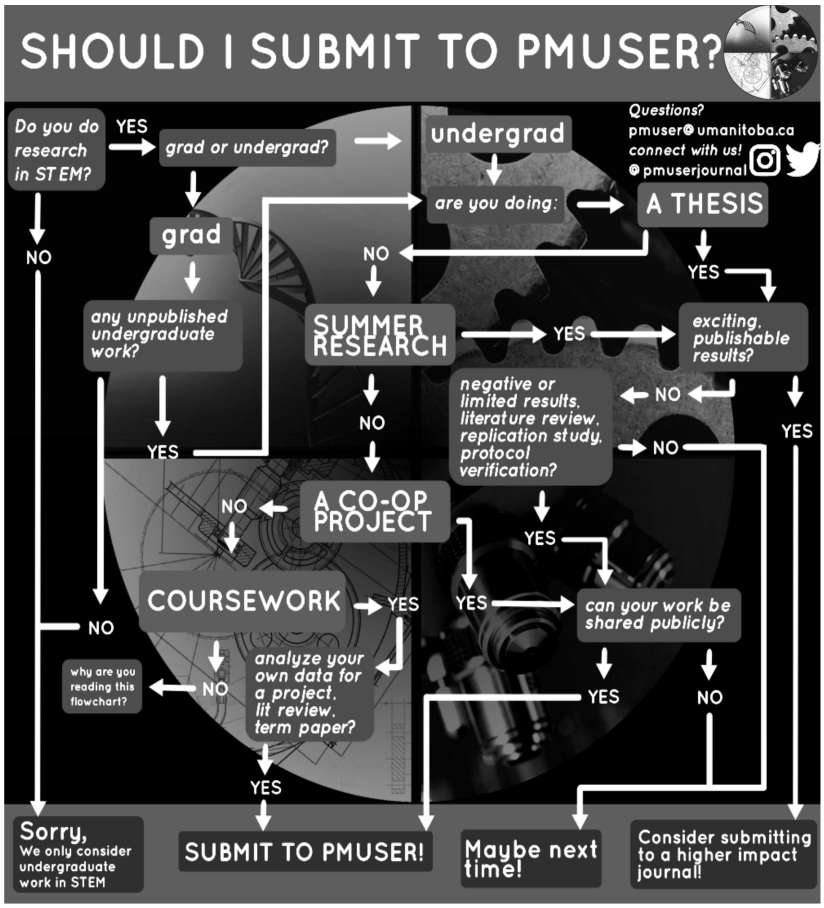

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