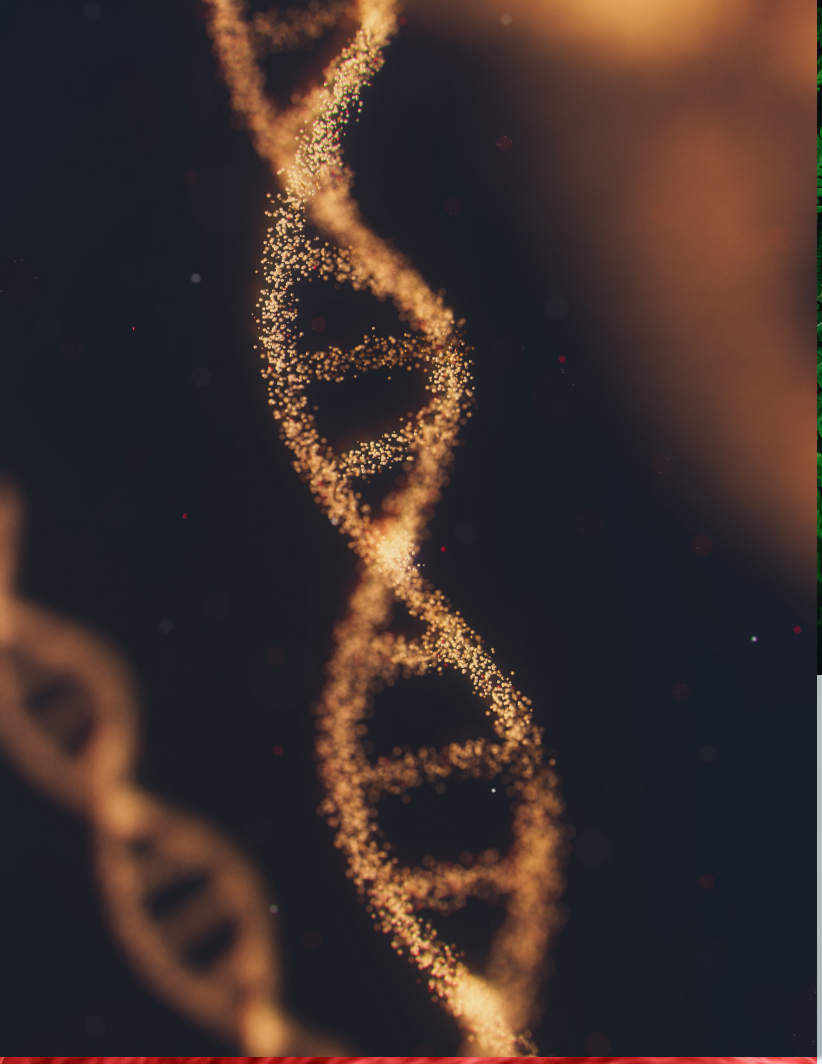

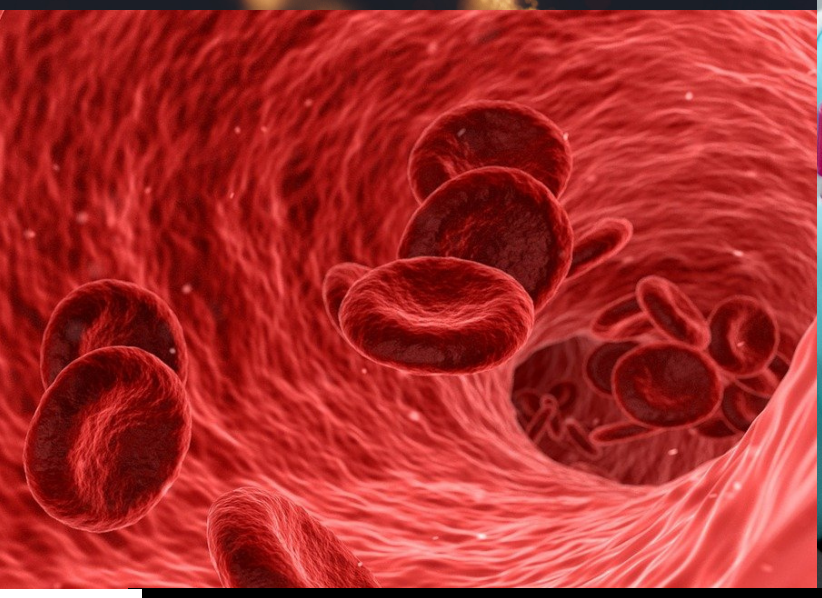

2021

BSURP

The BioScientific Undergraduate Research Publication

BSA BIOLOGY STUDENTS' ASSOCIATION

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What is BSURP?

The BioScientific Undergraduate Research Publication (BSURP) is a collection of summaries detailing research projects that were undertaken by Biological Sciences undergraduate students at the University of Calgary. As one of the top research universities in Canada, the University of Calgary provides many students with the incredible opportunity to conduct innovative research in many diverse areas. Many students in the Biological Sciences have taken up research as part of summer programs or courses for their degree.

The Biology Students' Association (BSA) began the BSURP during the 2020-21 academic year in order to highlight the amazing research being done by our fellow Biological Sciences students. Each one of the six programs in Biological Sciences (Biochemistry; Biological Sciences; Cellular, Molecular and Microbial Biology; Ecology; Plant Biology; and Zoology) is represented in our publication. Projects were conducted in research labs both within and outside the Biological Sciences department, covering a diverse range of interesting and ground-breaking topics.

We hope this publication not only showcases the fantastic work of Biological Sciences students and encourages other students to get involved in research, but also provides insight into what research is about and what students can achieve.

Eligibility

To be eligible to submit to BSURP, applicants had to be undergraduate Biological Sciences students at the University of Calgary for the duration of the project. The project had to take place between May 2020 and May 2021, and both research courses (507/528/530) and non-credit projects were acceptable. Research could occur in any department so long as the student was a Biological Sciences student.

Evaluation and selection

Every project submitted to BSURP was reviewed and lightly revised by the BSURP team. All applicants received feedback on their entry based on the provided guidelines

and reviewer impressions of the writing and science. Evaluation was done by fellow upper-year undergraduate students at the University of Calgary.

Contributors

The following University of Calgary undergraduate students and alumni are featured in this publication:

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Evaluation of entries: Joan Chu (2021-2022 BSA Vice-President Academic) and Manuella Nsangou (2020-2021 BSA Vice-President Academic)
Publication design: Joan Chu and Manuella Nsangou
Light revision of entries and assembly of publication: Joan Chu

The 2020-21 Academic team (Manuella Nsangou, Joan Chu, Candace Elisha Chan and Shubh Patel) collaborated to determine submission criteria and evaluation methods.

Acknowledgements

Firstly, we would like to thank all applicants for taking the time to participate in BSURP and contributing your research to this publication. The BSURP wouldn't be possible without you!
Additionally, we would like to thank the BSA 2020-2021 and 2021-2022 executive councils for helping shape this publication and assisting with logistics, feedback and marketing.

Contact us

The BSA is a Student Union sanctioned, departmental club at the University of Calgary that provides community engagement opportunities, academic resources, professional development events, and more. Our club resources and initiatives are designed to help Biological Sciences students thrive academically, connect with peers and enhance their student experience. Learn more about the BSA at **bsaucalgary.ca**

For inquiries about BSURP or the BSA, please feel free to reach out to our team at **contact@bsaucalgary.ca**.

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BSURP Entries

CerebroFlow: A web-based visualization tool for large-scale functional human brain networks

Type of project: Prototype Building
Student colleagues: Braedyn Au, Mahsa Faryadas, Ganga Poudel, Rian Opperman, Nicholas Lee, Ayush Mandwal
Supervisor(s): Dr. Emma Towlson
Department: Computer Science, Physics and Astronomy

About the researcher

Tanya Abraham

I am currently a fourth year Biological Sciences major. During my undergrad, I worked in a pediatric pain research lab as I love working with kids and volunteering with the Alberta Children's Hospital. At the moment, I am working on a project exploring inner experience modalities and cognition within a cognitive neuroscience lab. In my spare time, I enjoy reading, playing video games and watching YouTube documentaries.
The prototype project below was designed within an interdisciplinary team. This was such a great learning opportunity as I had the chance to collaborate with students from various academic backgrounds, resulting in the perfect culmination of skill sets. I would highly recommend getting involved in interdisciplinary projects during undergraduate studies as it is a chance to bring your unique set of talents to the table while acquiring knowledge from outside of your faculty. I also believe that the future of research environments is heading towards one featuring diverse disciplines.

Background

The human brain is the largest and most complex system which we know. Modern neurotechnologies provide us with unprecedented windows into its structure and function, and network neuroscience is well poised to make sense of and exploit the resulting data (Mishra & Gazzaley, 2015). Electroencephalography (EEG) is one such non-invasive electrophysiological monitoring method that is used to record brain-wide neural activity. EEG signals acquired from the brain can provide a characteristic representation of human physiological and pathological states (Gruzelier, 2014). Indeed, the networks built from correlations between regional activations describe dynamic brain states and hold the ability to diagnose brain disorders and diseases, including depression, schizophrenia, and epilepsy (Van Wijk et al., 2010). Yet, there remains a gap between this knowledge and clinical practice.

Methods

Typically, EEG data is visualized as line charts or as 2D/3D networks using various software choices which place undesirable constraints on the Operating System and have consequences for broader accessibility. To address this issue, we have developed a web-based application using open-source modules from the Python and Javascript programming languages to provide a user-interactive visualization of brain activity displayed as a time-varying 3D network. Nodes correspond to the recording sites of EEG cap electrodes on the head, while the links represent connection strengths (functional correlations) between brain areas at the recording sites.

Results

Our tool is able to pre-process raw EEG data and compute various graph-theoretic quantities, including but not limited to degree, clustering coefficient, and path lengths. It can generate functional and effective connectivity from a range of methodologies, including partial correlation and Granger causality. Users can adjust all computational parameters, and display network properties as varying node sizes and links as a function of time (Figure 1). Further testing would prove beneficial for assessing the accuracy of both EEG and network data.

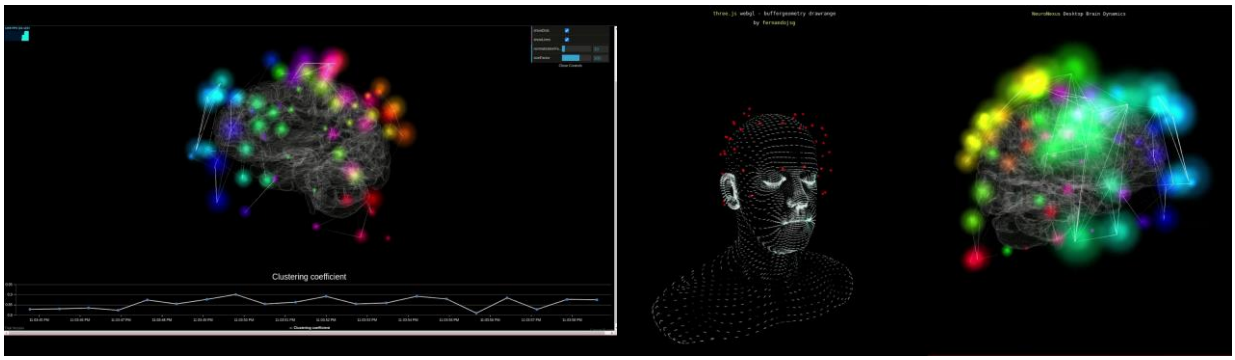

Figure 1. CerebroFlow. (a) View of the whole brain network and clustering coefficient evolving over time. (b) View of the whole brain network and the corresponding recording sites on the human head.

Significance

Network neuroscience is being employed for both medical purposes such as early diagnosis of neuro-pathologies in patients as well as accelerating neuroscience research in general - and with great success. Developing a web application enables increased portability of the setup and allows anyone with EEG data to access the computing capability on any web browser and on any device. Our tool can be used to accelerate discoveries (such as neural markers of disorder and disease), and also to apply them. Importantly, the tool holds future potential towards curating highly individualized diagnostics for brain and mental health illnesses that practitioners may use in real-time with their patients (Van Wijk et al., 2010).

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Small-flowered sand verbena (Tripterocalyx micranthus) species distribution modelling

Type of project: 530
Supervisor(s): Dr. Jana Vamosi
Department: Biological Sciences

About the researcher

Rory Armstrong

I'm a geography and biology combined major graduating in 2021 after 8 years of study at the UofC. I'm interested in researching with geography and remote sensing software. Although I will be pursuing further academic goals in geography, studying Arctic applications of satellite radar, I am also interested in plants and geographical modelling of species. This research project was an interesting way to expose myself to the world of species distribution modelling, and it was great practice using geographical software in a biology context. It was interesting learning the different software packages available, particularly Wallace, which worked well for me, despite some challenges. I didn't expect to run into so many software and server hiccups with the different software tools, and learning to work around these problems was good experience. It was also interesting learning about a local plant and learning about sand dunes in the Canadian prairies. I was excited to learn about the small-flowered sand verbena could fare in the future, as not much information on this topic was available and I didn't know what to expect.

Background

The small-flowered sand verbena (*Tripterocalyx micranthus*) is a member of the 'four o'clock' family' (Nyctaginaceae) residing on sand hills across western North America. The species grows to 20-50 cm tall, forms taproots, and bears flowers lacking petals and veiny paper-like pink-coloured 'winged' fruit (Government of Canada, 2012). The species has experienced substantial declines in habitat extent and habitat suitability as agriculture, infrastructure and the encroachment of weeds have driven the stabilization of dune fields across the Canadian prairies (Hugenholtz et al., 2010). Human activity and the spread of vegetation has substantially reduced windblown erosion in the Canadian prairies, threatening the continuation of many dune-dependant

species, including the small-flowered sand verbena (Hugenholtz et al., 2010). The small-flowered sand verbena has been declared 'endangered' in Canada since 2005, and its current range is expected to be at risk in the near future (Government of Alberta, 2012).

Species distribution models (SDMs) allow researchers to use species occurrence datasets alongside layers of environmental data to estimate habitat suitability, potentially across time periods, locations and differing environmental conditions (Elith & Lethwick, 2009). Locations of species occurrences are evaluated with respect to environmental data using an algorithm tailored to the ecological traits of the species in question. Both maximum-entropy (Maxent) model algorithms and BIOCLIM model algorithms have been shown to be particularly useful algorithms for SDM development. A number of software platforms have been released for SDM development, including some GUI-based systems that have emerged recently, such as the R-based program 'Wallace'. Wallace is capable of producing SDMs based on either BIOCLIM or maximum-entropy (Maxent) algorithms. Wallace is also capable of projecting such models into the future using a variety of different global circulation models (GCMs) and emissions scenarios. Application of Wallace-developed SDM estimates can help researchers evaluate future climate suitability and can help inform monitoring and management decisions (Kass et al., 2018). Due to its precarious position in the face of substantial climatic changes expected in the Canadian prairies, application of SDMs to model the small-flowered sand verbena is expected to provide considerable information on future prospects. BIOCLIM and Maxent models are produced using Wallace, estimating habitat suitability currently and in 2050 and 2070. These estimates are analyzed for potential insights into management practices or future ecology, and the models are used to assess Wallace as an SDM development tool.

Methods

The GBIF database, supported by iNaturalist data, was used to obtain points for occurrence mapping (Van Horn et al., 2018). Records for *Tripterocalyx micranthus* were queried from GBIF, and points with erroneous metadata or erroneous locations were removed. Additional Canadian points were added by including all mapped small-flowered sand verbena occurrence locations specified by the Government of Alberta (2012). A 20 km spatial thinning procedure was applied to the point dataset to address uneven point distribution (Figure 1). 20 km thinning essentially eliminated risk of 'double counting' individual populations while retaining as much ecological information as possible. WorldClim bioclimatic variables were used for environmental data layer inputs (Marchi et al., 2019). Bioclimatic variable 15, precipitation seasonality, was removed from all model inputs due to negligible and inconsistent species response curves

observed during Maxent model testing. This ineffectiveness and inconsistency of precipitation seasonality was also observed in models constructed with Chelsa environmental data, which was used to verify model parameter effectiveness (Karger et al., 2017a; Karger et al., 2017b). Each of the remaining 18 default WorldClim bioclimatic variables were inputted into the model (WorldClim, 2020). A 5° point buffer from occurrence points was used to restrict environmental data layer inputs. 5° point buffering ensured that the environmental data included was contiguous and inclusive of sufficient low-suitability locations.

Maxent model algorithms produced on Wallace were designed using an interactive testing process, whereby feature class and regularization settings were repeatedly tested and optimized. LQHP models with a regularization multiplier of '2' were found to be the most effective, consistently displaying minimized AICc (Figure 1). BIOCLIM modelling was performed using default BIOCLIM settings, as BIOCLIM model algorithms are provided 'as-in' in Wallace, without the need for adjustment. Three global circulation models are used for future model projections to 2050 and 2070: ACCESS 1.0, CCSM4, and MIROC-5. These global circulation models are well-supported in the literature and represent a variety of different assumptions about the future of earth's circulation patterns (Perez et al., 2014).

Results

Maxent models generally predicted a higher overall cumulative log-log suitability for the small-flowered sand verbena in the Canadian prairies than BIOCLIM models, although both approaches estimate the continuation of suitable range in 2050 and 2017 in Alberta (Figure 2, Figure 3). Suitability predictions for Saskatchewan were more variable and marginal, with some models predicting the continuation of only small patches of suitable habitat in 2050 and 2070. Maxent and BIOCLIM model results varied considerably across global circulation models, with the choice of circulation model heavily impacting predictions. Testing emissions scenarios indicated that the small-flowered sand verbena may be well-suited to dealing with high emissions, with range expansions possible.

Significance

Estimated continuation of suitable range across large parts of southern Alberta is promising for the future of the small-flowered sand verbena in Canada, particularly in Alberta. Suitability in Saskatchewan appears to be considerably lower and at greater risk in coming decades. Spatial variability across different model algorithms, global

circulation models and emissions scenarios lead to uncertainty in determining high-suitability locations for the small flowered sand verbena. Maintenance of numerous healthy populations in Alberta is suggested, as currently-available models do not agree on which areas may continue to provide suitable habitat in the future. Changes to drought risk associated with climate change may not be adequately modelled, and high-emissions scenarios may pose a greater drought risk to the small flowered sand verbena than estimated here. Wallace was found to be an effective means of developing and testing species distribution models.

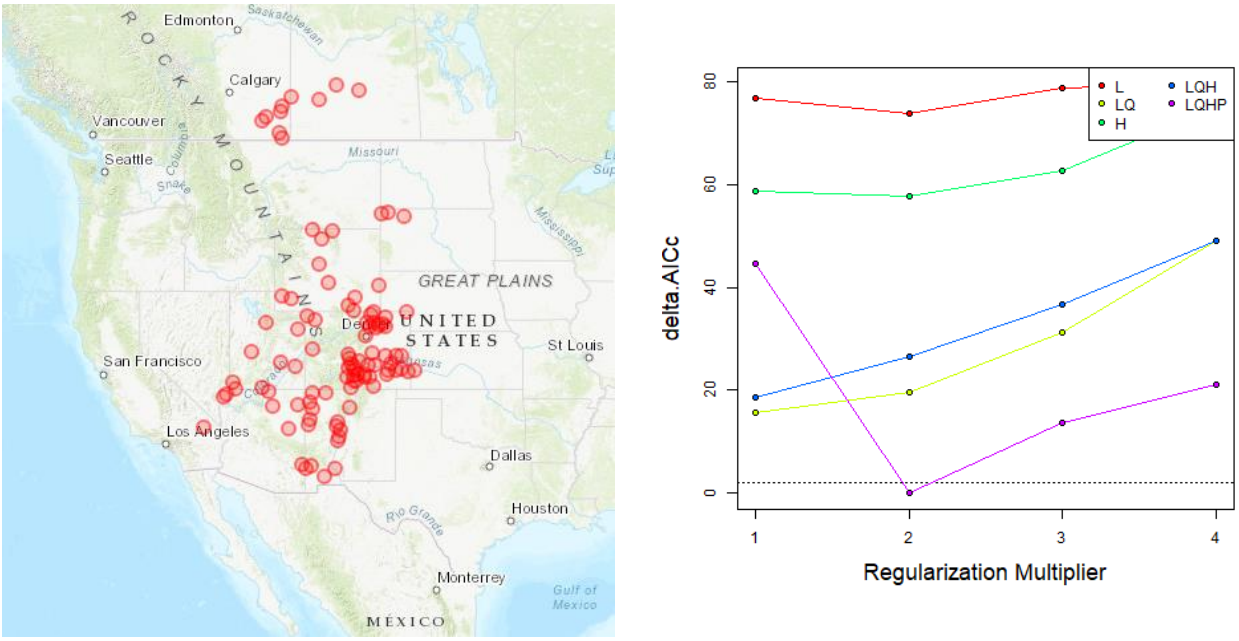


Figure 1. Occurrence point data, after data cleaning, input of additional Canadian points, and 20 km spatial thinning (left). Delta AICc across Maxent feature classes and regularization multipliers (right). Note that LQPH(2) (purple) minimizes AICc across tested model arrangements.

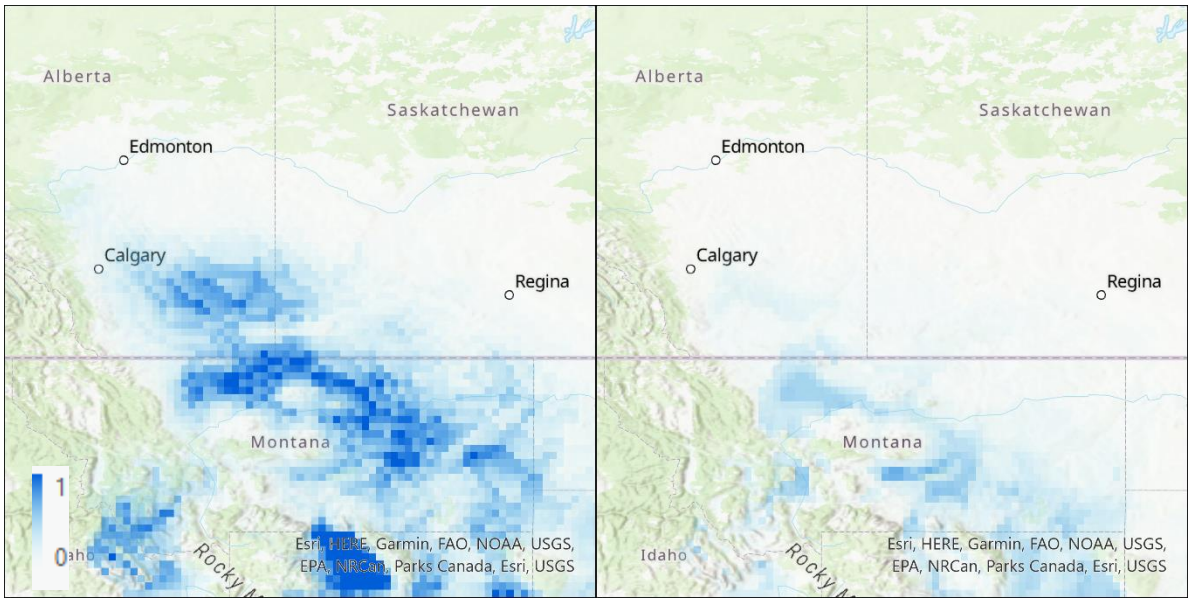
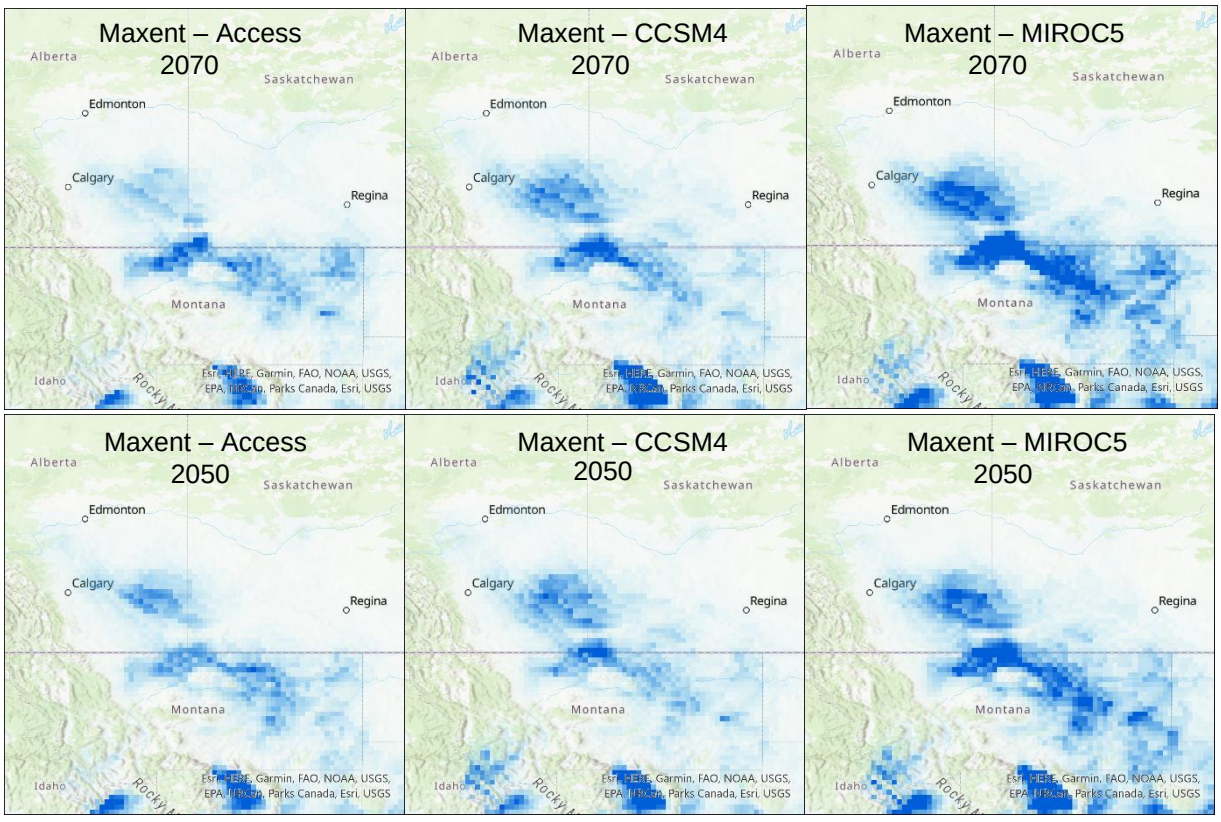


Figure 2. Current environmental suitability in cumulative log-log (cloglog) for Maxent-based model (left) and BIOCLIM-based model (right). Dark blue represents highly suitable environments.



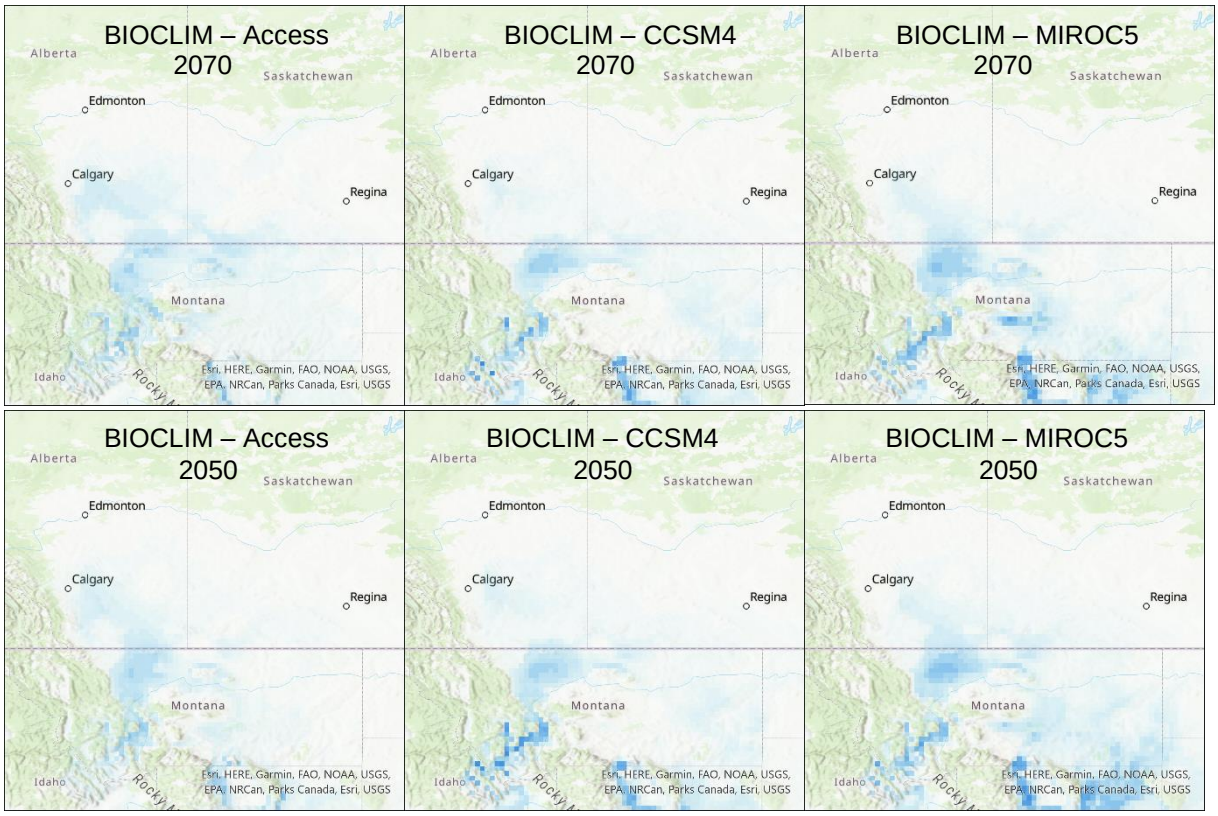


Figure 3. Future estimations of environmental suitability produced from Maxent and BIOCLIM Wallace models. Dark blue represents highly suitable environments with a value near '1'.

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Assessing natural sources of ethanol in wild primate foods: Testing key predictions of the dietary exposure hypothesis

Type of project: 530
Supervisor(s): Dr. Robert Barclay and Dr. Amanda Melin (co-supervisor)
Department: Biological Sciences

About the researcher

Julia Casorso

I am a sixth-year undergraduate student completing a combined degree in Honours Ecology and Honours Anthropology (BSc). My main interests are conservation biology, animal behaviour, and evolutionary anthropology, and I plan to go on to graduate school to study primate conservation. Outside of school, I grew up as a competitive dancer and enjoy teaching dance to kids. I also love to explore nature and find new ways to live sustainably.

I got involved in research because I needed help deciding what I wanted to do in life. I started my first research project in paleoanthropology, and while I enjoyed the work, I knew it wasn't for me. I then found my passion for primates and fieldwork when I attended a field school studying wild howler monkeys in Belize. Knowing I wanted a career in primatology, I wanted to get more research experience in this field to help prepare for graduate school and future jobs. To anyone wanting to get involved in research, don't be afraid to reach out to professors!

Background

Although human-directed ethanol fermentation began 12,000 years ago (Katz & Voigt, 1986), ethanol also occurs naturally in fruits and nectars (Spencer & Spencer, 1997). This suggests frugivorous animals, such as primates, have been consuming

ethanol over evolutionary time. Primates have been documented to seek out ethanol in the wild (Hockings et al., 2015; Wiens et al., 2008) and in captivity (Ervin et al., 1990; Juarez et al., 1993). They may use ethanol odours to optimize foraging decisions, given that primates are incredibly sensitive to ethanol odours (Laska & Seibt, 2002) and are often observed to sniff fruits before consuming them (Dominy, 2004). Most notably, humans and African great apes (chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*), and gorillas (*Gorilla gorilla*)) have a 40-fold higher efficiency for metabolizing ethanol relative to most other primates and mammals, due to a shared mutation in the alcohol dehydrogenase 4 (*ADH4*) gene acquired by our last common ancestor (LCA) approximately 10 million years ago (Carrigan et al., 2015). The selective pressures that facilitated enhanced metabolic efficiency for ethanol in our LCA are currently unresolved. The dietary exposure hypothesis posits that increased exposure to dietary ethanol selected for this adaptation in our LCA. For example, it predicts that fallen fruits are more likely to be in advanced stages of fermentation (i.e. higher ethanol content) and that fruits in Africa contain more ethanol than fruits in other primate habitats around the world, which our increasingly terrestrial hominid ancestors may have encountered more frequently (Carrigan et al., 2015).

The study of living primates – our closest living relatives – and their foods is an effective way to test the dietary exposure hypothesis, as the diets of wild, frugivorous primates may be similar to those of our early ancestors (Carrigan et al., 2015). However, the relative amounts and sources of variation of ethanol that occur naturally in wild fruits are poorly known. To help fill this knowledge gap, our research team has investigated ethanol production in wild fruits in Costa Rica at a field site where we study white-faced capuchin monkeys (*Cebus imitator*). Here, I will present data on ethanol levels in these wild fruits and investigate associations between ethanol production and capuchin foraging behaviour – notably, whether capuchins consume ethanol in dietary fruits and whether they use ethanol as an odour cue to optimize foraging decisions.

Methods

Spanning a full year, habituated capuchins at Sector Santa Rosa in Área de Conservación Guanacaste, Costa Rica were followed and the different fruit species they consumed were collected. To measure ethanol in fruits, a fruit sample was placed in a plastic bag blown up with air and left to incubate for over one hour to allow for any ethanol vapours to equilibrate with the air. The air within the bag was then collected with a syringe and pushed through a Mark V breathalyzer adapted with a plastic tube to obtain a blood alcohol content (BAC) reading. A series of known ethanol standards (0.1%, 0.5%, 1%, and 2% alcohol by volume (ABV) in water) were simultaneously

measured to create a standard curve, which was used to convert the BAC of each fruit sample to %ABV. To investigate associations with capuchin foraging behaviour, long-term data previously collected on the diets (Melin et al., 2014) and sniffing behaviour while foraging (Melin et al., 2019) of these capuchins were used.

Results

Ethanol production was present in 81.0% of dietary fruits (n = 42 species) and 87.1% of preferred fruits (n = 31 species), or those that capuchins consumed more frequently than expected given their abundance. Ethanol levels could get as high as 2.02%, but ranged between trace amounts and 1% ethanol, on average, for most fruits. Furthermore, 92.3% of fruit species that capuchins sniffed prior to eating or rejecting them (n = 26 species) produced ethanol. However, there was no significant relationship between mean ethanol content and the proportion of fruits capuchins sniffed out of all fruits they investigated for a species (Zero-inflated generalized linear model, $p > 0.05$).

Significance

These results indicate that capuchins consume ethanol at relatively low concentrations. Capuchins may consume and prefer ethanol-producing fruits because ethanol is a reliable indicator of sugar content (Pesis, 2005) and the caloric value of ethanol is almost twice that of carbohydrates (Dominy, 2015), providing a substantial reward. Conversely, there was no relationship between ethanol content and sniffing frequency. Given that fruits produce an array of volatiles upon ripening (Nevo et al., 2018), capuchins may be assessing the overall scent profile of fruits to identify ripeness rather than focusing primarily on ethanol. Regardless, capuchins sniffed many ethanol-producing fruits, suggesting they still make use of ethanol odours. For instance, capuchins also rely on tactile (Melin et al., 2009) and visual (Melin et al., 2014) signals of fruit ripeness, so they may use ethanol odours in combination with these other foraging strategies.

Overall, these results provide preliminary support for the dietary exposure hypothesis. Capuchins are arboreal, Neotropical primates that preferentially consume ripe fruits in trees (Melin et al., 2014), while African great apes are more terrestrial frugivores that have been observed to collect and consume dropped fruits (Amato et al., 2021). According to the dietary exposure hypothesis, this would imply that African great apes are consuming higher levels of ethanol and their *ADH4* gene is under greater selective pressure relative to capuchins. The relatively low concentrations of ethanol in capuchin diets and the fact that they do not possess the *ADH4* mutation for enhanced

ethanol metabolism (Carrigan et al., 2015) supports this hypothesis. The next step for this project is to measure and compare ethanol levels in fruits consumed by primates in other locations, especially Africa, to better test the dietary exposure hypothesis.

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Where to find out more on the project?

Research presentation on ethanol production in commercial fruits and wild fruits consumed by capuchins at Sector Santa Rosa

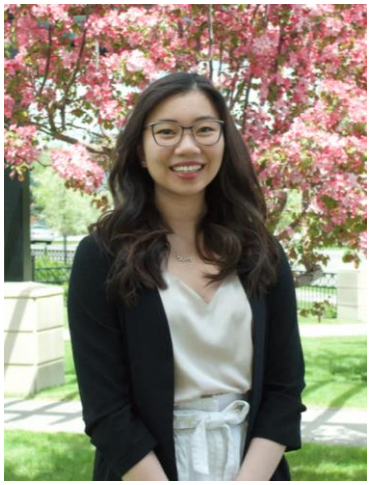
News article on the evolutionary foundations of ethanol metabolism in humans relative to other mammals

The utilization of biocides on sulfate-reducing bacteria to mitigate oil souring

Type of project: 528
Supervisor(s): Dr. Lisa Gieg and Dr. Gloria Okpala
Department: Biological Sciences

About the researcher

Bethany Chan



I am a 4th-year science student currently obtaining a degree in CMMB with a minor in Visual Studies & Art History. My favourite courses have been CMMB 431: Bacterial Pathogens and CMMB 527: Immunology due to their applications in the real world. Although they were difficult, I found them to be my favourite CMMB courses as they gave me a better understanding of the benefits and drawbacks of microbial presence in humans and the environment. During my free time, I enjoy acrylic painting and watching Korean dramas. I found that this microbial souring project helped me better understand what Alberta is currently up to in terms of their oil and gas resources, research, and problems. In addition, I found it interesting to learn about how such tiny microbes can wreak havoc and lead to companies losing billions of dollars due to their existence.

Background

Oil reservoir souring due to the activity of sulfate-reducing microbes (SRM) has become an increasing concern in the oil industry as the formation of sulfide by SRM is toxic, harmful to the environment, and corrosive to pipelines (Johnson et al., 2017). In

addition, the combustion of sulfur compounds can lead to the production of sulfur oxides, of which can react with moisture in the air and lead to acid rain, or low pH fogs that can have adverse effects on buildings and the environment (Johnson et al., 2017). The corrosive properties of sulfur also increase the corrosion rate of oil pipelines, costing companies billions of dollars to fix if the pipeline were to fail. As preventative measures, oil and gas companies have been testing ways to mitigate oil souring, increasing the overall project cost by an estimated 2% (Johnson et al., 2017). The process of sulfate reduction by SRM is well-documented, though optimal ways to mitigate SRM in oil reservoirs are still being researched today.

The objective of this study was to observe the individual effects of nitrate and the biocides glutaraldehyde (GLUT), benzalkonium chloride (BAC), and sodium nitroprusside (SNP) on their ability to inhibit the souring of oil by targeting SRM. Thus, based on the literature, it is hypothesized that the addition of nitrate or biocides to oil will be able to reduce sulfide levels through the targeting of SRM.

Methods

Anoxic Coleville synthetic brine medium K 2 sulfate (CSBK-2S) was made using a procedure from Callbeck et al. (2013). Sulfide was added in last to help establish a sour environment in the bioreactors. Volatile fatty acids (VFA) were added to the medium as an electron donor. The procedure for creating ten anoxic, sand-packed bioreactor systems was taken and modified from Xue & Voordouw (2015). A three-way Luer-Lock valve was assembled to the lower and upper openings of the bioreactor columns, where the bottom valve was the inlet, and the top was the outlet to allow for sampling. Pore volume (PV) was calculated by subtracting the wet weight of water-flooded columns from the dry weight of the bioreactors. The bioreactors were then inoculated with SRM that were enriched from pipeline sludges and were left to incubate under closed, anoxic conditions at room temperature, until approximately equal amounts of growth were observed. Nitrate and the biocides GLUT, BAC, and SNP were used as treatments to inhibit sulfide production by SRM. Nitrate was tested using the concentrations of 170 ppm, and 340 ppm, GLUT and BAC were individually tested at 50 ppm, and 100 ppm, and SNP was tested at 7.5 ppm, and 15 ppm. A sulfide assay (Trüper & Schlegel, 1964) was performed on each sample. High performance liquid chromatography (HPLC) was performed to determine sulfate, nitrate and nitrite concentrations in each sample obtained.

Results

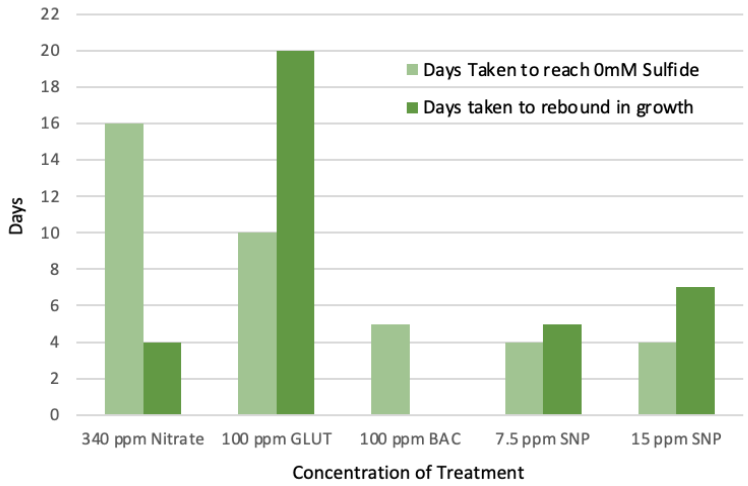


Figure 1. Summary bar graph comparing 15 ppm SNP and the lowest treatment concentrations that inhibit sulfide production (340 ppm nitrate, 100 ppm GLUT, 100 ppm BAC, 7.5 SNP). Light green bars represent the number of days taken for the treatment to reach ~ 0 mM sulfide. Dark green bars represent the number of days taken for the SRM to rebound back to pre-treatment sulfide concentrations after the respective treatment was removed.

The results obtained showed that the fastest to slowest acting treatments were: 100 ppm of BAC, 7.5 ppm of SNP & 15 ppm of SNP, 100 ppm GLUT, and 340 ppm of nitrate (Figure 1). More specifically, there was a 3.2-fold difference between the rates of the fastest (BAC) and slowest (nitrate) acting treatments as well as a 3.4-fold difference in concentration where nitrate was higher (Figure 1). In contrast, when the treatments were removed, the fastest to slowest rebound growth was seen in the 340 ppm nitrate, 7.5 ppm SNP, 15 ppm SNP, GLUT, BAC (no growth) bioreactors (Figure 1). Lastly, the highest to lowest concentration of biocide used was 340 ppm nitrate, 100 ppm GLUT & 100 ppm Bac, 15 ppm SNP, and 7.5 ppm SNP, where there was a 45-fold difference between the highest and lowest treatment concentrations used in this study (Figure 1). Overall, the 100 ppm BAC treatment worked the fastest, 100 ppm BAC took the longest to rebound (being that no growth was seen), and 7.5 ppm SNP was the lowest concentration of a biocide used that successfully inhibited sulfide.

Significance

This research is being used to help determine the best treatment that an oil and gas company can use to reduce oil souring in pipelines and oil reservoirs. Specifically, this research tested the abilities of nitrate and the biocides GLUT, BAC, and SNP at

varying concentrations on their ability to mitigate sulfide production by targeting SRM. Overall, it can be said that the best treatment to use is 100 ppm BAC if the full eradication of SRM is desired. However, if a partial biocidal effect is desired, the next best treatment would be 15 ppm of SNP. If no biocidal effects are desired, the best treatment would be 100 ppm of GLUT. In contrast, although nitrate was the slowest acting and had the quickest rebound time after the treatment was removed, it would be the best suited treatment if microbial biocide resistance (MRB) was a concern. This is because nitrate can target SRM sulfide production via 3 main mechanisms (production of nitrite, competition for the same electron donors, and sulfide oxidation). Future work should go towards observing the lowest concentrations of biocides that can be used to inhibit sulfide production, observing which synergistic pairing is most efficacious, and if these treatments have bactericidal or inhibitory effects on SRM. A cost analysis of the various treatments may also be useful for helping oil and gas researchers determine which treatment is the best option for a certain oil reservoir.

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The roles of Glyoxalase 1 and maternal diabetes in neuronal migration and its implications in autism spectrum disorder

Type of project: 528
Supervisor(s): Dr. Guang Yang and Dr. Elmar Prenner (co-supervisor)
Department: Biochemistry and Molecular Biology

About the researcher

Michelle Hua

Hello! I was a Biochemistry major and graduated in May 2021. I believe that my experience as a post-secondary student has also given me the opportunity to develop strong verbal and written communication skills and the ability to multitask and work well in a multidisciplinary team environment.

In my spare time, I like to try out new restaurants and watch horror movies. In the 2020-2021 academic year, I held the role of President for the BSA. In this role, I aimed to promote a sense of community within the department of Biological Sciences and to help foster an environment for like-minded students.

Advice I would give to someone looking to start a research project is to be persistent and not to get discouraged by lack of results!

Background

Autism spectrum disorder (ASD) is a neurodevelopmental disorder hallmarked by different levels of cognitive and functional impairment, restricted interests, and repetitive behaviour. While ASD presents characteristic features at the behavioral level, its underlying causes are heterogeneous and still largely unknown. Genetic and environmental factors – namely, the interplay between the GLO1-methylglyoxal pathway and gestational diabetes has been implicated in the etiology of ASD. Genetic deficiencies in the *GLO1* gene or the presence of metabolic disorders such as gestational diabetes results in elevated levels of MG precursors, thus resulting in the accumulation of MG. Elevated MG levels lead to the production of advanced glycation end products (AGEs) which act to induce oxidative stress and mitochondrial dysfunction.

Previously, studies have reported that perturbations of this pathway may affect neuronal migration and neural circuitry in the early stages of development (Figures 1, 2, 3). Aberrant neurodevelopment has long been associated with ASD and it is thought to be a potential causative factor underlying ASD, leading to the functional and cognitive impairments commonly observed in patients.

We hypothesize that perturbations of the GLO1-methylglyoxal pathway will result in an increase in intracellular methylglyoxal levels, which in turn, will negatively impact neuron migration and their morphological transition from multipolar to bipolar (Figures 3, 4, 5, 6). The objective of this study is to test this hypothesis by investigating if disruption of the ASD-susceptible gene *GLO1* via RNA interference regulates early neuronal development - specifically migration and the morphological transition of migrating neurons in the mouse cortex. This will also provide a greater understanding of the mechanisms underlying developmental anomalies in neurodevelopmental disorders such as ASD.

Methods

CD1 mouse embryos at E14.5 were used for *in utero* electroporation (performed by Dr. Guang Yang). Brains were then analyzed at P5 to verify GFP expression, cryosectioned and immunostained as described previously (Yang et al. 2016) (Figures 7, 8, 9). For immunostaining, sections were incubated with the primary antibodies rabbit anti-Tbr1 (1:500), mouse anti-Satb2 (1:1000) and chicken anti-EGFP (1:1000 and the secondary antibodies, AlexaFluor 488, 555 and 647 conjugated with donkey antibodies to chicken, rabbit and mouse IgG (Invitrogen). Nuclei were stained with Hoechst 33342 (1:5000, Invitrogen). A western blot analysis of HEK293 cells co-transfected with FLAG-tagged mouse *GLO1* with the *GLO1* shRNA or control shRNA was previously carried out to verify knockdown of *GLO1* expression (Figure 10). For quantification of distribution of cells, z–stacked images of the somatosensory cortex were collected with a 20X objective. Representative images of the somatosensory cortex used for quantifications were selected from three independent experiments for each condition (Figure 12, 13, 14). The number of EGFP-positive cells in was counted in columns within the cortex using Image J (NIH). Data was analyzed using Microsoft Excel (Microsoft, WA). Hoechst staining was used to define the boundaries of the CP and VZ which allowed for the division of the cortex into ten equally spaced bins (Figure 11). The distribution of Hoechst- and GFP-positive cells was determined by counting the number of neurons in each bin and represented as an average proportion (N=3). The proportion of EGFP-positive cells that were also immunostained positive for Satb2 and Tbr1 were counted in the same manner. All images were processed with Fluoview software (Olympus) and

ImageJ (NIH). All data were presented as bar plots with mean with standard deviation. Statistical analysis was performed with Microsoft Excel (Microsoft, WA). Two-tailed Student's t-test was used to determine statistical significance.

Results

In *GLO1* knockdown brains, most EGFP-positive cells were observed to be in layers V/VI rather than II-IV as seen in the controls (Figure 12, 13, 14). Statistically significant differences were observed in bin 1 (*p = 0.038), bin 2 (*p = 0.038), bin 3 (*p = 0.034), bin 6 (*p = 0.042), bin 7 (*p = 0.020) and bin 10 (*p = 0.049) (Figure 15). To further test this hypothesis, the proportion of EGFP-positive, Satb2-positive and EGFP-positive, Tbr1 positive cells was counted, and a statistical analysis was performed. This analysis revealed that there was a significant difference between the proportion of Tbr1/EGFP-positive (*p=0.031) cells between the GLO1 knockdowns and control groups, with the knockdowns having a significantly greater number of cells that are positive for both markers. Similarly, a significant difference was also measured between the control and knockdown Satb2/EGFP-positive cells with the control having a significantly greater number of cells that are positive for both markers (*p=0.031). In Figure 12 and 13, it can be observed that Satb2/EGFP-positive cells are abnormally located in layers V/VI rather than in layers II-IV (Figure 15). Thus, these analyses showed that Glo1 knockdown caused an altered distribution of EGFP-positive cells with decreases in total number of EGFP-positive cells in the layers II/IV.

Significance

Results obtained from this study exhibit preliminary evidence of the interactions between the GLO1-methylglyoxal pathway and the regulation of early neuronal development and its implications in ASD. It is thought that GLO1 capabilities are overwhelmed by increased methylglyoxal due to *GLO1* deficiency or the elevated presence of methylglyoxal precursors due to metabolic disorders – resulting in tissue damage. Data presented here is consistent with this idea. Proper GLO1 activity was found to play a critical role in maintaining proper neuronal migration in the developing cerebral cortex. GLO1 knockdown neurons were dispersed throughout the lower layers of the cortex rather than localized in layers II-IV as observed in the control. This is further supported by the statistical difference in the number of layer VI marker, Tbr1-positive/GFP-positive cells and upper/intermediate layer marker Satb2-postive/GFP-positive cells between the knockdown and control groups. This evidence suggests that this pathway is involved regulates neuronal migration. Thus, in light of epidemiological findings, this study provides insights into cognitive disorders such as ASD and

schizophrenia caused by human genetic variants of GLO1 that result in reduced enzyme activity (Junaid et al., 2004, Barua et al., 2011) and increased methylglyoxal as a result of metabolic disorders like gestational diabetes.

Figures

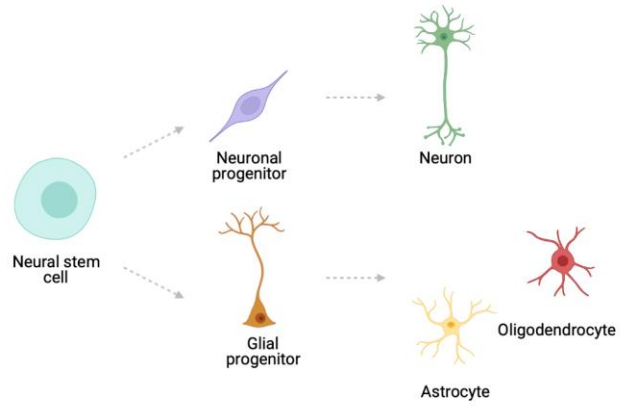

Figure 1. *Neural stem cell differentiation*. Glial progenitors have the potential to give rise to glial cells such as astrocytes or oligodendrocytes. Neuronal progenitors give rise to neurons. Figure generated in Biorender and adapted from Gines et al., 2009.

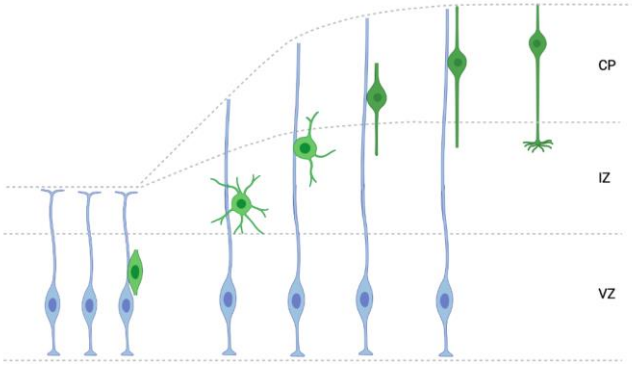

Figure 2. *Morphological transition of from multipolar to bipolar in neurons*. As neurons develop and become polarized, their morphology shifts from a multipolar shape characterized by multiple short projections to a bipolar morphology, whereby the neuron develops a leading process that will become the axon. Abbreviations: cortical plate (CP); intermediate zone (IZ); ventricular zone (VZ). Figure generated in Biorender and adapted from Kon et al., 2017.

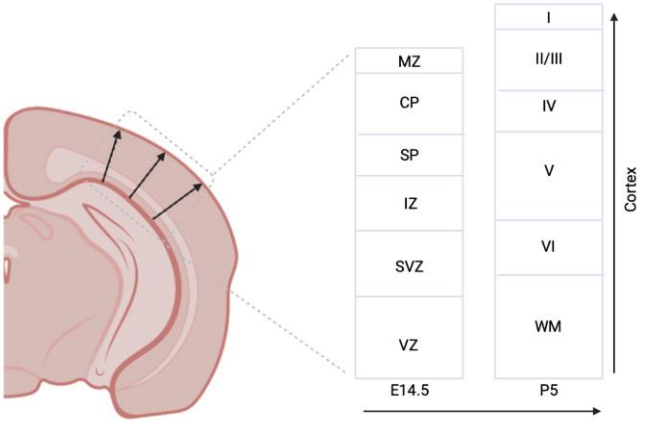

Figure 3. *Cortical development*. Schematic representation of the developing cerebral cortex at E14.5 and P5 in mice. Abbreviations: marginal zone (MZ); cortical plate (CP); subplate (SP); intermediate zone (IZ); subventricular zone (SVZ); ventricular zone (VZ); white matter (WM). Figure generated in Biorender and adapted from Gleeson and Walsh (2000).

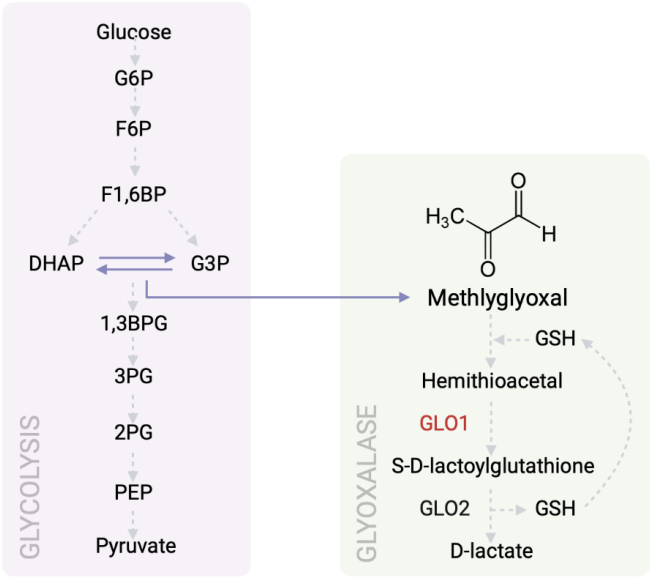

Figure 4. *Glycolysis and the GLO1-methylglyoxal* pathway. Methylglyoxal, a cytotoxic metabolite, from glycolysis (by-product) and other extracellular sources are detoxified through two sequential enzymatic reactions catalyzed by glyoxalase-1 (GLO1) and glyoxalase-2 (GLO2) using glutathione as a cofactor. GLO1 functions to convert hemithioacetal to the non-toxic compound, S-D-lactoylglutathione which is then converted into D-lactate. GLO2 recycles glutathione in this process, allowing for its reuse in following reactions. Abbreviations: glucose-6-phosphate (G6P); fructose-6-phosphate (F6P); fructose 1,6-bisphosphate (F1,6BP); dihydroxyacetone phosphate (DHAP); glyceraldehyde 3-phosphate (G3P); 1,3-bisphophoglycerate (1,3BPG); 3-phosphoglycerate (3PG); 2-phosphoglycerate (2PG);

phosphoenolpyruvate (PEP); glutathione (GSH); glyoxalase 1 (GLO1); glyoxalase 2 (GLO2). Figure adapted from Yang et al. (2016).

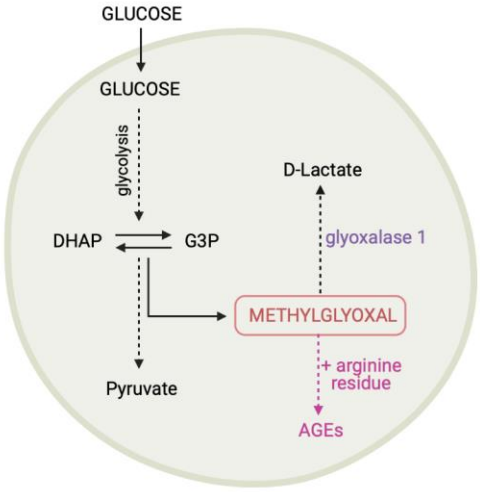

Figure 5. *Simplified overview of the formation and degradation of methylglyoxal and the production of AGEs*. Methylglyoxal (MG) is formed via the spontaneous degradation of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) formed as intermediate products of glycolysis. Methylglyoxal is detoxified via glyoxalase 1 (Glo1) activity. It can also form advanced glycation end products (AGEs) through modification of the amino acid arginine (and to a lesser degree lysine – this process is not shown in figure). Dashed lines indicate processes that were omitted. Abbreviations: dihydroxyacetone phosphate (DHAP); glyceraldehyde 3-phosphate (G3P). Figure generated in Biorender and adapted from Schalkwijk and Stehouwer (2020).

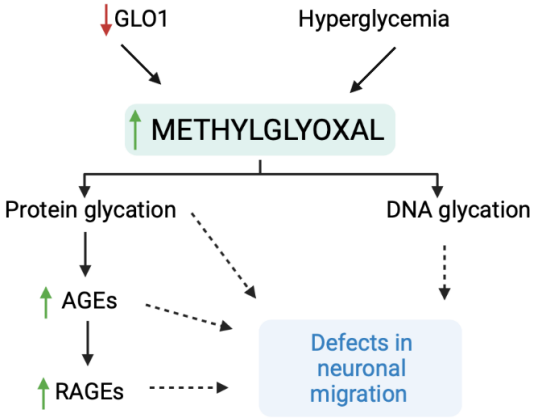

Figure 6. *Factors that potentially contribute to defects in neuronal migration and ASD pathogenesis*. An accumulation of methylglyoxal (MG) results from deficient glyoxalase a (GLO1) enzymatic activity or elevated levels of precursors as a result of metabolic disorders

such as hyperglycemia. Excess MG results in protein and DNA glycation, the former of which leads to the production of advanced glycation end products (AGEs) and receptors for advanced glycation end products (RAGEs). This has been found to result in defects in neuronal migration, however the molecular mechanism by which this occurs is unknown (as represented by dashed lines). Figure generated in Biorender and adapted from Ramasamy et al. (2006).

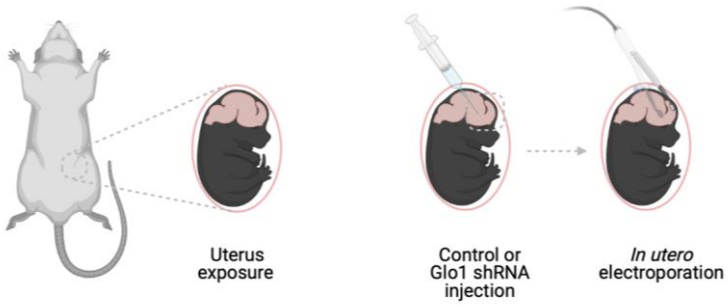

Figure 7. *In utero* electroporation**.** Schematic representation of the *in utero* electroporation procedure. Cortex was *in utero* electroporated on E14.5 with plasmids expressing EGFP. pEF1α-EGFP (1ug/ul) with control or Glo1 shRNA plasmids (3ug/ul) (1:3 ratio) for a total of 4 µg/µL was injected. 0.5% Trypan blue was used as a tracer. Figure generated in Biorender.

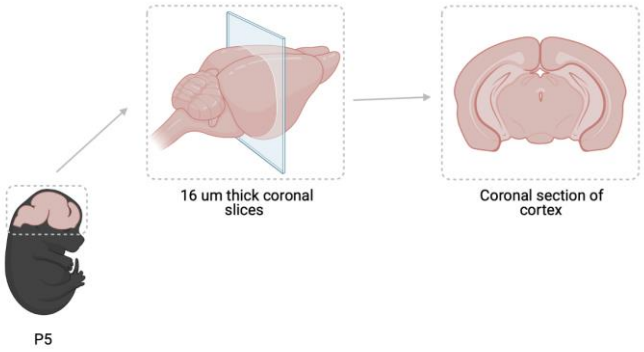

Figure 8. *Cryostat sectioning*. Schematic representation of cryostat sectioning procedure. P5 embryonic brains were dissected in ice-cold HBSS. The brains were then fixed in 4% PFA in PBS overnight, cryoprotected using 30% sucrose overnight and cryosectioned coronally at 16 µm and embedded in OCT (Leica). Figure generated in Biorender.

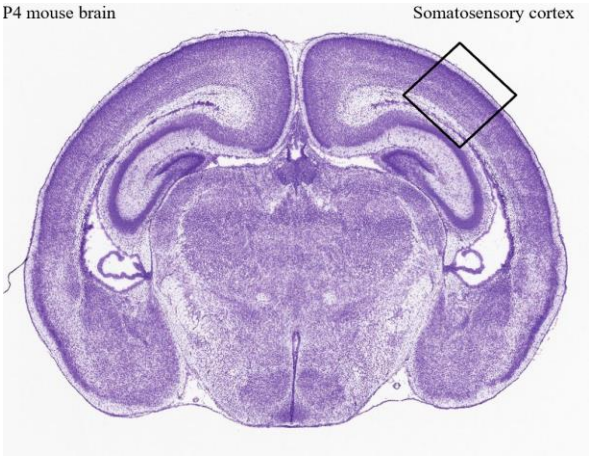

Figure 9. *Coronal section of somatosensory cortex in P4 mouse brain*. Figure from Allen Developing Mouse Brain Atlas.

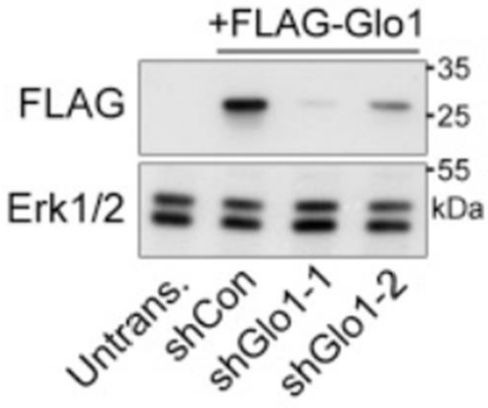

Figure 10. *Confirmation of Glo1 knockdown via RNA interference by Yang et al. (2016)*. Western blots of HEK293 cells co-transfected with FLAG-tagged mouse Glo1 encoded plasmids without one of two Glo1 shRNAs (shGlo1-1 and shGlo1-2) or a control shRNA (shCon), probed with anti-FLAG and reprobed with anti-Erk1/2. Untransfected (Untrans.) cells served as a control. Figure and caption taken with permission from Yang G. *et al.,* 2016.

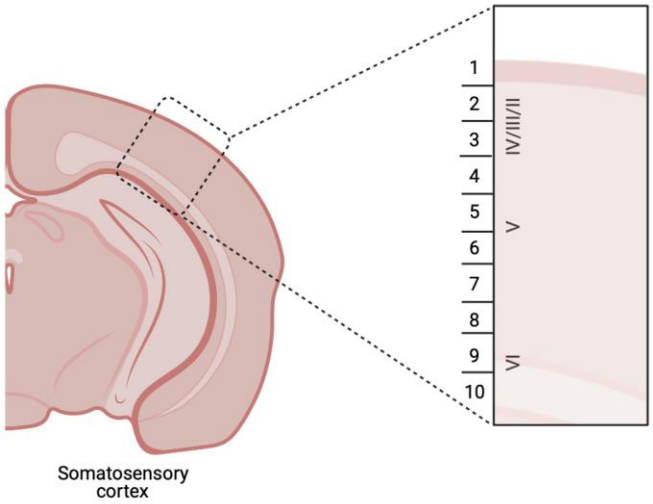

Figure 11. *Somatosensory cortex and division into bins for statistical analysis*. Box is indicative of area imaged (z–stacked images were collected with a 20X objective on an Olympus FV3000 confocal microscope). Hoechst staining was used to define the boundaries of CP (minimum) and VZ (maximum) which allowed for the division of the cortex into ten equally spaced bins. Approximate spacing of layers is indicated. Figure generated in Biorender.

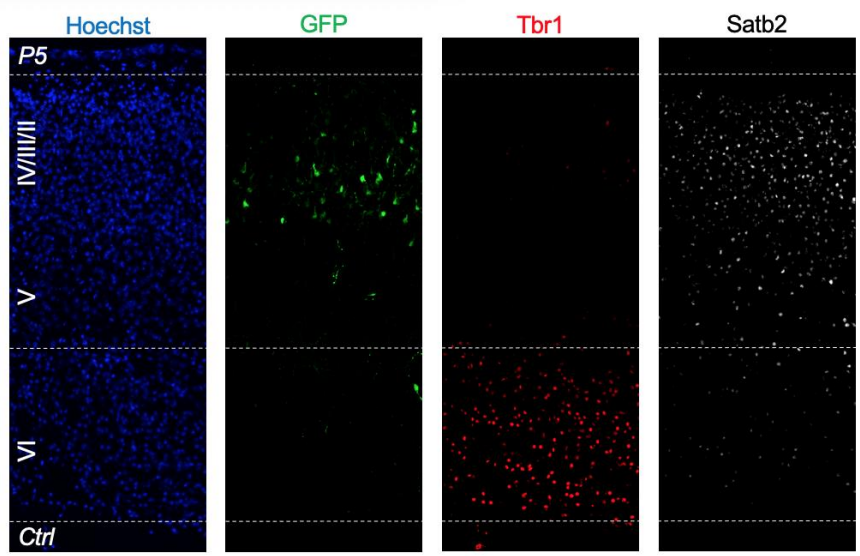

Figure 12. *Representative images of P5 mouse somatosensory cortex electroporated with control vector and GFP*. Left to right: Hoechst33342, anti-GFP; Alexa Fluor 488, anti-Tbr1; Alexa Fluor 555, anti-SATB2; Alexa Fluor 647. Z–stacked images were collected with a 20X objective on an Olympus FV3000 confocal microscope and projected with maximum intensity using ImageJ Software.

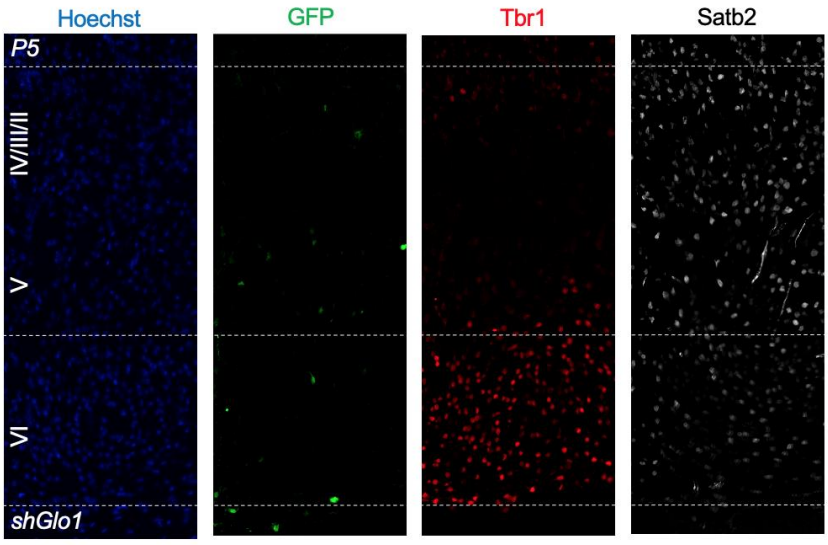

Figure 13. *Representative images of P5 mouse somatosensory cortex electroporated with shRNA against Glo1 and GFP*. Left to right: Hoechst33342, anti-GFP; Alexa Fluor 488, anti-Tbr1; Alexa Fluor 555, anti-SATB2; Alexa Fluor 647. Z–stacked images were collected with a 20X objective on an Olympus FV3000 confocal microscope and projected with maximum intensity using ImageJ Software.

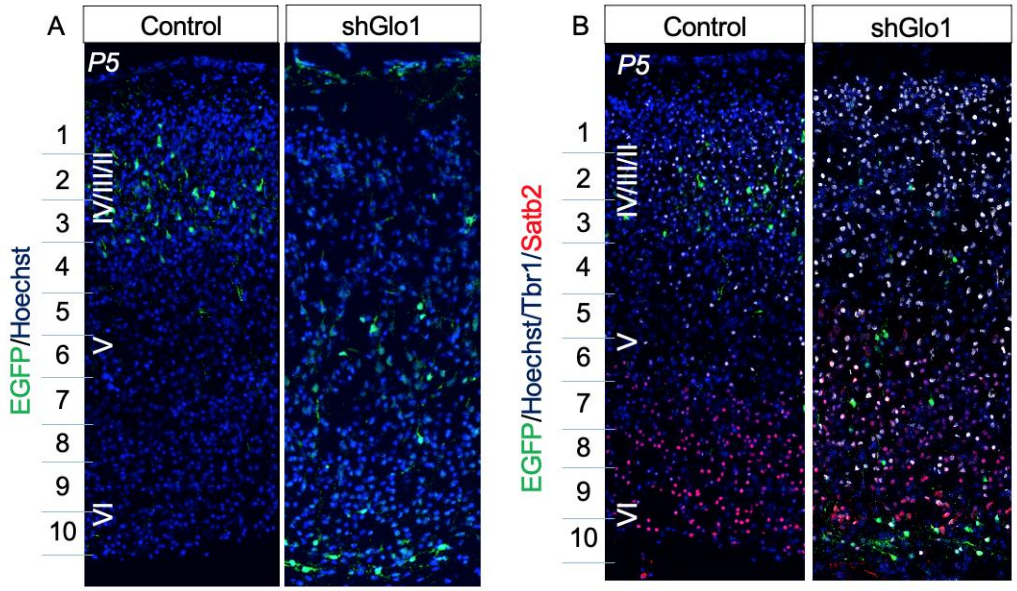

Figure 14. *Representative images of P5 mouse somatosensory cortex electroporated with control vector and GFP (left) or with shRNAs against Glo1 and GFP (right).* Z–stacked images were collected with a 20X objective on an Olympus FV3000 confocal microscope and projected with maximum intensity using ImageJ Software. **(A)** Hoechst33342 and anti-GFP; Alexa Fluor 488 channels were merged to generate image using ImageJ Software. **(B)** Hoechst33342, anti-

GFP; Alexa Fluor 488, anti-Tbr1; Alexa Fluor 555 and anti-SATB2; Alexa Fluor 647 channels were merged to generate a composite image using ImageJ Software.

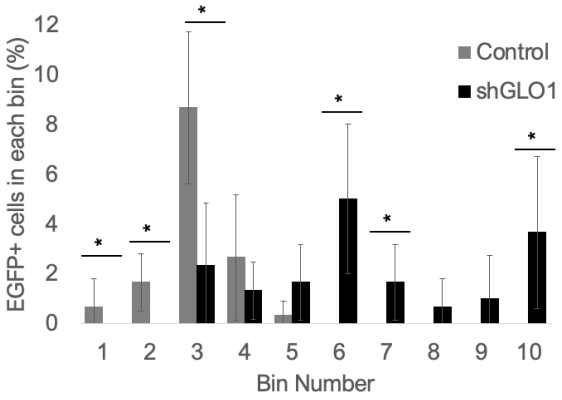

Figure 15. *Distribution of Hoechst/EGFP-positive cells in GLO1 knockdown and control*. Graphs represent the average proportion of GFP-positive cells in each bin. Grey bar represents Control: N = 3 brains. Black bar represents Glo1 knockdown: N = 3 brains. The cortex in the images were divided into 10 equally spaced bins (bin 10, deepest; bin 1, most superficial). Statistically significant differences were observed in bin 1 (*p = 0.038), bin 2 (*p = 0.038), bin 3 (*p = 0.034), bin 6 (*p = 0.042), bin 7 (*p = 0.020) and bin 10 (*p = 0.049). Error bar indicates mean ± SEM. Student's Two-ended unpaired t test: *p < 0.05.

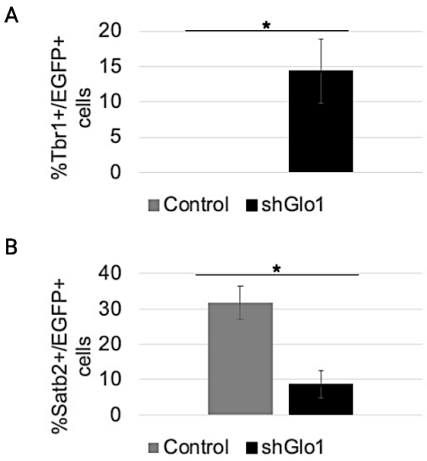

Figure 16. *Distribution of Tbr1/EGFP-positive and Satb2/EGFP-positive cells in GLO1 knockdown and control*. (A) Graph represents the average proportion of Tbr1/EGFP-positive cells. Statistically significant differences were observed (*p = 0.031). (B) Graph represents the average proportion of Satb2/EGFP-positive cells. Statistically significant differences were observed (*p = 0.031). Grey bar represents Control: N = 3 brains. Black bar represents *GLO1*

knockdown: N = 3 brains. Error bar indicates mean ± SEM. Student's Two-ended unpaired t test: *p < 0.05.

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Microplastics partitioning between biosolids and final effluent in a Calgary tertiary wastewater treatment plant

Type of project: 528
Supervisor(s): Dr. Sean Rogers and Dr. Leland Jackson (co-supervisors)
Department: Biological Sciences

About the researcher

Paige V. Jackson

I am in my fifth year completing my Bachelor of Science Biological Sciences Honours degree. My scientific and research interests include plastics in the environment, sustainability and conservation practices, and microplastics in freshwater environments. On my own time, I enjoy reading, playing squash, hiking, and being in nature. My independent research project focused on microplastics in wastewater, a topic of great interest to me. Over the course of this project, I enjoyed having an opportunity to explore my interest in plastics and gain new research experiences from concept, through design and execution, to results. This project taught me about the immense opportunities possible through research and the vast variety of follow-up questions that derived from a single research topic. This project was exciting for me as this is a novel area of research and I learned new things daily from not only my own data and results, but also from emerging literature throughout the course of this project.

Background

Plastic has been classified as an emerging contaminant of concern as mass amounts of plastic are accumulating on ocean surfaces and incorporated into aquatic sediments (Urbanek et al. 2018). Microplastics are defined as pieces of plastic 250 µm – 5 mm in size (Ma et al. 2019; Ryan, 2015) and are classified as fibres, films, foams, fragments, nurdles, and microbeads (Edo et al. 2019). Microplastics negatively affect

aquatic organisms that mistake plastic for food and lead to alterations in feeding and reproductive behaviour, leaching of chemicals from the plastics such as phthalates, adsorption of chemicals and pollutants onto their surfaces and transportation, and promotion of biofouling (Cole et al. 2015; Gallo et al. 2018; Qu et al. 2018; Verla et al. 2019). Microplastics are present in marine and freshwater environments; however, most research has been conducted in marine systems, leaving little known about the extent, fate and impacts of freshwater microplastics (Zbyszewski & Corcoran, 2011). Wastewater treatment plants (WWTPs) are a point of concentration of microplastics (Kang et al. 2018). Samples were collected from a tertiary WWTP in Calgary to quantify and categorize freshwater microplastics in wastewater coming from municipal and industrial sources in influent, final effluent, and biosolids. The aim was to understand how the plastics are being partitioned between the two outflows and quantify unremoved microplastics.

Methods

There is no standard method to sample, quantify and analyse microplastics. Therefore, a method was devised that used aspects from previously published methods appropriate for wastewater samples (Conley et al. 2019; Kang et al. 2018; Li et al. 2018; Mahon et al. 2016). Influent and final effluent wastewater and biosolid samples were collected weekly for eight weeks and frozen until sample processing. Three samples of Milli-q water were collected to serve as methodological controls to determine if any contamination existed from the method. Glassware was used where possible but was not always an option. Frozen samples were thawed in a refrigerator, then sieved through a 250 µm sieve to obtain the correct size fraction. The collected material underwent a wet peroxide digestion with 50% H_2O_2 to digest all organic matter in the samples. Following digestion, samples were vacuum filtered onto glass fibre filters (47 mm, 1.2 µm pore size) and dried. A dissecting microscope was used to count and categorize the microplastics. A Pearson's Chi-Squared test for homogeneity was performed to determine any difference in the proportions of plastics. An analysis of variance (ANOVA) was performed to determine differences in the plastic categories between the sampling locations, with significant ANOVA results followed by a Tukey's Honestly Significant Differences to determine the location of significance.

Results

Based on calculations from the study period, the total number of microplastics exiting this WWTP was estimated to be 6.23 trillion pieces daily. Of the microplastics identified in the influent, 71% were identified in the final effluent and biosolids. Of the

microplastics leaving the plant, 99% were found in the biosolids and less than 1% were found in the final effluent. A mass balance analysis revealed that 29% of the microplastics detected in influent were 'missing' and removed elsewhere in the wastewater treatment process. Influent and final effluent samples were dominated by fibres (88,820/L in influent; 48/L in final effluent) followed by fragments (18,550/L in influent; 30/L in final effluent). Biosolid samples were dominated by fragments (43,322/L) followed by films (22,861/L). All Chi-Squared comparisons were significant, indicating a high degree of sample heterogeneity. All ANOVA comparisons were significant, with significant Tukey's HSD results in many cases with exception of the final effluent-biosolid comparison for fibres.

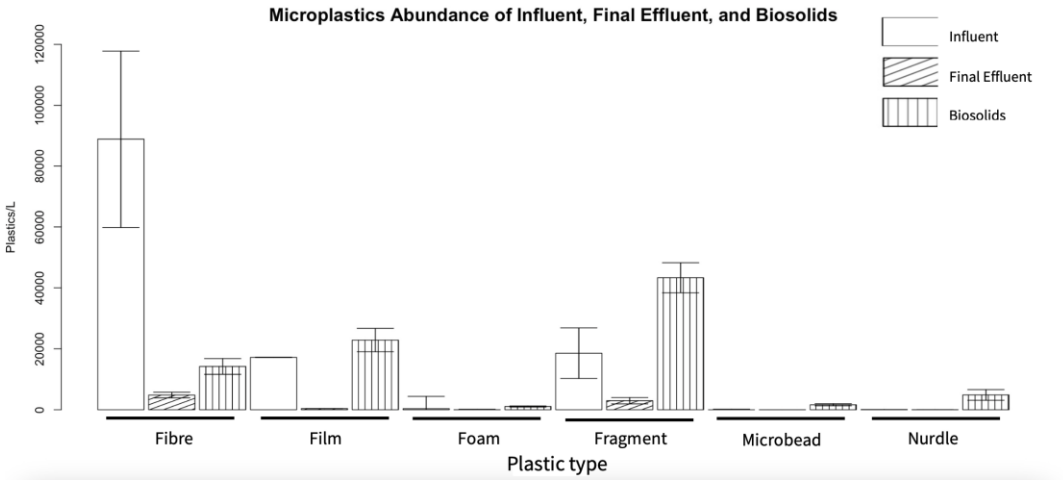

Figure 1. Average number of microplastics/L of influent, final effluent, and biosolids. Bars represent 1 standard error of the mean, n=8. Final influent is multiplied 100x to allow visualization.

Significance

Microplastics entering and exiting the WWTP in the influent, final effluent, and biosolids were quantified while providing an estimate of the plant's microplastic removal efficiency and identification of some dominant polymers. The microplastics leaving the WWTP may have implications for receiving environments since final effluent is discharged into the Bow River and biosolids are applied to agricultural fields as fertilizer (City of Calgary, 2021). Although the total number of microplastics in the final effluent was only 82/L, this may still be ecologically significant over time and these particles are likely highly bioavailable. The results show that microplastics are being discharged to aquatic and terrestrial environments and are not all being removed during the wastewater treatment process, and are being concentrated primarily into the biosolids.

The primary plastic type removed was fibres, and the dominance of plastic types differed between the influent, final effluent, and biosolids further showing removal. Organisms can consume microplastics in aquatic and terrestrial environments, causing issues. Microplastics have even been implicated in gene sharing between antimicrobial resistant bacteria at WWTPs (Sun et al. 2021). Future research could determine how microplastics are partitioned and removed within wastewater treatment processes and whether there might be options, for example with filtration, to target and optimize removal.

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Characterization of the sexually dimorphic on the Y-chromosome (sdY) locus in Coho Salmon (Oncorhynchus kisutch)

Type of project: 530
Supervisor(s): Dr. Sean Rogers
Department: Biological Sciences

About the researcher

Katie Jones

I am in my fifth and final year of my Bachelor of Science undergraduate degree, majoring in biological sciences with honours. I am very interested in the conservation of wildlife, specifically in aquatic systems. Some of my favourite scientific topics include the colour changing ability of cephalopods and the genetic and evolutionary relationships between different species. I have a passion for animal care and research involving animals, large and small. My interests outside of science include scuba diving, travelling, camping, and motorcycle riding. I also enjoy spending time with my dogs, rabbits, and lizards! Throughout conducting my research, I learned that ideas are constantly changing and adapting. I learned that sometimes the project you complete is not the exact same as the one you set out to complete because different questions arise, and unforeseen problems need solving. During this project, my collaborative abilities grew, and I was able to experience the amount of teamwork and cooperation that is present in biological research.

Background

Oncorhynchus kisutch (Coho salmon) are an important food and economic resource and their conservation is aided and managed by captive environments (hatcheries) (Bendriem et al., 2019). *O. kisutch* spend their juvenile lives in freshwater

and then undergo physiological and morphological changes (smoltification) as they migrate to salt water, and later return to freshwater streams as adults to spawn (NOAA Fisheries, 2020; McMahon & Holtby, 1992). Although the sex of returning adult fish can be determined by seeing if there are eggs or sperm inside, juvenile fish (smolts) are too young to see a phenotypic difference between males and females (King & Stevens, 2019). To effectively manage *O. kisutch* populations in hatcheries and the wild, it is important that we are able to genetically determine their sex since we cannot see if phenotypically in smolts (King & Stevens, 2019). The sexually dimorphic on the Y-chromosome (*sdY*) locus is likely the master sex-determining gene in salmon (Royle et al., 2018) and is highly conserved among salmonids (Yano et al., 2013). This locus has been used to determine genetic sex in many Pacific salmon species, including archeological samples (Royle et al., 2018). The presence of this marker indicates genotypically male and the absence indicates genotypically female (Royle et al., 2018). The aim of this study was to characterize the *sdY* locus in *O. kisutch*. To do this, this study had two main objectives: (1) to test the effectiveness of previously designed primers on *O. kisutch* to see if they accurately identify males and females and (2) to design and test new primers for the *sdY* locus in *O. kisutch* that are more effective at determining genetic sex.

Methods

Isolated DNA from adult *O. kisutch* (35 female and 21 male) from the Nitinat River hatchery on Vancouver Island, BC were used for PCR analysis. The *sdY* locus was amplified using previously designed *sdY* primer sequences alongside primers for the autosomal *clock1a* gene as an internal positive control (IPC) (Royle et al., 2018):

sdY-F19 (5′-CCCAACACCCTTCCTATCTCC-3′) and
sdY-R20 (5′-CCTTCCTCCCTAGAGCTTAAAAC-3′)
Clk1a-F50 (5’-TAGCCATGTCTGTGTGTTTACTTGC-3’) and
Clk1a-R60 (5’-GCAGCCAGCTAATTKGATTTG-3’)
New primers were also designed to target the exon 2 region of the *Oncorhynchus mykiss sdY* gene (Figure 1), since the *sdY* locus is not annotated in *O. kisutch*:
Set 1: Omy_sdY_KJ1F (5’-GCCCACAAGACCTCCCTAAT–3’)
and Omy_sdY_KJ2R (5’-CATCACAGGGTCCACATCAC-3’)
Set 2: Omy_sdY_KJ3F (5’-GGGTGATGTCAGAATTGCCC-3’)
and Omy_sdY_KJ4R (5’-TCACGTGCATCTCATCTCCG-3’)
Set 3: Omy_sdY_KJ5F (5’-GTGATGTCAGAATTGCCCACA-3’)
and Omy_sdY_KJ6R (5’-CTCCATCACGTGCATCTCATC-3’)

All PCR reactions had a total volume of 10 µL and consisted of water, forward and reverse primers, the sample DNA, and a master mix containing buffer solution, deoxyribonucleotide triphosphates (dNTPs), and *Taq* polymerase. All PCR reactions consisted of 5 steps adapted from Royle et al (2018). Step 1: Run for 15 minutes at 95˚C. Step 2 (denaturation): Run for 30 seconds at 95˚C. Step 3 (annealing): Run for 30 seconds at 54˚C. Step 4 (extension): Run for 40 seconds at 72˚C. Steps 2-4 were repeated 30 times before proceeding to step 5. Step 5: Run for 10 minutes at 72˚C. Negative PCR controls were included to monitor for contamination. The results obtained by PCR were then visualized by electrophoresis on a 3% agarose gel and compared against a 100bp ladder.

Results

The primers from Royle et al. (2018) had about a 50% accuracy at identifying genetic sex in the Nitinat River *O. kisutch* samples (Figure 2). With these primers, both false males and false females were obtained (Figure 2).

Initially, all three newly designed primer sets demonstrated the same result: all males amplified a clear *sdY* band, 1 out of 4 females also amplified this *sdY* band, and the remaining females had no amplification. Overall, the new primers demonstrated good amplification of *sdY* in all males, and only slight misidentification of females.

When primer set 1 was tested on a larger sample set, all males and all females amplified an *sdY* band, however the female bands were all less intense than the males (Figure 3). When primer set 2 was tested on the same large sample set, all males amplified an *sdY* band, but only 2 out of 24 females amplified this band (Figure 3). Overall, primer set two was 93% effective at identifying genetic sex, with very few incorrect assignments.

Significance

The Royle et al. *sdY* primers demonstrated non-specific amplification in the *O. kisutch* samples. If they had worked as expected, all males would amplify two bands (one for *sdY* and one for *clock1a*) and all females would amplify one band (for *clock1a*) (Royle et al., 2018). The most likely explanation for this abnormal amplification pattern is that the primers were not effective at amplifying *sdY* in *O. kisutch* because they were designed for salmonids in general using an alignment of sequences, and not specifically a single salmon species (Royle et al., 2018).

Newly designed primer set 1 had low levels of amplification in all females, indicating low levels of contamination which could be resolved by optimizing the PCR protocol. Primer set 2, however, was extremely effective at identifying genetic sex in *O. kisutch*. This indicates that *sdY* is still highly linked with sex in *O. kisuch* and that the primers designed in this study are more effective than previously designed primers at identifying sex in this population.

Other less likely explanations for this discordance include a non-functional copy of *sdY* being present in females, another locus determining sex in this population or other environmental or genetic factors influencing sex that are not yet understood. This study is important for understanding salmon evolution, improving hatchery practices, and in determining sex-specific survival differences in the future.

Figures

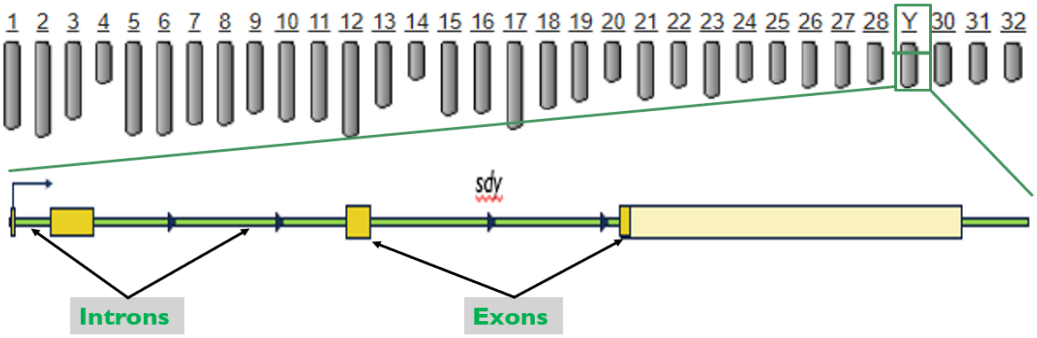

Figure 1. Male rainbow trout (*O. mykiss*) karyotype and schematic of the *sdY* gene (NCBI Resource Coordinators, 2018; Yates et al., 2020).

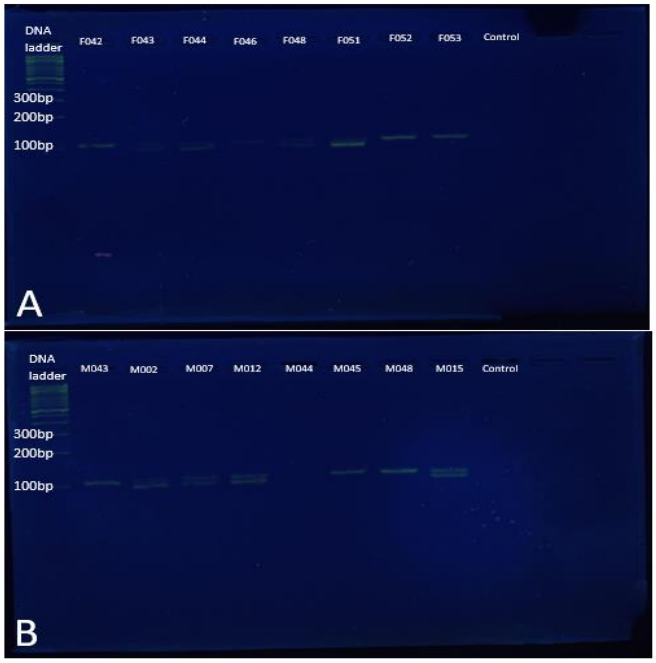

Figure 2. (A) 3% agarose gel containing all female samples and utilizing the *sdY* primers from Royle et al., 2018 (B) 3% agarose gel containing all male samples and utilizing the *sdY* primers from Royle et al., 2018.

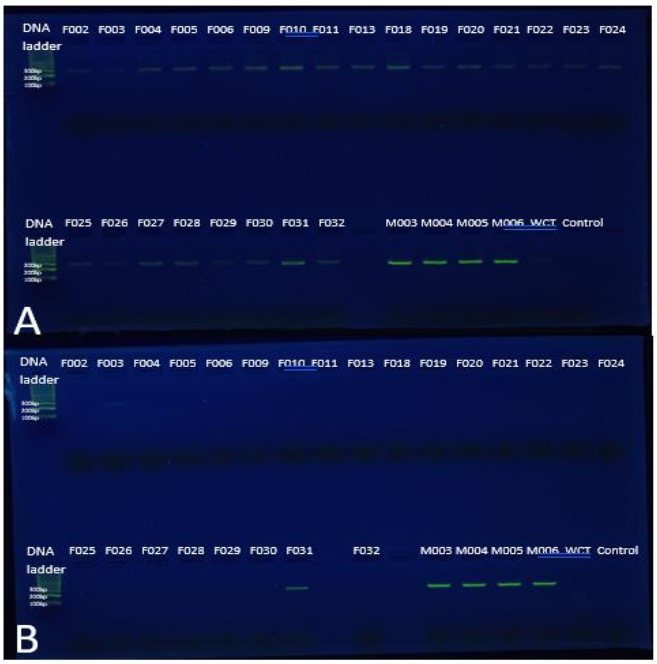

Figure 3. (A) 3% agarose gel utilizing the *sdY* primers from primer set 1. (B) 3% agarose gel utilizing the *sdY* primers from primer set 2. No *clock1a* control was visualized on gels A-B.

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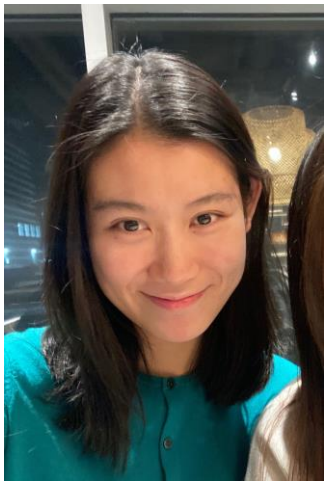
Using picture storybooks to communicate complex parasite lifecycles with undergraduates

Type of project: 530
Supervisor(s): Dr. Constance Finney and Dr. Mindi Summers (co-supervisor)
Department: Biological Sciences

About the researcher

Nikki Cheuk-Kei Kong



I am a fourth-year student at the University of Calgary studying towards an Honours Bachelor's degree in Zoology. I am particularly interested in the field of science communication as I believe it is important for scientists, academics and scholars to communicate their achievements with the general public. Not only do scientifically informed citizens make better decisions, but scientific misconceptions can often be reduced and addressed through effective communication.
I enjoy watching movies and TV shows outside of science and my current favourite Netflix series is Girl from Nowhere.
Through this project, I honed my teamwork skills through valuable relationships with my mentors and colleagues. The challenging work also enabled me to grow as a person, enhancing my problem-solving and communication skills.

Background

Stories have been around for a long time and the way humans communicate stories has evolved from cave drawings, oral traditions to the use of multimedia digital stories (Wang & Zhan, 2010). One thing that has remained constant is the power of stories to disseminate knowledge and to build shared understanding (Wang & Zhan, 2010). By presenting information in a familiar format, stories increase engagement and

the stimulation of emotions (ElShafie, 2018). ElShafie (2018) argues that an emotionally compelling story creates meaning for broad audiences which in turn makes the content intelligible. As a result, storybooks can be effective educational tools for communicating and learning complex scientific concepts. It is currently unknown if storybooks can advance what students in upper division Zoology courses at the University of Calgary think about this educational tool and whether or not there is a positive impact on students' conceptual knowledge before and after reading a storybook. As a result, a storybook about parasites was created as complex life cycles are common to many parasite taxa (Benesh, 2016). It is also important to communicate knowledge about parasites as parasitism is the most popular animal lifestyle (Lafferty et al., 2006) on Earth and parasites are an important public and veterinary health issue (Seville et al., 2004).

Methods

Story elements

The structure of the storybook followed the five-part framework, also commonly referred to as "Freytag's Pyramid", that was devised by Gustav Freytag (ElShafie, 2018). This framework includes an exposition to give readers context, a rising action to build tension, a climax to act as the turning point of the story, a falling action to ease tension and finally a resolution to end the story. In order to communicate science effectively with readers, the story also contained five essential elements, including protagonist, obstacle, stakes, inciting incident, and broad theme (ElShafie, 2018).

Implementation in the classroom

Testing was carried out with undergraduate students from two different Zoology courses at the University of Calgary in the 2021 Winter term. ZOOL401 is an introductory invertebrate course that introduces the natural history and diversity of invertebrates while ZOOL581 is an upper-level parasitology course that introduces students to parasitism. There were a total of 96 students in ZOOL401 across six lab sessions and 19 students in ZOOL581 across one lab session. The labs were held online via ZOOM and a time slot of approximately one hour was given to the storybook activity during a 2 hours and 50 minutes lab period. In each lab session, students completed a pre-survey on their knowledge of parasites and attitudes toward using storybooks to learn about science prior to the storybook activity. The students were then put into breakout rooms to complete the storybook activity. After the activity, students discussed book club questions on a laboratory exercise sheet, took turns sharing their thoughts and recorded their discussion on Google Jamboard. By the end of the storybook activity, students completed a post-survey that contained the same set of

questions as the pre-survey but included additional questions such as their learning experience and improvements for the storybook. Undergraduates completed the surveys with their student IDs to match up the pre- and post-surveys but data produced were de-identified to preserve the privacy of research participants. Data were only collected from students that agreed to participate in the study.

Results

Nick the tick storybook

Nick the tick is a 35-page educational storybook that was designed, illustrated, and written by Nikki Kong. The storybook features four local parasites (*Dermacentor albipictus*, *Dicrocoelium dendriticum*, *Myxobolus cerebralis*, and *Giardia duodenalis*) and explains their lifecycles in a linear process rather than a cyclic process.

Students' conceptual knowledge of parasites

Students in ZOOL401 and ZOOL581 were able to name more parasites that were specific to the storybook after reading *Nick the tick*. Moreover, the average number of parasites each student could name had a percentage increase of 20.5% in ZOOL401. More students were also able to describe the lifecycles of the four different parasites in *Nick the tick* and performed better on knowledge-based questions after the storybook activity.

Students' attitudes toward parasites

Students in ZOOL401 and ZOOL581 associated parasites with less negative words and more positive and neutral words. Students expressed high agreement with positively-worded attitudinal statements and described learning about parasite lifecycles to be less intimidating after the storybook activity. More students also expressed their desires to share what they know about parasites with others.

Students' attitudes toward storybook as a learning tool

Students expressed high agreement with positively-worded attitudinal statements and high disagreement with negatively-worded statements. After reading *Nick the tick*, 99% of ZOOL401 students and 100% of ZOOL581 students expressed their agreement that storybooks can be used to visualize complex lifecycles. 58.3% of ZOOL401 students and 84.2% of ZOOL581 students disagreed and strongly disagreed that *Nick the tick* oversimplified scientific concepts. After reading *Nick the tick*, students mostly considered the activity to be "fun" and "engaging".

Significance

Students scored higher on knowledge-based questions after reading *Nick the tick*, demonstrating the effectiveness of storybooks in communicating complex scientific concepts. More students expressed their positive attitudes toward parasites and storybooks after the activity, demonstrating the effectiveness of storybooks as learning tools in undergraduate classrooms. Results and data from this study can provide a useful framework for the design and development of future storybooks for public outreach. Indeed, *Nick the tick* will be donated to the Host Parasite Interactions group at the University of Calgary to be used in future outreach activities.

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Where to find out more on the project?

Nick the tick storybook

Full manuscript

Game-based learning in science: The use of an educational game in parasitology

Type of project: 530
Supervisor(s): Dr. Constance Finney and Dr. Mindi Summers
Department: Biological Sciences

About the researcher

Christina Nykyforuk

I am currently completing my undergraduate Honours degree in Zoology. I am fascinated by the natural world and enjoy learning about a wide range of topics. These include ornithology, paleontology, entomology, and parasitology. Outside of my major, I enjoy taking courses in a variety of subject areas, including archaeology, zooarchaeology, anthropology, and geology. I also have a passion for creating artwork, hiking throughout the Rocky Mountains, and exploring the badlands. In my free time, I often volunteer at animal shelters and foster rescue dogs. I was drawn to the opportunity to conduct undergraduate research to gain hands on experience with a creative project, allowing me to learn more about the scientific process. I also valued the mentorship that I received from my knowledgeable supervisors. This experience piqued my interest regarding the creative methods that can be utilized to make the learning of complex biological subjects more engaging and accessible for learners.

Background

Parasitology is a complex topic full of intricate lifecycles and highly technical terms, making the subject difficult for many students to comprehend (Menendez et al., 2020; Raimondi, 2016). Parasites are often viewed in a negative light, being associated with disease and as threats that need to be eradicated. Parasite education is critically important for creating an informed population that can recognize how parasites are

diverse, impact their lives, and interact with the world (Acka et al., 2010). The primary mode of instruction for teaching complex scientific topics in the classroom uses traditional methods, such as passive lectures and rote memorization (Pinhatti et al., 2019). The aim of this study was to investigate how students' understanding and attitudes towards parasites changed after playing an educational game.

Well-designed educational games can be used to teach learning outcomes through game-play, while creating a fun, engaging, and enriching experience (Qian & Clark, 2016). Currently, there are relatively few studies investigating the perspectives and attitudes of students towards the use of game-based learning in undergraduate biology (Spiegel et al., 2009). The creation and implementation of a strategically designed educational game, *Parasite Patrol,* was explored in two upper-level zoology courses at the University of Calgary, and students were surveyed before and after playing the game.

This research provides insight into how game-based learning can impact students' understanding and attitudes towards parasites and their lifecycles. Is game-based learning capable of increasing students' understanding of concepts in parasitology? Can playing an educational game improve students' attitudes when learning about parasites? What are students' opinions of using an educational game to learn about parasitology?

Methods

Parasite Patrol was strategically designed to create an immersive learning experience. The game was drawn using Adobe Photoshop and hosted online using Conceptboard and Zoom. *Parasite Patrol* contains a combination of design criteria such as challenge, fantasy, and curiosity elements, with the aim of increasing student engagement and understanding (Malone, 1980). To assess the effectiveness of *Parasite Patrol,* the game was played by undergraduate students during their weekly lab session in two upper-level zoology courses at the University of Calgary: An Introduction to Invertebrate Zoology (ZOOL 401), and Principles in Parasitism (ZOOL 581).

Parasite Patrol was used to investigate the impact of game-based learning on students' understanding of parasitology concepts, attitudes towards parasites, and attitudes towards educational games. To test the effectiveness of the game, a pre and post survey experimental design was utilized containing open and closed questions. 96 students in ZOOL 401 and 18 students in ZOOL 581 consented to the use of their data

for the study. Changes between student's pre-survey and post-survey responses were analyzed and descriptive statistics were performed.

Results

After playing *Parasite Patrol,* students were able to name an average of 33.5% (ZOOL 401, n=43) and 24.1% (ZOOL 581, n=10) more parasites that were specific to the game. The students' abilities to accurately describe the lifecycles for the parasite species included in the game also increased by 21-47% (ZOOL 401, n=43) and 0-60% (ZOOL 581, n=10). Students demonstrated a high level of performance on knowledge-based questions, testing their understanding of parasitology concepts.

When describing parasites, students in both courses used fewer negative words and more neutral or positive words after playing *Parasite Patrol.* More students also agreed that they wanted to share what they know about parasites with others, learning about parasites is interesting, and learning about parasite lifecycles is less intimidating. More students in ZOOL 401 (n=43) were afraid of parasites after playing the game.

Students in both courses described their experience playing *Parasite Patrol* using positive words, such as "fun", "engaging", "interactive", and "educational". 88.5% (ZOOL 401, n=96) and 83.3% (ZOOL 581, n=18) of students agreed that the game was helpful to their learning. After their experience playing *Parasite Patrol*, more students indicated that they enjoy playing games about science, games make learning about science more interesting, and that they would like games to be included in more of their classes.

Significance

The impact of playing *Parasite Patrol* on undergraduate students' knowledge of parasitology, attitudes towards parasites, and attitudes towards educational games was assessed. Students in both courses had an improved performance on parasite knowledge questions after playing the game, more positive attitudes towards parasites, and enjoyed using the game as a learning tool. These findings imply that *Parasite Patrol* offered an effective, interesting, and enjoyable learning experience, and that future efforts should be made to investigate the use of educational games in other courses and settings. Going forward, improvements can also be implemented to further enhance the benefits of *Parasite Patrol*.

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Where to find out more on the project?

Full project write-up

Inoculum-related reduced susceptibility to beta-lactam antibiotics in Staphylococcus aureus isolated from cystic fibrosis airways.

Type of project: 507
Supervisor(s): Dr. Michael Parkins and Dr. Douglas Storey (co-supervisor)
Department: Microbiology and Infectious Disease

About the researcher

Julianna Svishchuk

I am a recent graduate from the Faculty of Biological Sciences, having majored in Cellular, Molecular and Microbial Biology and pursued a minor in Mathematics. I was introduced to the beauty of scientific research rather spontaneously throughout my degree, hearing of a professor studying the microbial dynamics within the cystic fibrosis (CF) lung environment. Owing to my fascination with the topic, I began my experience as a student researcher in the Parkins Lab in 2019, and have since embarked on a graduate degree with Dr. Parkins, studying the cellular effects of cytomegalovirus infections in CF. Outside of my research, I am very passionate about the arts, and devote this passion to painting, writing, music, dance and photography. It is a privilege to be recognized by the Biology Students' Association, and I'm honored to take part in the BSURP, given my immense gratitude for the Faculty of Science in leading me to where I am today!

Background

Cystic fibrosis (CF) is an autosomal recessive disorder characterized by aberrant transport of mucus along the surface of epithelial tissue throughout the body (Brown et al., 2017). While CF affects numerous organ systems, the lungs are the site of the greatest attributable morbidity and mortality. In fact, recurrent airway infections associated with mucosal airway obstruction lead to chronic airway infection (Lyczak et al., 2002). Despite *Staphylococcus aureus* recently becoming the most prevalent pathogen within the CF airway (Canadian CF Registry, 2019), very little CF-specific knowledge exists. An area of particular concern relating to methicillin-sensitive *S.*

aureus (MSSA) is its reduced susceptibility to many beta-lactam antibiotics when tested at higher bacterial inocula than used during routine clinical laboratory testing ($\geq 10^5$CFU/mL). This phenomenon has since been termed the *inoculum effect* (IE) and has predominately been studied with the cephalosporin, cefazolin – currently the preferred agent for treating MSSA infections (Song et al., 2019). Individuals with bloodstream MSSA infections caused by isolates with the cefazolin IE exhibit higher mortality when treated with cefazolin (Miller et al., 2018). Given that mucus from CF airways contains MSSA at concentrations of $\geq 10^8$CFU/mL (Esposito et al., 2019), understanding the prevalence and dynamics of the IE in CF is crucial to optimize antibiotic therapy development, and predict long-term treatment outcomes.

Methods

Drawing from a prospectively collected biobank, all adults attending the Calgary CF Clinic with at least one MSSA-positive bacterial culture between 2014 and 2016 were identified. Isolates were tested with antibiotics targeting MSSA, as well as those used during pulmonary exacerbations. Susceptibility testing was performed with standard (SI: $5x10^5$ CFU/mL) and high (HI: $5x10^7$ CFU/mL) inoculum to determine the minimal inhibition concentrations (MICs) to prevent bacterial growth. All 238 isolates were tested by broth microdilution with cefazolin (CFZ), piperacillin-tazobactam (TZP) and meropenem, while only the first isolate from each patient was screened against cefepime, ceftazidime and cloxacillin. As done in the literature, two definitions for the IE were employed: i. *IE*, defined as an isolate having a ≥4-fold difference between MICs at SI and HI, and ii. *pronounced IE (pIE),* defined as an isolate being susceptible to an antibiotic at SI based on CLSI established breakpoints, but non-susceptible at HI. The MICs required to inhibit 50% and 90% of bacterial growth are reported for standard and high inoculum as MIC_{50} and MIC_{90} below.

Results

A total of 100 adults with MSSA were included; 27 of which had only 1 isolate, while 73 had chronic infections with isolates screened yearly for a total of 238 MSSA samples. The CFZ IE was observed in 21.4% (51/238) of isolates while the pIE was noted in 5.0% (12/238). The range of CFZ MIC increased with inoculum; SI: MIC_{50}=0.5µg/mL and MIC_{90}=1µg/mL vs. HI: MIC_{50}=1µg/mL and MIC_{90}=4µg/mL. TZP showed an IE prevalence of 42.0% (100/238) and a pIE prevalence of 38.7% (92/238). The MIC range for TZP was also increased with inoculum; SI: MIC_{50}=4µg/mL and MIC_{90}=8µg/mL vs. HI: MIC_{50}=8µg/mL and MIC_{90}=128µg/mL. Minimal inoculum effects were found in cefepime (IE, 2.0% and pIE, 4.0%), ceftazidime (IE, 5.0% and pIE, 0%)

and cloxacillin (IE, 7.0% and pIE, 2.0%) while no inoculum effects were observed for meropenem.

Significance

To our knowledge, this study is the first to assess for the prevalence of inoculum effects in beta-lactams used to treat MSSA in CF lung infections. Our data suggest that this phenomenon is particularly common for CFZ and TZP. Given that previous studies have confirmed a positive correlation between IE-positive MSSA infections and poor patient outcomes, future work to analyze the effect of the IE/pIE on CF outcomes is imperative.

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Utilizing low-throughput screening to identify morphology of hypothalamic primary cilia

Type of project: 507
Supervisor(s): Dr. Jiami Guo
Department: Cell Biology and Anatomy

About the researcher

Jerome Timbol

My name is Jerome Timbol, and I am a fourth year Biological Sciences student at the University of Calgary. I got involved with research, specifically medical research as I enjoyed applying my knowledge gained from the classroom to the research lab and knowing that my contributions will one day benefit the world. I found satisfaction in the process of the scientific method, from being presented with a question, formulating a hypothesis and testing that hypothesis through multiple experiments! Through the guidance, mentorship and collaboration with other members within the lab, I enjoyed every interaction I have encountered. These interactions helped influence my interests by expanding my knowledge to other disciplines such as neuroscience. Outside of the lab, I love spending my free time volunteering for various organizations around my community, skateboarding in the summer, snowboarding in the winter and of course, eating ice cream! Ultimately, these extracurriculars reduce any stress I may be feeling and help with keeping my mind clear and fresh.

Background

The role of primary cilia in neurons is very understudied especially when looking at specific areas of the brain such as the hypothalamus (Zeng et al., 2015). Most work to date on primary cilia has focused on lower organisms or in cell lines. The hypothalamus

interacts with various hormones within the endocrine system such as estrogen (Bear et al., 2021). Drugs such as gefitinib and paroxetine hydrochloride are found in prescription drugs that are used to treat certain breast/lung cancers and depression respectively (Khan et al., 2016). Understanding how these hormones and drugs affect primary cilia can help understand the importance these hormones play in affecting the structure of primary cilia and ultimately the structure of the neuron (Banks et al., 2012). Gaining a larger understanding of how these hormones interact with primary cilia can help benefit by providing potential treatments of neuronal disorders such as autism in children (Hyman et al., 2020). This project will pair with another project currently in the lab attempting to identify receptors and ion channels that make up the primary cilia membrane. These two projects in tandem will help establish a neuronal primary cilia interactome. This interactome will provide insight into all signalling occurring within primary cilia and will help identify key signalling pathways that ultimately dictate neuronal development.

Methods

Neuron culture was the primary scientific technique used. Following a mouse dissection, the hypothalamic embryonic neurons were isolated through microdissection's, cultured and treated with 100nM, 1µM and 10µM concentrations at 30 minutes and 48 hours. A low-throughput drug screen was utilized using the drugs from the food and drug administration (FDA) approved drug library. The neurons were plated on 24 well plates with glass cover slips that were coated with laminin. The treated neurons were then stained, analyzed, and quantified using a MATLAB based software for automated cilia detection (ACDC) to determine the structure of cilia (Lauring et al., 2019). This helped aid in determining whether the drugs used showed impacts in cilia structure which may potentially lead to determining whether cilia function will be impacted as well.

Results

The hypothalamic cells were quantified and measured in micrometers (uM) using the ACDC software (Lauring et al., 2019). One of the drugs tested was estrogen which interacts with the hypothalamus and was found to impact cilia length. Estrogen at 30 minutes presented an overall increase in cilia length when exposed to 100nM, 1µM and 10µM concentrations but with higher concentrations the length started to show a decreasing trend. With the neurons exposed to estrogen for 48 hours, an increase in cilia length at 100nM and 1uM was present but a decrease in cilia length for the 10uM. Gefitinib being another drug that was tested mimicking the same concentrations and

time points as estrogen showed increasing cilia lengths. When the concentration increased for the 30-minute time point, an increase in cilia length was observed. At the 48-hour time point, cilia length was still increased but at the highest concentration (10uM) the length was slightly smaller than the 100nM and 1uM. Paroxetine hydrochloride presented an overall increase in cilia length at the 30-minute time point for increasing concentrations as well as in the increasing concentrations of paroxetine hydrochloride for the 48-hour time point. A slight decrease in length at the 10uM concentration was present when compared to the 100nM and 1uM.

Significance

Estrogen affected the length of primary cilia by interacting with various parts involved in ciliogenesis such as membrane bound receptors and the production levels of cAMP and calcium. Tyrosine kinase inhibitors such as gefitinib enhanced the cilia length. This may be because an increase in ciliogenesis took place by gefitinib inhibiting ligand-dependent activation for cell cycle activation or also by regulating signaling events that cause cilia growth. Initial increase in the cilia length found at the 30-minute mark when compared to the 48-hour time point may be due to initial interaction with membrane bound receptors or local signaling with other receptors found on the membrane of the cell. The 48-hour time point showed a smaller increase in cilia length and could be due to transcription/translational events that can only occur later in time. Paroxetine hydrochloride is a selective serotonin reuptake inhibitor (SSRI) and acts by blocking the serotonin reuptake transporter (SERT) resulting in an increase in concentration of synaptic serotonin. Paroxetine binds to the pre-synaptic serotonin transporter complex which then results in blocking reuptake of serotonin by the pre-synaptic transporter. This then causes an accumulation of serotonin in the synaptic cleft which leads to downregulation of serotonin. Paroxetine hydrochloride interacts very close to a neuronal cell as it acts directly within the synapse of a neuron. This may lead to indirect association with cilia causing a deformity with the length or function. Although the structure of the cilia has changed through exposure to various drugs, the functionality may have not been affected. To determine the functionality of the affected cilia, a calcium drug screen needs to be done to determine the intracellular levels of calcium as the intracellular levels of calcium determine ciliary function. By pursing further investigation and determining whether these drugs affect functionality or not, future treatments for neurodevelopment and disorders such as autism can arise.

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Are evolutionary associations diminishing over time?

Type of project: 528
Supervisor(s): Dr. Jeremy Fox
Department: Biological Sciences

About the researcher

Mykaela Tucker

I am a 4th year student majoring in Biology, with a strong focus in genetics. I began my undergraduate degree at Quincy college in Boston, Massachusetts. I transferred during my second year, to the University of Calgary. Outside of academia, I enjoy travelling, hiking, and spending time with my daughter. I decided to become involved in undergraduate research in order to gain real life experience in a subdiscipline of biology that I was interested in. This experience helped me narrow down my own passions within the field of biology. Participating in research helped me gain new skills and confidence in academic writing and study design. Some advice I would give to students interested in research would be to get involved in an area they are interested in, and to be flexible!

Background

Publication bias has been well documented in many fields of science through systematic reviews of meta-analyses. These biases include high impact factor journals publishing “more exciting”, higher effect size studies first and more often, while “less exciting”, lower effect size studies are published later, or not at all (Jennions & Moller 2002). Additionally, journals have been known to publish positive effect sizes more often than null or negative effect sizes. Both of these factors contribute to a decline effect trend, where initial results on a novel topic will show a strong relationship, and over time

when more studies are published, a weaker relationship is often reported (Jennions & Moller 2002). There is a lack of studies published on the impacts of publication bias in evolutionary biology.

Methods

Meta-analyses within the field of evolutionary biology were screened using PRISMA standard for eligibility into the study. Studies excluded were missing information including effect size, variance and publication year. 33 meta-analyses were included in the study. To test if the magnitude of effect sizes had changed over time, a correlation coefficient was calculated for each meta-analysis, and a single sample t-test was performed for all 33 correlation coefficients to determine if the mean correlation coefficient was significantly different from zero. Additionally, a linear regression was performed in R studio to determine if the relationship between effect size and meta-analysis publication year had significantly changed over time.

Results

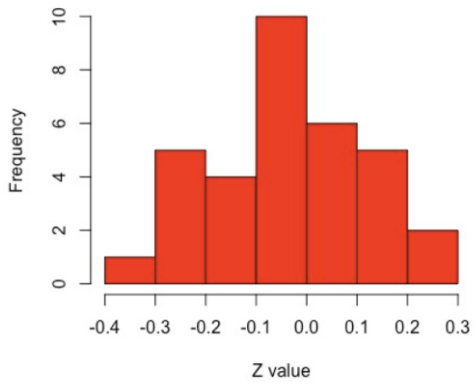

Figure 1. The frequency of the value of the Z-transformed correlation coefficients, for 33 meta analyses. The Z-transformed correlation coefficients were calculated from the absolute value of effect size and study year for each meta analysis (single sample t-test, t= -1.6389, p=0.111, df=32).

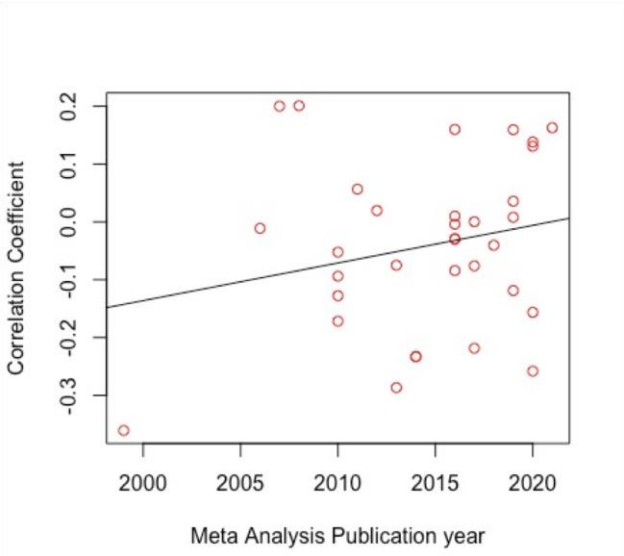

Figure 2. The correlation coefficient for 33 meta analyses, plotted as a function of Meta analysis Publication Year. The correlation coefficients were calculated from absolute value of effect size over study year, for each meta analysis. This regression not be significantly different from zero ($F(1,31) = 1.596$, $p = 0.2159$) and the meta analysis publication year explains 4.89% of the variation in the data by the relationship $y=(0.006502x) – 13.14$.

The t-test of the 33 correlation coefficients was not found to be significantly different from zero (Figure 1). The linear regression of the correlation coefficients over the meta-analysis publication years was not found to be significantly different from zero (Figure 2). Overall, no decline effects were found throughout evolutionary biology, and trend has been consistent over time.

Significance

While no decline effects were found in this study, this is not a direct indicator of the lack of publication bias in evolutionary biology. Unpublished studies, lack of evolutionary meta-analyses available and meta-analyses with a broad range of topics could all be contributing to this trend. Therefore, publishing studies with null or low effect sizes within this field, along with consistently publishing data, could aid in a clearer view on the effects of publication bias in evolutionary biology.

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