UTM
Biology

2022

2023

44TH ANNUAL BIO481 SYMPOSIUM

COURSE COORDINATOR Prof. Ted Erclik

APRIL 10 AND 11, 2023

RECEPTION & AWARDS,
APRIL 11, 4:00PM, CCT ATRUM

SCHEDULE OF EVENTS

APRIL 10, 2023

Set-up	8:45 - 9:10 AM
Opening Remarks: Ted Erclik	9:10 AM
SESSION 1	**9:15-10:30 AM**
BREAK	**10:30-10:45 AM**
SESSION 2	**10:45-12:05 PM**
LUNCH BREAK	**12:05-1:05 PM**
SESSION 3	**1:05-2:20 PM**
BREAK	**2:20 - 2:35 PM**
SESSION 4	**2:35 - 4:05 PM**

APRIL 11, 2023

Set-up	8:45 - 9:10 AM
Opening Remarks: Ted Erclik	9:10 AM
SESSION 1	**9:15-10:30 AM**
BREAK	**10:30-10:45 AM**
SESSION 2	**10:45-12:00 PM**
LUNCH BREAK	**12:00-1:00 PM**
SESSION 3	**1:00-2:00 PM**
BREAK	**2:00 - 2:15 PM**
SESSION 4	**2:15 - 3:15 PM**

Biology ROP Poster Day, CCT Atrium	3:00PM
Reception & Awards, CCT Atrium	4:00PM
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APRIL 10 EVENTS

APRIL 10, 2023

APRIL 11 EVENTS

APRIL 11, 2023

Set-up	8:45 - 9:10 AM
Opening Remarks: Ted Erclik	9:10 AM
SESSION 1	**9:15-10:30 AM**
Shirley Liu	**25**
Kevin Iizuka	**26**
Ahmad Kubbar	**27**
Sona Tissington	**28**
Daniela Cobo	**29**
BREAK	**10:30-10:45 AM**
SESSION 2	**10:45-12:00 PM**
Melvin Chelvanayagam	**30**
Nawal Faisal	**31**
Andy Lu	**32**
Fatima Nagi	**33**
Andrew Yu	**34**
LUNCH BREAK	**12:00-1:00 PM**
SESSION 3	**1:00-2:00 PM**
Samantha Bestavros	**35**
Kortni Kindree	**36**
Simran Rakhra	**37**
Daphne Arguelles	**38**
BREAK	**2:00 - 2:15 PM**
SESSION 4	**2:35 - 3:15 PM**
Eunhye Lee	**39**
Krista Kueviakoe	**40**
Margaret Anderson	**41**
Alexis Konopny	**42**
Biology ROP Poster Day, CCT Atrium	3:00PM
Reception & Awards, CCT Atrium	4:00PM

April 10, 2023

Naumana Basharat

Bryan Stewart

9:15am

The Effects of Lasp and Brahma Knockdown on Synaptic Bouton Growth at the *Drosophila melanogaster* NMJ

Retrograde signalling, from the postsynaptic to the presynaptic cell, influences synaptic growth at the neuromuscular junction (NMJ) of *Drosophila melanogaster*. Proteins involved in generating retrograde signals can influence the number of axonal varicosities (boutons) present at the NMJ. Lasp, a *Drosophila* protein previously linked to postsynaptic vesicle trafficking, is suspected of influencing retrograde signalling pathways to regulate bouton growth at the NMJ. Furthermore, in a preliminary yeast two-hybrid screen, Brahma (Brm), a subunit of the Brahma chromatin-remodelling complex, was discovered to interact with Lasp. Therefore, it is possible that Brm and Lasp also interact in *Drosophila* and have a synergistic effect on synaptic bouton growth at the NMJ. In the present study, we investigated the effects of postsynaptic knockdown of Lasp and Brm via RNA interference (RNAi) on the number of boutons formed at the NMJ. Additionally, by crossing amorphic alleles to create a trans heterozygous genotype at the Lasp and Brm loci, the synergistic effect of the proteins was also investigated. Preliminary results show that postsynaptic RNAi knockdown of Lasp or Brm has no significant effect on the number of boutons that develop. In contrast, double heterozygotes of Brm and Lasp exhibited an increase in the number of boutons observed at the NMJ compared to the wildtype. We plan to compare the phenotype of double heterozygotes to that of single heterozygotes to determine whether the effect observed is in fact synergistic. Ultimately, the findings of this study provide a foundation upon which future experiments to elucidate the roles of Lasp and Brm in synaptic bouton development at the *Drosophila* NMJ can be performed.

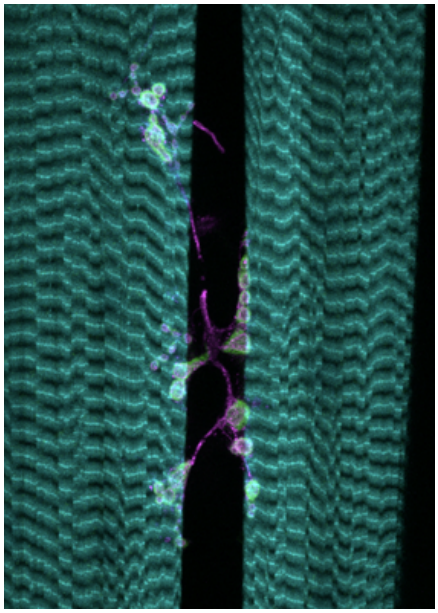


Figure 1. The *Drosophila melanogaster* NMJ at muscle 6/7 in the third abdominal hemisegment. Actin is visible in turquoise, discs large-1 (dlg) in green, and horseradish peroxidase (HRP) in magenta. The bright round structures are synaptic boutons. NMJ was visualized using a Zeiss LSM800 Confocal Microscope.

Sofia de Carvalho Neves Pinto 9:30am
Bryan Stewart

The Drosophila Neuromuscular Junction: An Evaluation on the Effect of Botulinum Toxin A on Non-Muscle Myosin II present at Type 1B Boutons

This study aims to evaluate whether there is a significant relationship between Botulinum Toxin A and Non-muscle Myosin II. NMMII is a motor cytoskeletal enzyme emerging as a target of pharmaceutical interest due to its involvement in many cellular activities including vesicle dynamics. Furthermore, recent papers have demonstrated this protein to be a critical regulator of homeostasis. As such, understanding how NMMII is regulated becomes essential. The Rho/ROCK pathway has a key role in this. Particularly, RhoA has been identified as one of the most important promoters of NMMII activity. Though this relationship has been established, there is lack of research on how factors influencing RhoA could lead to altered NMMII activity. In this regard, Botulinum Toxin A emerges as a possible factor, considering that Park *et al* has shown that BoT-A upregulates RhoA gene expression. The question then arises: could Botulinum Toxin A affect Non-Muscle Myosin II activity?

In this study, stocks of GFP-tagged Zipper (NMMII) gene flies will be crossed with UAS-BoNT-A flies, at 25ºC. Emerging third instar larvae will be dissected in HL3 buffer. In parallel, relevant controls will be dissected under the same conditions. Confocal microscopy will be used to assess for differences in localization and quantity of protein at the NMJ. It is expected that NMMII presence in *Zip-GFP / UAS-BoNT-A* Drosophila is higher comparing to controls, since it is hypothesized that BoT-A upregulates NMMII. If so, this will be the first work that identifies a significant relationship between BoT-A and Non-muscle Myosin II.

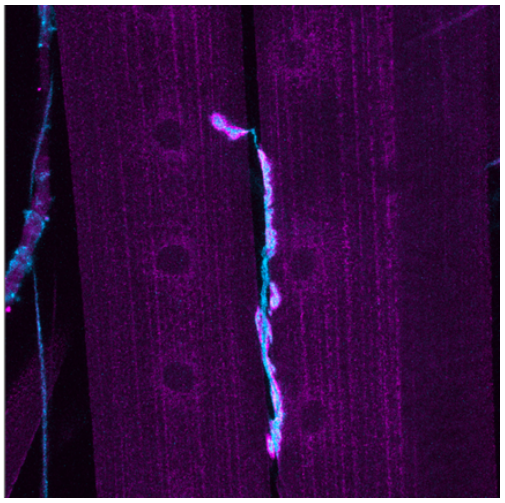


Figure 1: The *Drosophila melanogaster* neuromuscular junction (NMJ). Imaging was done for muscles 6 and 7 at the A3 segment. Highlighted in blue is a motor neuron and the muscles are colored in magenta.

Keywords Non-muscle myosin II; zipper gene; botulinum toxin A; rhoA; neuromuscular junction

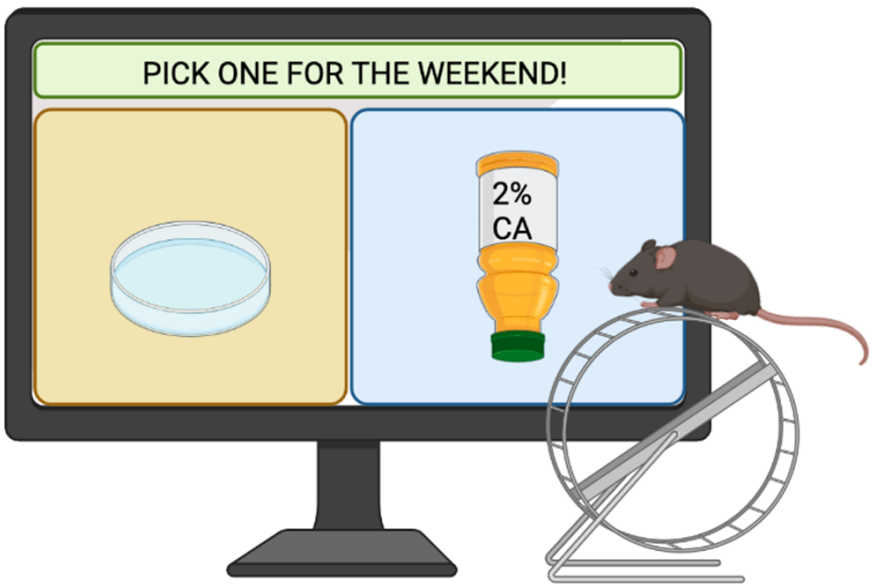
Apurva Singh
Arbora Resulaj

9:45am

Assessing the Effects of a Citric Acid Protocol on Visual Orientation Discrimination Task Performance in Mice: An Ecologically Valid Study on Learning Curves and Behavioral Analysis

This paper evaluates the effectiveness of a free access 2% citric acid water restriction protocol in motivating behavioural performance in a visual orientation discrimination task using C57/BL6 mice (n=8) as animal models. A case-by-case analysis approach was used to assess the outcome of the restriction protocols in terms of rewards attained and discrimination accuracy. Results showed inconsistent effects of the free access protocol given the hesitance of some mice to consume citric acid as contrasted with others drinking too much, leading to decreased performance. Among the mice that drank citric acid, there was a drop in the number of rewards attained and performance for the first few days of the weeks. Conversely, mice that did not drink citric acid were hyper-motivated to attain the rewards, leading to a drop in performance.

This thesis argues that a fine-grain behavioural analysis approach rather than overall performance would allow for better understanding of motivational states in animal subjects. Additionally, this project discusses the importance of appropriate handling methods and statistical analysis that stray away from learning curves – in the context of this experiment. The thesis concludes that citric acid protocol is not an effective alternative to conventional water restriction for the orientation discrimination task and recommends further refined studies using fine-grain behavioural analysis.

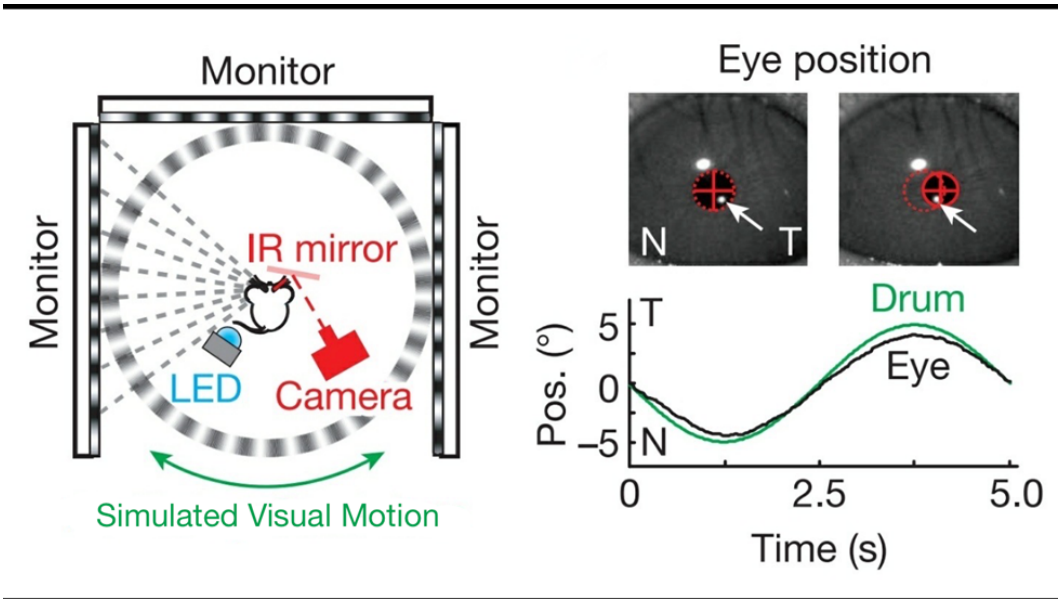


Valentyn Sobolenko
Baohua Liu

10:00am

Mapping how the Optokinetic Reflex is Differentially Responsive to Motion that is in Different Areas of Visual Space.

The optokinetic reflex (OKR) functions as a visual stabilizer for forward visual motion, such as head motion, body movement or other stimuli passing through the visual field. It is a highly conserved reflexive eye movement among most animals. The OKR response stabilizes vision through involuntary repeated nystagmus. The eyes follow the visual stimulus and snap back to continue following the direction of motion. So far, the OKR was understood to be a response to global or large-field motion. There have been no studies investigating whether the OKR responds differentially based on retinotopic position of the visual movement. This pilot study presented visual motion at different locations throughout the mouse's visual field, and mapped the strength of the OKR. This study observed that visual motion near the horizontal meridian of the eye triggered the strongest OKR response, peaking at 10 degrees below the horizontal meridian and sharply decreasing as the stimulus moves away from the center; with an overall stronger response in the lower visual field. It was also found that there was an increased OKR response when motion is presented over 20 degrees nasally on the horizontal axis. With narrow vertical bands of motion reliably stimulating the OKR. This study lays the foundation that the OKR responds preferentially to visual motion in specific areas of the visual space, rather than being a global response, leading future investigations to continue uncovering the possible retinotopic organization of this reflex.

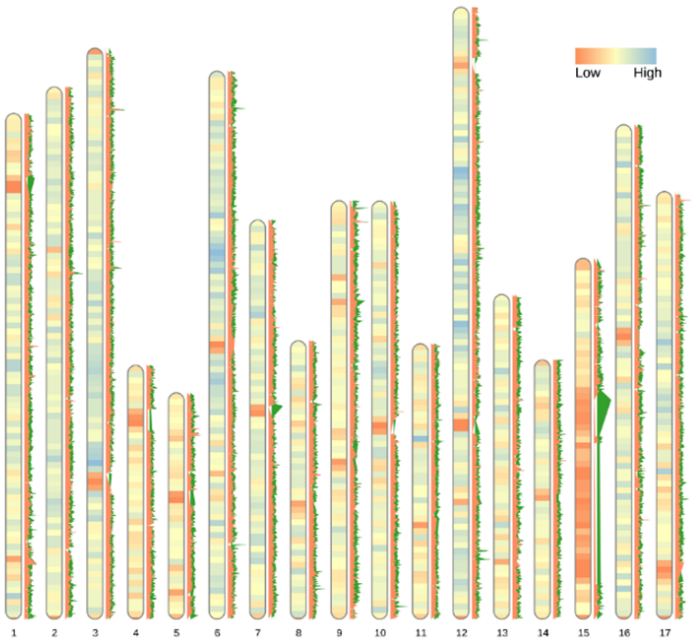


Jimmy Issa
Rob Ness

10:15am

Population structure and selective sweep analysis of North American isolates of the green algae *Chlamydomonas reinhardtii*

Microbial eukaryotes are thought to have large reproducing populations and ease of geographic migration compared with larger multicellular counterparts. However, microbiologists are currently in debate about how these properties impact microbial population structure. The ubiquity model suggests that these properties result in these microbes spreading worldwide. The endemicity model which suggests that many (but not all) microbes only form endemic populations, and microbes with cosmopolitan distributions are composed of different, genetically diverging population lineages. *Chlamydomonas reinhardtii* is a unicellular, cosmopolitan soil microbe and model organism which has been used to study plant cell biology, photosynthesis, and more. The large number of sequenced strains of *C. reinhardtii* now allow for an unprecedented, fine scale analysis of potential signals of fine or broad scale signals of population structure and local adaptation in this soil microbe. The new isolates also allow an investigation into whether urban versus rural environments structure the genetic variation in populations of *C. reinhardtii*, giving insights onto the impact of urbanization on soil microbial populations. Ecological divergence is expected with geographic separation, and so selective sweep analysis can accompany findings of the population structure of these isolates and identify how and to what degree separated populations of *C. reinhardtii* have evolved in parallel and lineage-specific manners. These findings are expected to produce crucial evidence to help resolving the longstanding ubiquity vs endemicity debate, and produce one of the highest resolution pictures of how geography structures populations of soil microbes to date.



Colour gradient representing gene density along each chromosome of *Chlamydomonas reinhardtii.* Nucleotide diversity levels at each position of the chromosome are also depicted using polygon labels, with the orange polygon representing the NA1 population of *C. reinhardtii* and the green representing the NA2 population. The NA2 label has been right-shifted to allow a comparison of its trend relative to the NA1 diversity levels at the same points in the genome.

Celina Micaela Javier & Veronica Venditti
Robert Gerlai

10:45am

Appetitive associative learning & memory: A new fish model in behavioral neuroscience with *Mikrogeophagus ramirezi*

Despite the numerous sophisticated behavioral tools created for, and the genetic tractability of the zebrafish, there is growing evidence that the zebrafish may not be the most suitable species for learning & memory tasks employed in neurobehavioral research. Their proneness to exhibiting fear and anxiety-like behaviors paired with their innate preferences for staying in a social group often make implementing sensitive learning tasks difficult. The present study examines whether the German ram cichlid can perform an appetitive learning task. German ram cichlids were able to show a reversed preference for the colour green, which was the least preferred colour in a colour preference task, after training with a food reward. This suggests that adult German ram cichlids have the behavioral plasticity to override natural environmental preferences when provided sufficient motivational rewards and provides a new, easily adaptable model for neurobiologists interested in studying cognitive function with fish in the laboratory.

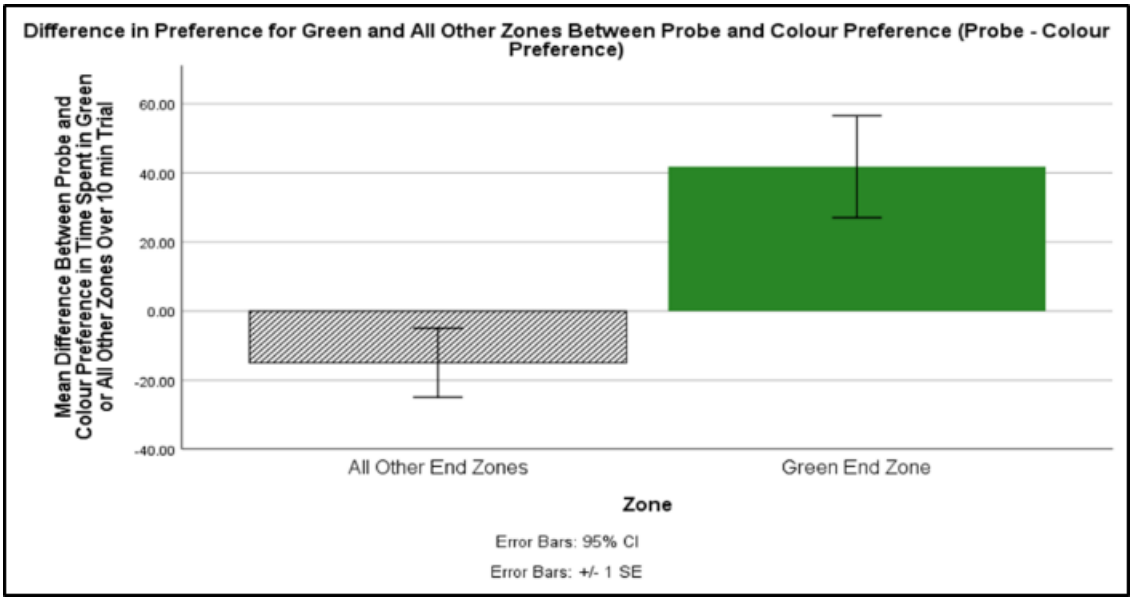


Figure: The difference in preference for the green end zone and all other end zones from the Colour Preference test (Experiment 1) to the Probe test (Experiment 2). The preference for all other end zones non-significantly ($p > 0.05$) decreased from the Colour Preference test to the Probe test; in contrast, the preference for the Green End Zone significantly ($p < 0.05$) increased.

Rachel Dass & Chuanyu Wu
Robert Gerlai

11:10am

The Effect of Housing Densities on Zebrafish Shoaling Preferences

Social behaviour is an important aspect of zebrafish's behavioural repertoire. It is also important in laboratory settings from an animal welfare standpoint, as both solitary fish or fish kept in overcrowded tanks may be stressed. Unintentional stress exposure can act as a confounding variable in experimental paradigms and can lead to false positive or negative inferences. Shoaling is a spontaneous social behaviour that occurs in zebrafish when exposed to stressors such as novel environments. Previous research has found that zebrafish prefer the numerically larger shoal to the smaller one in spontaneous binary choice tasks. Additionally, research has found tank size and housing density to have interactive and additive effects on other swim path parameters, including immobility, speed, turn angle, distance to the bottom of the tank, and distance to stimuli. However, the impact of housing conditions on the zebrafishes' demonstrated shoal preference has not been studied. In this study, we investigated the effect of housing density on the shoaling preference of zebrafish. We randomly assigned zebrafish to be housed in either high-density (3.3 f/l) or low-density (0.7 f/l) conditions for 1 week. We used a spontaneous binary-choice task to measure test fishes' preference for swimming in the proximity of a large (8 fish) versus a small (2 fish) stimulus shoal. As predicted, we found that zebrafish housed under high-density conditions showed the least preference for the larger stimulus shoal, indicating reduced motivation to shoal compared to fish housed in low-density conditions. Our results suggest that providing sufficient space and social opportunities may be important for maintaining the welfare of zebrafish. These factors may also be necessary to ensure the validity of experimental results, especially if the experimental paradigm utilises social behaviour as a behavioural parameter.

Figure 1. Experiment setup of a binary-choice task we used to measure test fish's (green) preference for swimming in the proximity of a large (8 fish) versus a small (2 fish) stimulus shoal.

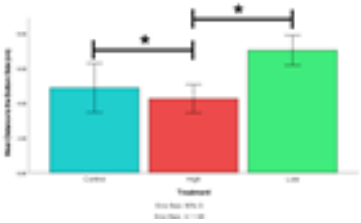


Figure 2. Distance to the bottom of the testing tank. The blue, red and green bars show data from the control (n=27), high (n=30), and low-density (n=28) groups respectively. The X-axis shows the 3 treatment groups. The Y axis shows the mean distance from the larger stimulus shoal in cm during the 5-minute trial. * Represents significant data differences across groups.

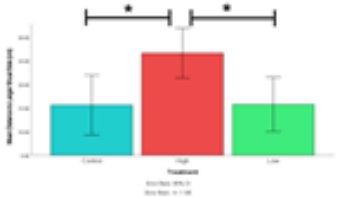


Figure 3. Distance from the larger shoal. The blue, red and green bars show data from the control (n=27), high (n=30), and low-density (n=28) groups respectively. The X-axis shows the 3 treatment groups. The Y axis shows the mean distance from the larger stimulus shoal in cm during the 5-minute trial. * represents significant data differences across groups.

Sara Asim
Robert Gerlai
11:35am

Preconception Ethanol Exposure of Zebrafish: Is it the father's or the mother's drinking?

Zebrafish (*Danio rerio*) serve as model organisms for human diseases due to evolutionarily conserved features. Likewise, zebrafish have been used to study fetal alcohol spectrum disorders (FASDs), one of the many life-threatening conditions resulting from alcohol abuse. Literature suggests significant behavioural alterations including reduced frequency and increased duration of immobility, increased intra-individual variance of turn angle and increased turn angle in offspring of ethanol exposed zebrafish (Suresh et al., 2021). However, whether the behavioural effects were due to exposure of the mother, father or both to alcohol remain unknown. This study was designed to dissociate maternal from paternal alcohol exposure effects on their offspring. We assigned adult wildtype zebrafish (40M, 40F) to one of four experimental conditions (maternal exposure, paternal exposure, biparental exposure, or no exposure). Based on the assignments, the parental fish were exposed to either 0.0% (control) or 0.5% (vol/vol) alcohol chronically for 7 days. After alcohol exposure, the parental fish were bred, and their offspring were behaviourally tested using a video tracking system. The following swim path parameters were quantified; velocity (cm/min), duration of immobility (min), frequency of immobility (count), turn angle (degree), thigmotaxis (cm) and intra-individual variances of these parameters.

The results of this study aim to explore preconception alcohol effects, that is whether exposure of mother, the father or both parents to alcohol leads to lasting behavioural and/ brain function related changes in the offspring. Our ultimate goal is to use discoveries about zebrafish to better understand the development of fetal alcohol spectrum disorders in humans.

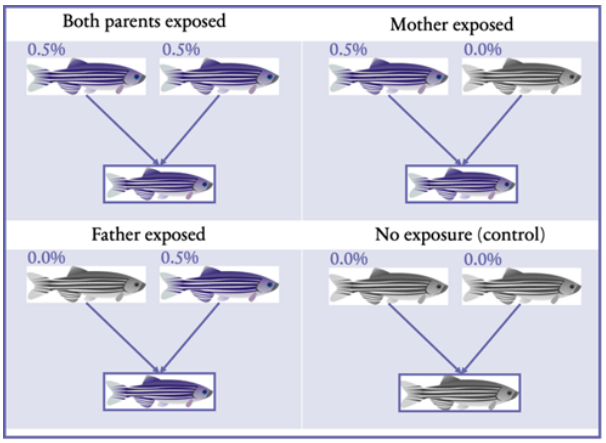


Figure 1. Experimental design for preconception ethanol exposure of zebrafish parents.

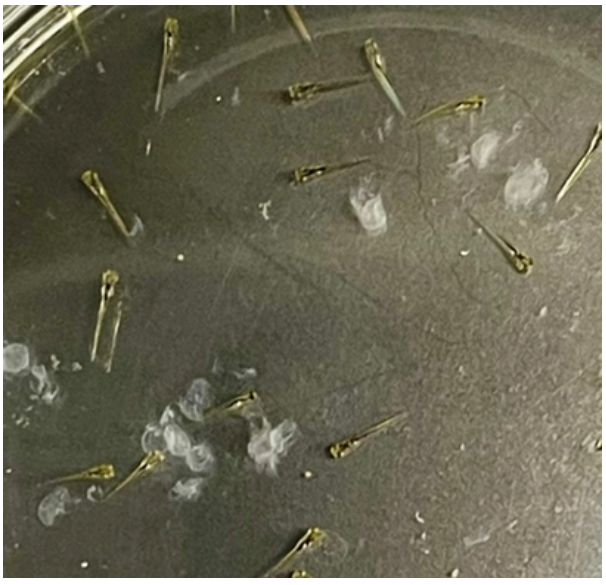


Figure 2. 5-7 days post-fertilization old zebrafish hatchlings were behaviourally tested.

Omer Syed
Robert Gerlai

11:50am

Time-of-day-dependent effects of caffeine administration on zebrafish (*Danio rerio*) behavior

Caffeine is a widely used stimulant that is consumed by billions of people on a daily basis. The psychoactive compound elicits a biphasic effect in humans, increasing alertness and motor performance at low doses, and inducing considerable levels of anxiety at higher doses. These dose-dependent effects have also been replicated in rodents and zebrafish. Nevertheless, although there is a vast literature examining the influence of caffeine on the circadian rhythm in humans and other animal models, the zebrafish (*Danio rerio*), an increasingly popular model in behavioral neuroscience, has not been extensively employed to study this interaction. In particular, the time-of-day-dependent effects of caffeine in zebrafish, or in any other animal model, have not been previously examined. Here, we investigate whether caffeine elicits differential effects when administered in the morning versus the evening. We exposed zebrafish to caffeine for 30 minutes between 8:00 and 11:00 or between 16:00 and 19:00, and analyzed the effects of this treatment by comparing the swimming behavior of 20 mg/L and 100 mg/L caffeine-treated and caffeine-naive (control) zebrafish. We found a complex dose- and time-of-day-dependent effect of caffeine on zebrafish behavior, indicating that caffeine does indeed differentially affect behavior when ingested in the morning versus the evening.

Tejal .

1:05pm

Sanja Hinic-Frlog & Helene Wagner

The biology behind the high diabetes rates in some Indigenous communities

Diabetes is a long-term health condition resulting from impairment of the kidneys and their sub-optimal functioning. Type-2 diabetes is a form of diabetes in which the body builds an insulin resistance and there is a surplus of glucose circulating in the body. This can result in worsening of an individual's health and damage their blood vessels leading to complications such as heart attack and stroke. Although each Indigenous community is affected by type-2 diabetes to various degrees, the high rates of this disease in some communities have been linked to colonization. Here, I identify theories discussed in the scientific literature as possible explanations for a biological link between colonialism and type-2 diabetes. I conducted a scoping review of the scientific literature and focused on 14 review papers based on inclusion criteria related to community and diabetes descriptors. The four most prominent theories based on studies conducted with Indigenous communities within Canada, the USA, Greenland, Australia, Taiwan, and the UK are: (1) epigenetics; (2) lack of access to healthcare; (3) food insecurity; and (4) the thrifty gene hypothesis. By comparing the two most common and opposing theories, epigenetics, and the thrifty gene, I show how the thrifty gene hypothesis has been used as a dehumanizing approach to health problems within Indigenous communities. I discuss these findings in the context of my own positionality as a biology student in Canada and outline their potential use in biology courses to help students learn about diabetes in the context of Truth and Reconciliation.

Key words: Indigenous, Type-2 Diabetes, Biological theories, Literature review, Epigenetics, Thrifty gene, Decolonization

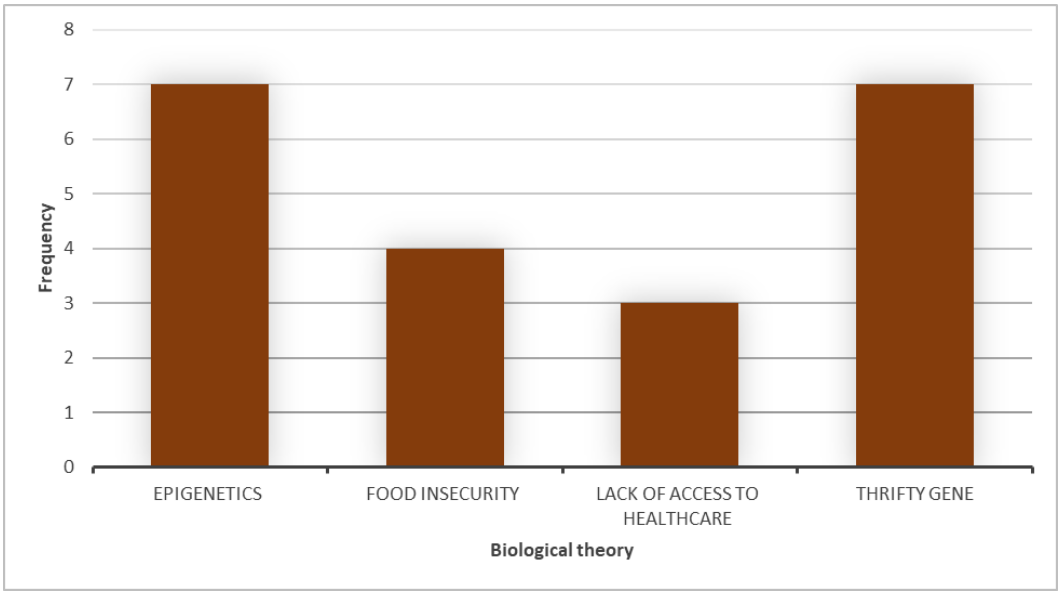


Figure 1. Bar graph of frequencies of four biological theories found in the scoping review of literature in Web of Science and Google Scholar. These four theories potentially explain the high rates of diabetes in some Indigenous communities. Out of the 14 eligible studies, every study discussed at least one of these theories in some detail.

Thacze Kuganesan
Helene Wagner & Sanja Hinic-Frlog

1:20pm

Decolonization in Epidemiology: How Does Health Canada Misrepresent Indigenous Peoples?

Epidemiology is the study of the incidence, distribution, and possible control of diseases and other factors relating to health, in populations of humans. In countries founded upon settler-colonialism, like Canada, the methods used to determine these population health statistics may systematically misrepresent and potentially exclude Indigenous communities and their health-related needs. Thus, this study aims to explore the statistical discrepancies in the data collected from Indigenous health surveys against those collected at the national scale, relating these differences to colonial practices conventionalized in epidemiology. The study began with a literature review that yielded 8 papers, discussing the role of Canadian Indigenous communities in epidemiological study design and data collection. This review found major criticisms of western epidemiology, regarding the disencouragement of Indigenous participation, with specific criticisms of study design and data usage in survey-based and census-based epidemiology. Next, I made descriptive statistical comparisons in the distribution of different chronic health conditions, through the comparison of data from the "First Nations Regional Health Survey" against those from the "Canada Health InfoBase". This resulted in a list of prevalence rates that were directly comparable between the two surveys, which showed important discrepancies. Further, parts of the two data sources were not directly comparable, due to differences in age groups and a larger focus on patient agency variables in the Indigenous survey. These findings are discussed in terms of the literature search findings, and in terms of my own learnings and positionality as a Canadian biology student. I have also adapted my findings into a course video, for use in undergraduate biology courses.

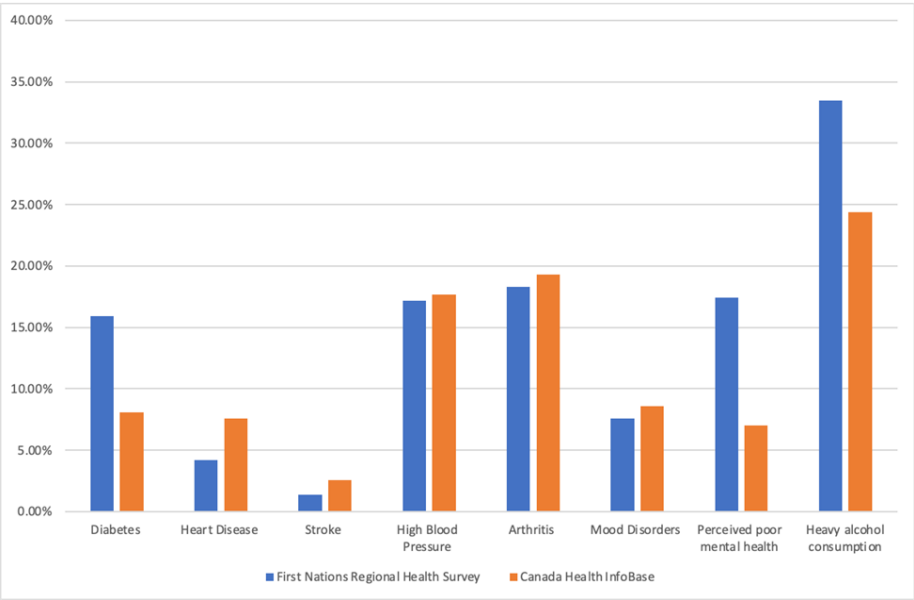


Figure 2 - Comparison of the distribution of various chronic health conditions, between the prevalence rates found in the First Nations Regional Health Survey and the prevalence rates found in the Canada Health InfoBase (national census data). Only direct comparisons (i.e. matching health conditions and population age ranges) are presented here. All data is obtained from 2017.

Affan Ahmed
Marcus Dillon

1:35pm

The Effects of Temperature Variation on Virulence of Pseudomonas syringae in *Arabidopsis thaliana* Col-0

Pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) are the two main bifurcations of plant immunity. PTI works by recognizing general microbe (or pathogen)-associated molecular patterns (MAMPs or PAMPs) via pattern recognition receptors (PRRs) which can be found on the surface of plant cells. Microorganisms, though, have developed effectors that are inserted into cells via the type III secretion system (T3SS) that help them to overcome the PTI arm of plant immunity thereby increasing their virulence. Plants, in turn, have developed ETI to counter this effector threat; deployed nucleotide-binding leucine-rich repeat receptor (NB-LRR) proteins are able to recognize effector proteins. ETI is a stronger immune response that will often lead to localized cell death called the hypersensitive response (HR). Temperature changes have been known to either increase or decrease the robustness of PTI and ETI in *Arabidopsis thaliana*. Temperature changes (specifically higher temperatures) have also been shown to be associated with greater virulence of *Pseudomonas syringae* through amplified movement of effectors in terms of movement across plasma membranes. However, there are also studies that have shown that higher temperatures are associated with lower virulence of *P. syringae* by virtue of lower secretion of avirulence gene products. In this study, I use a syringe infiltration protocol to infect *A. thaliana* Col-0 plants with four *P. syringae* strains of varying virulence: PmaYM7930, PmaES4326, PmaES4326 hopAR1 and PMAES4326 EV. I specifically test the effect of higher and more stressful temperatures on the virulence of these strains on *A. thaliana*. My results show that the *P. syringae* strains have greater *in planta* concentration at 32◯C compared to 23◯C, suggesting that pathogenicity between the strongest and weakest strains becomes more similar at higher temperatures.

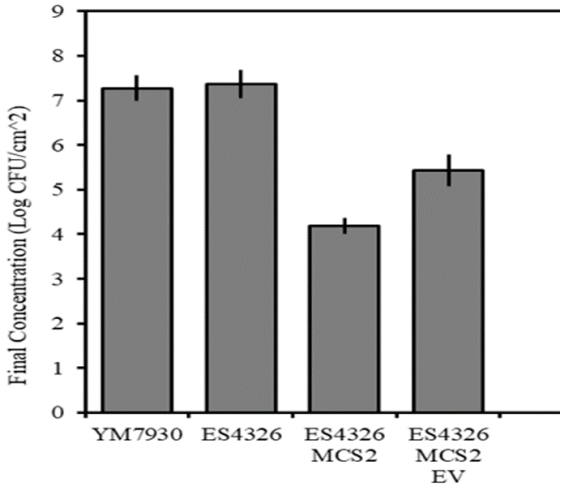


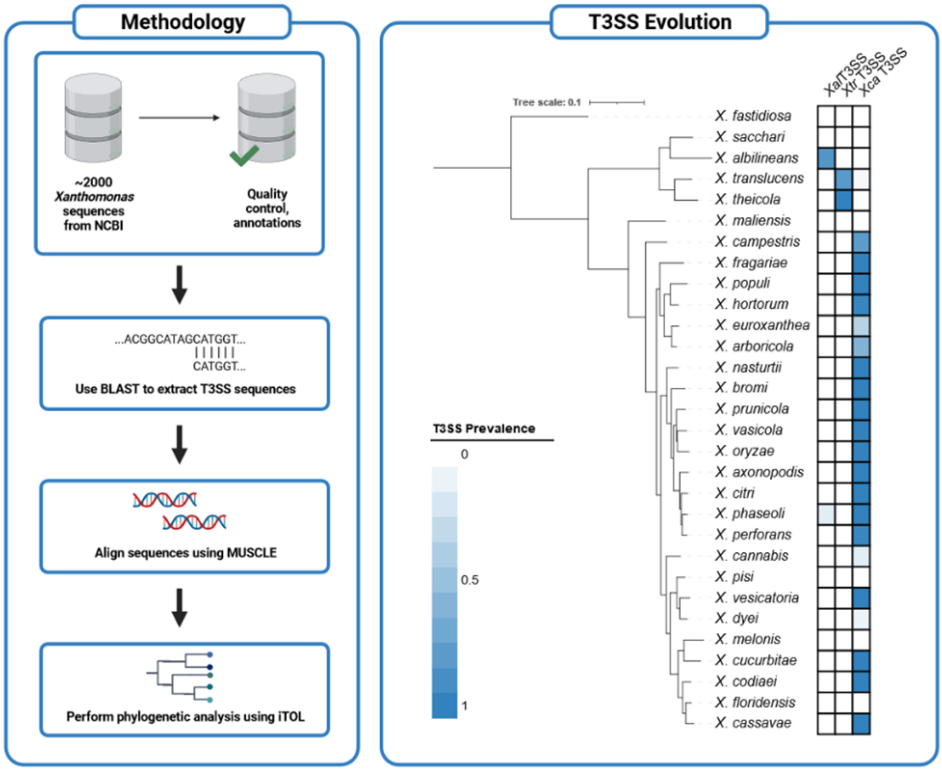
Figure 1. In planta concentration of different *P. syringae* strains in *A. thaliana* hosts. Concentration was quantified by counting colonies grown on KB+rif+chx plates. Error bars represent standard deviation.

Zain Nassrullah
Marcus Dillon

1:50pm

The Molecular Evolution of the Type III Secretion System in the *Xanthomonas* Pathosystem

The *Xanthomonas* genus harbours a diverse array of plant pathogenic bacteria that infect and cause disease on over 400 economically important hosts, including tomato, rice, cabbage, and cassava. Remarkably, individual strains of *Xanthomonas* are known to possess a very high specificity for their target host, likely due to the specific plant-microbe interactions facilitated by the type III secretion system, which delivers immune-modulating proteins (effectors) into the host's cytoplasm. We investigated the evolution of the type III secretion system using a collection of 1910 quality controlled and annotated *Xanthomonas* genome assemblies and a database of 12 core genes associated with the type III secretion system complex. Three different forms of the type III secretion system with divergent genetic architectures were identified: a *campestris*-like system (88% of strains), a *translucens*-like system (3% of strains), and an *albilineans*-like system (1% of strains). Furthermore, 8% of strains had no type III secretion system at all. While most strains that possessed a type III secretion system only contained one copy, 33 *X. phaseoli* strains were found to possess both a *campestris*-like system and an *albilineans*-like system, suggesting the possibility of horizontal gene transfer of the type III secretion system within *Xanthomonas*. The results obtained here highlight the complex nature of host-pathogen evolution at the molecular level and shed light on how the infection strategies used by xanthomonads can be manipulated to modulate host range.

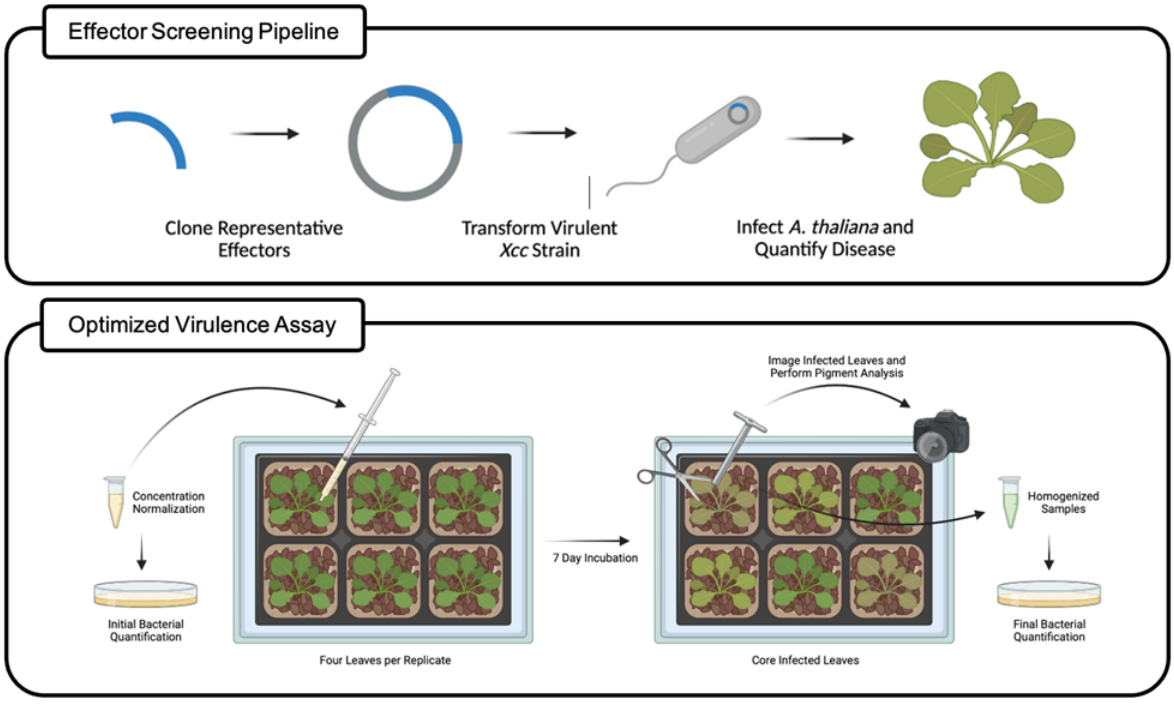


Mateja Perc
Marcus Dillon

2:05pm

Developing a Virulence Assay to Characterize *Xanthomonas-Arabidopsis* Type III Secreted Effector Interactions

Xanthomonas is a bacterial genus which consists of many agronomically important phytopathogens capable of infecting a wide range of crops. Despite this host diversity, a restricted host range is observed for individual strains. Type III secreted effectors (T3SEs) are known to contribute to host specificity in many phytopathogens via both modulation of the immune response (leading to effector-triggered susceptibility) and host recognition of individual effectors (leading to effector-triggered immunity (ETI)). Better characterization of the impact of individual T3SEs in *Xanthomonas* is critical to understanding host specificity in this genus. We have developed and optimized a high-throughput virulence assay that can be used to screen hundreds of T3SE in order to identify the breadth of ETI interactions driven by *Xanthomonas* effectors. The background strain for this pipeline is *X. campestris* pv. *campestris* CN05 ΔxopAC, which is a strong pathogen of *Arabidopsis* with a relatively low background number of effectors that can cause confounding effector-effector interactions. Inoculation procedures mimicking natural modes of entry of *X. c.* pv. *campestris* (i. e. surface inoculation) tended to require longer incubation periods and did not produce significant differences in phenotypes associated with effector triggered immunity. We therefore leverage stomatal infiltration as an alternative, making several changes to general plant pathogenicity protocol performed infiltrations. Specifically, an incubation period of seven days, an infiltration concentration of 6 log CFU/mL, and pigment analysis of individually infiltrated leaves were all introduced as optimization measures for this virulence assay.

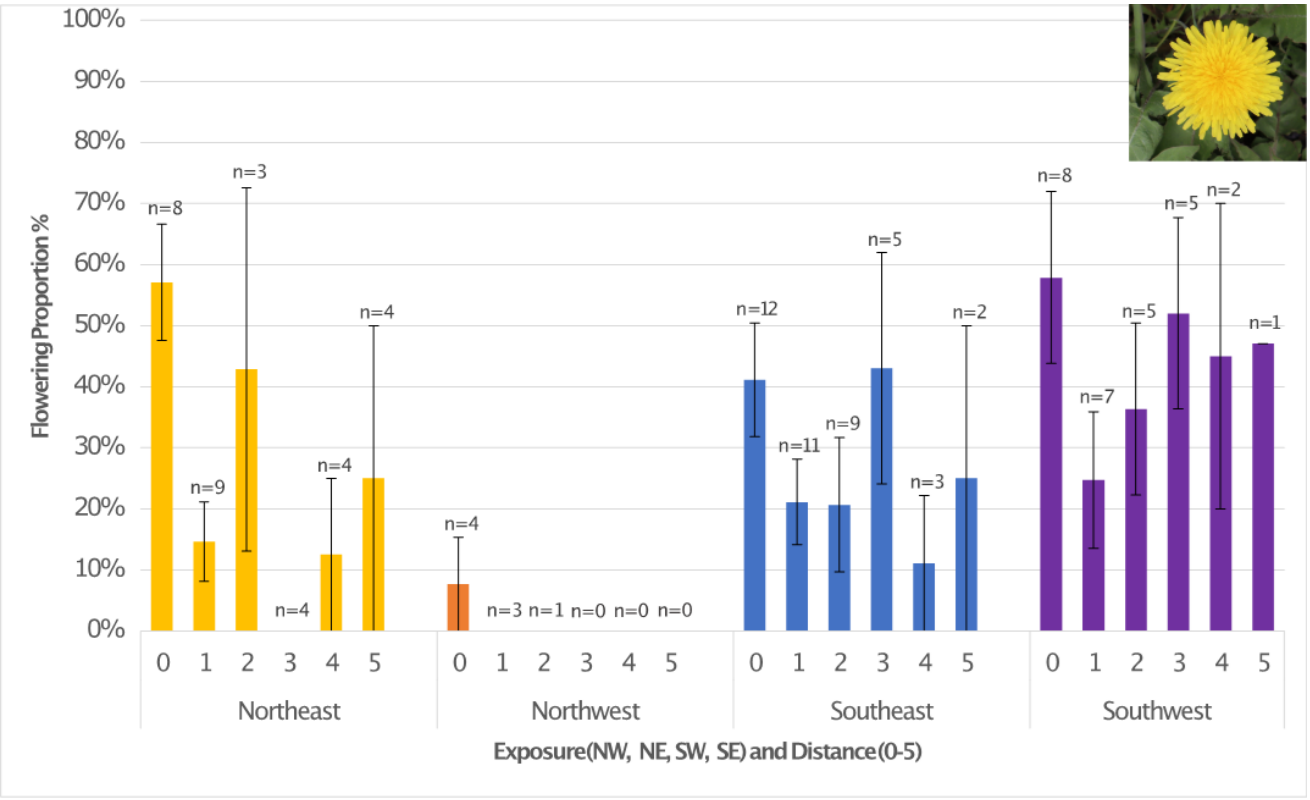


Brianna Wong
Peter Kotanen

2:35pm

The influence of buildings on invasive common dandelion performance in its northern range limit

Invasive plants are rare at high latitudes. One exception, the Eurasian common dandelion (*Taraxacum officinale*), is established in the subarctic region in the Town of Churchill in Manitoba. One factor contributing to its local success may be the shelter provided by buildings. I tested this possibility with a systematic field survey in which I used transects to quantify the proportion flowering, longest leaf length, and number of leaves per plant around buildings at different exposures and distances. Preliminary results suggest that the proportion flowering is the most greatly influenced out of the three performance measures, with higher values in the southeast and southwest exposures and at closer distances. This is important as this higher proportion in flowering increases the chances of reproduction through seed dispersal, potentially contributing to *T. officinale's* continued persistence and success. Determining the effect of buildings on T. officinale therefore may help in predicting the consequences of growing human settlement in the subarctic.

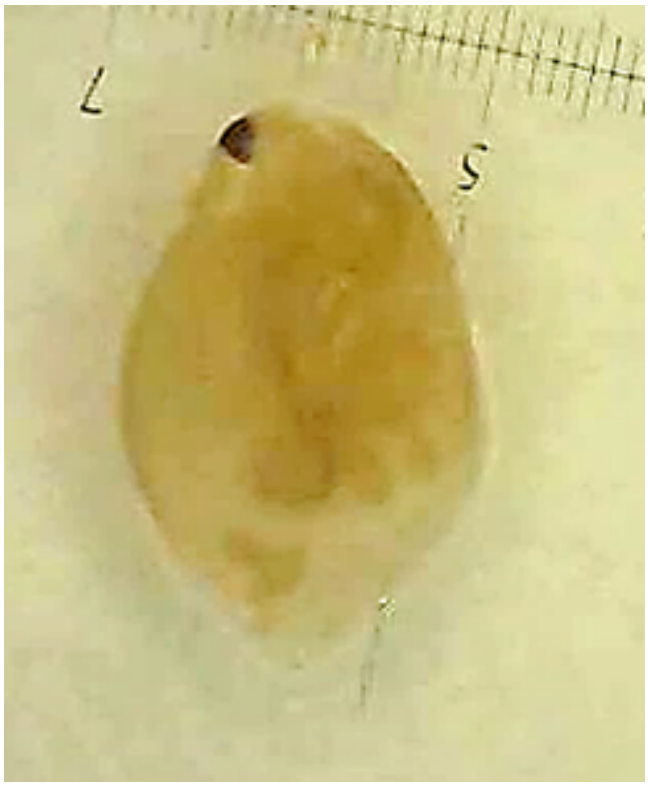


Ahmed Elsaifi
Rosalind Murray

2:50pm

The effect of road salt runoff on zooplankton abundance

Aquatic ecosystems are threatened by many anthropogenic activities that introduce pollutants. These pollutants have serious negative impacts on aquatic communities. Road de-icing salt is a source of pollution that has captured the attention of scientists and environmental organizations. While several studies have predicted an increase in the proportion of salinized freshwater ecosystems due to the intensification of anthropogenic pressures and climate change, it is yet unknown how different drivers of salinization, such as road de-icing, can affect freshwater ecosystems in the future. Daphnia are freshwater organisms sensitive to changes in salt concentration, which makes them useful as a bioassay for salinity pollution. They provide insight into the impact of salt on aquatic life and the mechanisms by which it affects organisms. In this study, I investigated the effects of salt concentration on the abundance of daphnia, a vital component in the aquatic food chain. Using samples taken from an artificial pond set up consisting of mesocosms with different levels of salinity (0 g/L, 1g/L, and 3g/L added salt), I counted the number of daphnia to determine their abundance in each salt concentration. The results indicate that increased salt concentration has a negative effect on the abundance of Daphnia, which could have cascading effects on freshwater food webs. The study provides valuable insights into how increased road salt could affect both daphnia abundance and the composition of aquatic communities. The results of this study could inform policies on road de-icing practices and their potential effects on aquatic ecosystems.

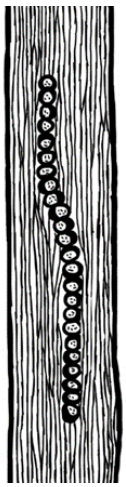


Joshita Sehgal
Rosalind Murray & Darryl Gwynne

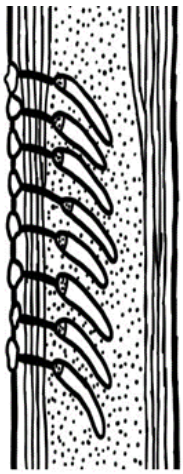
3:05pm

How do mowing regimes affect tree cricket hatching success?

Raspberry canes serve as important habitats for numerous insects, including hollow stems being used a retreat for spiders and nesting sites for larvae of some solitary bees, and stems being used as egg-laying (oviposition) sites for tree crickets (Oecanthus: family Gryllidae). To determine the impact of human activities/disturbances on egg hatching success in tree crickets, I examined the effects of mowing. Female tree crickets reproduce in select woody or herbaceous plant stems by drilling holes with their needle-like ovipositor; females drill a series of holes with their ovipositor into the pith of the cane and insert an egg into each hole, leaving a zipper-like scar on the substrate. I examined the effects of mowing on egg hatching success using 3 treatments: canes rooted in soil (the natural state), cut canes hanging upright against a fence simulating cut (mowed) stems in a natural orientation, and cut canes lying on the ground that simulated mowing where cut canes become covered with soil, leaves, and debris. I expect egg hatching success to be lowest in the mowing treatment because the dampness/moldiness of the canes may promote bacterial/fungal growth and prevent the eggs from surviving.

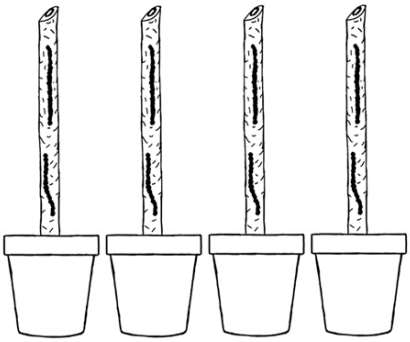


Egg Scar Segment

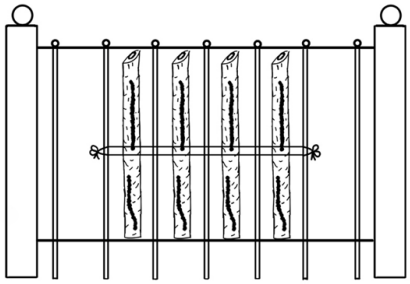


Longitudinal Scar Segment

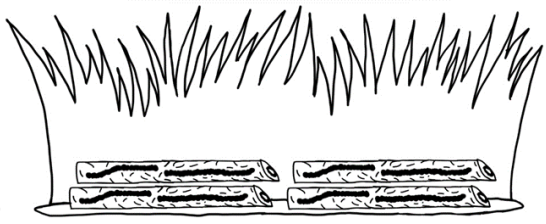
Egg



Treatment 1



Treatment 2



Treatment 3

Melody Yazdani
Rosalind Murray

3:20pm

Effects of Latitudinal Variation and Urbanization in Monarch Butterfly Larvae (*D. plexippus*)

Due to climate change, many habitats are experiencing an increase in temperatures, which ultimately affects thermoregulation and other aspects of their development. In this study, we are measuring the effects of latitudinal variation and urbanization on cuticular melanin in larvae of the *D. plexippus* (Monarch Butterfly). We look at the cuticular melanin because it is a vital thermoregulatory trait. Monarch butterflies are known to have a population in North American that tends to go across Canada, USA, and Mexico (depending on their stage of life and migration). Recently, the Monarch Butterfly was noted as an endangered species in Canada. This led to two research questions, (1) how does *D. plexippus* larvae cuticular melanin covary with latitude in central Canada and the eastern coast? (2) Do urban islands affect caterpillar melanization?

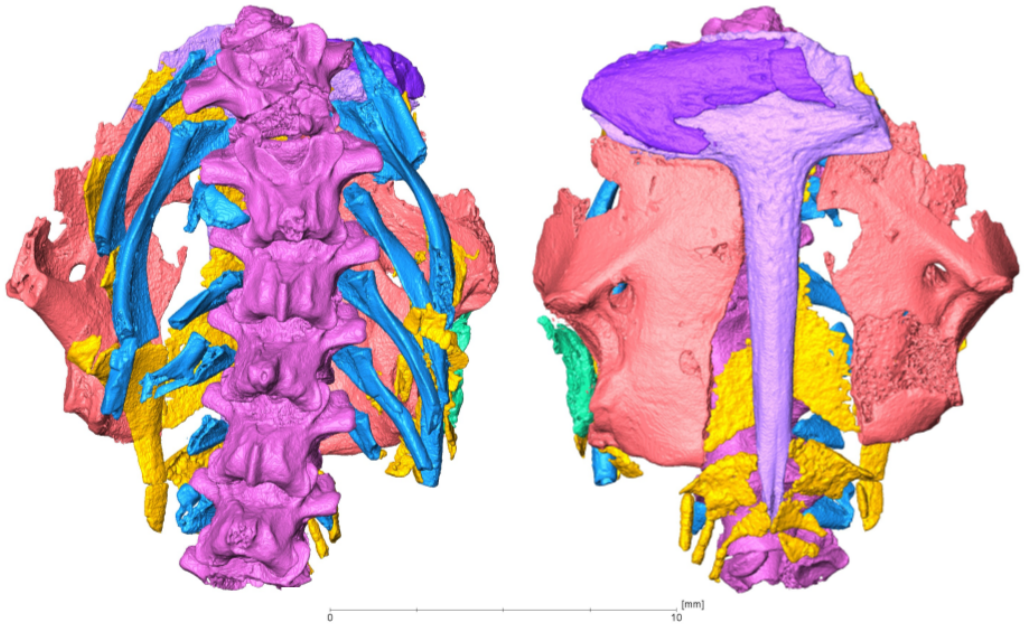
We predicted that latitude and urbanization will impact the thermoregulatory response of the Monarch larvae, and result in phenotypic variation in the proportion of cuticular melanin. Specifically, it was expected to see less melanin in area with high temperatures (cities and lower latitudes), and more melanization correlating with lower temperatures (rural areas and higher latitudes). Cuticular melanin is identified by measuring the width of each black band along the length of the fifth instar larval segments (A3-A5) from collected photographs. We found that urban conditions and latitudinal variation are separately statistically significant towards band width. However, there was a notable pattern that cities which tend to have higher temperatures than urban areas, displayed a greater band length. Since melanin is a thermoregulatory trait, our findings suggest that the protective properties of melanin against pollution and human interferences could be driving these results. This study provides insight into the potential adaptive strategies of species to urbanization and climate change.

Ethan Mooney
Robert Reisz

3:35pm

Exceptional Preservation of Cartilaginous Elements in Early Reptile Captorhinid and New Insights into Evolution of The Sternum and Costal Respiration

The success of early amniotes in terrestrial environments has been largely attributed to the amniotic egg, however the evolution of costal respiration (rib assisted breathing) and separation of the shoulder girdle from the skull, giving rise to the neck, are likely equally as important. Greater respiratory efficiency facilitated by costal respiration in conjunction with greater head mobility allowed for the adoption of more competitive active feeding strategies. During the early Permian, the time in which early amniotes became well established, this evolutionary pattern remains poorly understood. There were several modifications to the anterior skeleton and musculature to support the transition from buccal pumping (throat assisted breathing as seen in frogs) and cutaneous respiration (respiration through the skin) in anamniotes to more efficient costal respiration in amniotes. As such, it is with great excitement that we present the first substantiated evidence for costal respiration as the main respiratory mechanism in an early amniote. We have utilized high-resolution micro-computed tomography (mCT) to present articulated cartilaginous sternal structures, cartilaginous rib connections, and cervical rib extensions articulated within the shoulder girdle of a remarkably well-preserved subadult Captorhinid. These are the earliest articulated sternal structures and cartilaginous rib connections described in a tetrapod. Phenomenal preservation of this fossil can be attributed to the unique preservational conditions at the Richards Spur locality, in which impregnation of organic material with hydrocarbons was crucial for the diagenesis of cartilaginous and ossified material.

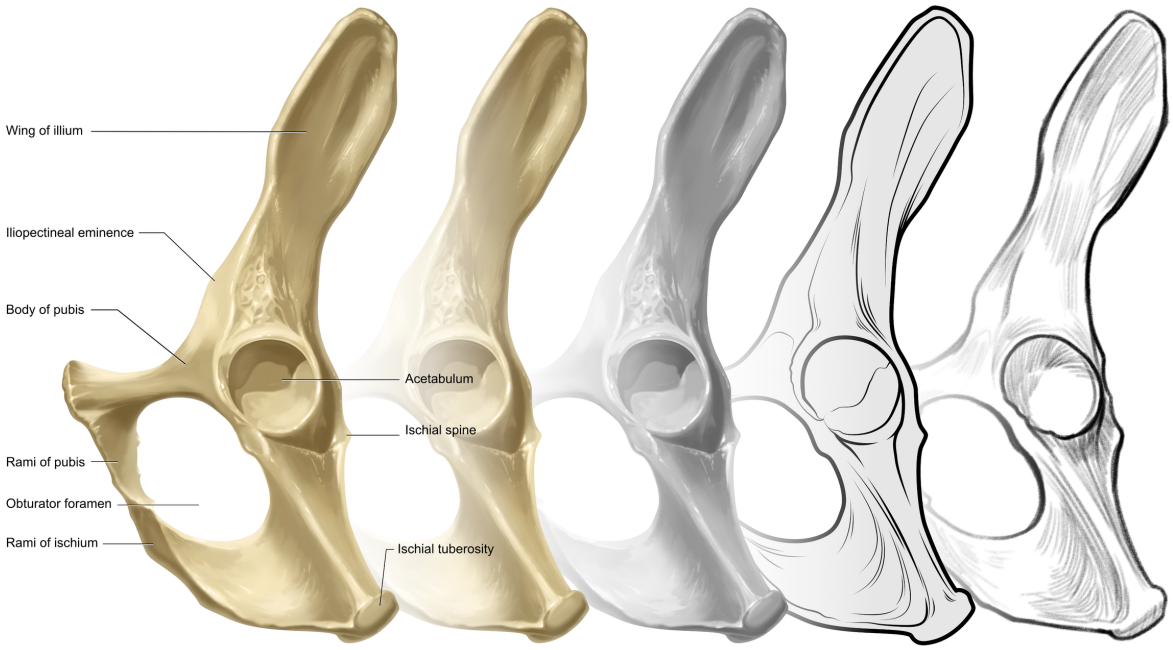


Anaiah Reyes
Robert Reisz

3:50pm

Visualizing vertebrate dissections for use in an interactive online lab manual and integrated lab manual textbook

Although supported by numerous studies, the implementation of interactive multimedia learning has been slow to present itself within the educational setting. The potential for multimedia within the biology curricula is especially evident in traditional anatomy laboratory settings, whereby students are required to conduct self-directed investigations of complex model organisms and may require resources outside of the classroom in preparation for in-lab practicals. Traditionally, the anatomy curriculum is supplemented by an illustrated lab manual, but little has been done to incorporate more interactive learning components outside the classroom. Moreover, traditional lab manuals have favored simplified figures over more descriptive and detailed renderings that are visually closer to in-lab specimens. To mediate this knowledge gap and further encourage interactive learning within traditional lab settings, the integration of a dissection manual and textbook was proposed to bridge the gap between lecture and lab content and provide updated life-like illustrations. Additionally, the Illustrated Interactive Guide to Anatomy Dissection (ILLIAD) was created to supplement the BIO354 Comparative Vertebrate Anatomy course at the University of Toronto, Mississauga with an interactive alternative to the traditional dissection manual. Currently, the manual includes interactive figures, videos, and quiz modules for the integument and muscular systems of the course's four vertebrate models: the domestic cat, dogfish shark, lamprey, and mudpuppy. This paper outlines the further development of the pre-existing interactive lab manual application ILLIAD and the contribution of specimen illustrations to the current library of figures and an integrated lab manual textbook.



April 11, 2023

Shirley Liu
Alana Ogata

9:15am

Developing an Impedance Bioresistor using Copper (II) Bipyridine Coordination Polymer

Low-cost and rapid diagnostic technologies are needed to reduce the strain on our healthcare system from patients waiting for disease diagnoses and treatments. Low-cost point-of-care (POC) biosensors detect disease biomarkers within minutes and are simple and portable for use by non-specialized people. I contributed to the development of a POC biosensor called the impedance bioresistor (IBR). On the IBR, I synthesized a copper (II) bipyridine (CuBpy) film that connected two gold electrodes into a circuit. My first objective was to determine the impedance of two types of CuBpy films: one made by incubating CuBpy precursors (4,4'-bipyridine and $CuCl_2{\cdot}2H_2O$) for four hours without replenishing and another made by incubating the precursors for four hours with replenishing halfway. I found that CuBpy synthesized with and without replenishing yielded similar impedance at voltage frequency 5 Hz, with replenishing resulting in 2472 ± 234 Ω in resistance and 16018 ± 666 Ω in capacitive reactance and no replenishing resulting in 2708 ± 365 Ω in resistance and 14878 ± 3223 Ω in capacitive reactance. My second objective was to see whether the CuBpy IBR could sense different concentrations of hydrogen peroxide (H_2O_2), a product of glucose oxidase. I measured the impedance of replenished CuBpy IBRs before and after H2O2 and tetramethylbenzidine (TMB) incubation (Figure 1). I created a calibration curve that shows 1M H_2O_2 decreased impedance by 96 ± 0.04% and capacitive reactance by 98 ± 0.03% and 10 mM H_2O_2 decreased impedance by 77 ± 1.08% and capacitive reactance by 83 ± 0.68%. Future work involves using the IBR to directly detect the activity of glucose oxidase and other disease biomarkers.

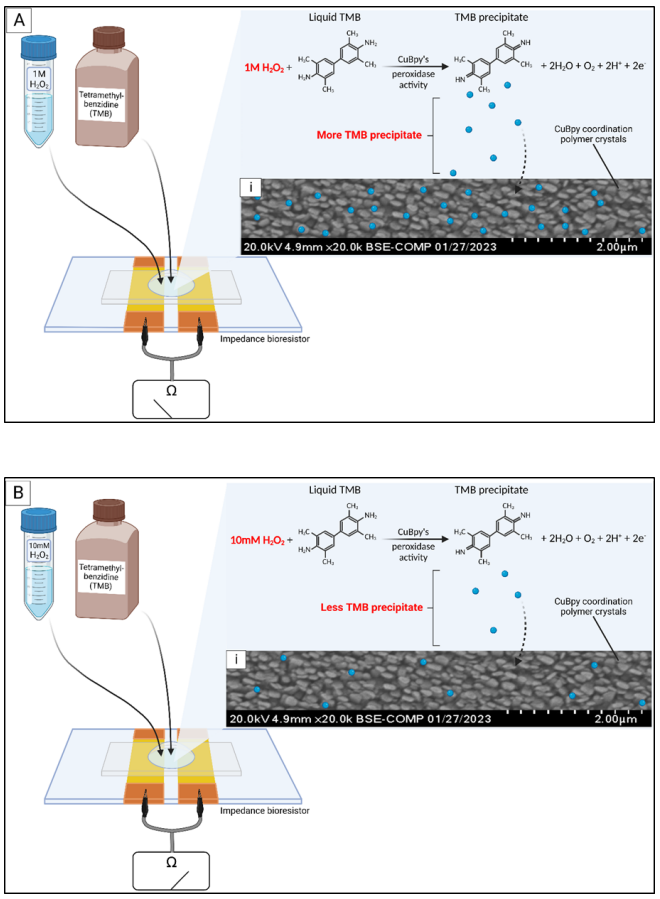


Figure 1. Figure 1. A schematic showing how the impedance bioresistor detects hydrogen peroxide concentration. (A) A greater concentration of H_2O_2 results in lower impedance (Ω) and (B) a lower concentration of H_2O_2 results in higher impedance (Ω). (i) A film of copper (II) bipyridine (CuBpy) synthesized with replenishing its precursors (4,4'-bipyridine and $CuCl_2{\cdot}2H_2O$). Image obtained using a Hitachi SU3500 scanning electron microscope with Bruker EDX with an accelerating voltage of 20kV. Figure created with BioRender.com.

Kevin Iizuka
David McMillen

9:30am

Dengue Antibody Detection via Agglutination of Serotype-Specific Antigens Surface-Displayed on Yeast Cells

Dengue virus is an RNA virus that spreads to human hosts through *Aedes* mosquitoes, inflicting the host with the disease called dengue fever. The virus exists in four possible serotypes, meaning a person infected may become reinfected by another serotype. Each reinfection is associated with increasingly exacerbated symptoms due to antibody-dependant enhancement (ADE). To control reinfections and inform mitigation processes, it is necessary to have a methodology that is affordable and of a production process that can be localized to dengue hotspots to test infection status. The main framework for the methodology central to this project was previously created by Dr. Kil in the McMillen Lab. It used two strains of Saccharomyces cerevisiae EBY100 cells that respectively displayed scEDIII, a synthetic consensus dengue antigen capturable by anti-DENV, and the z-domain of Protein A from *Staphylococcus* aureus, an antibody-binding protein to capture the Fc region of anti-DENV. In the absence of anti-DENV, the cells sediment, but when exposed, the cells agglutinate allowing for antibody detection. In this project, the aim is to further work on this previously used design by implementing antigens with serotype-specificity for the purpose of further reducing non-specific interactions in the detecting cells. To design the cells, the MoClo Yeast Toolkit protocol was used with the Aga1p-Aga2p yeast surface-display system to create yeast cells that displayed the respective antigens: P6 (Type 1), P7M (Type 2), DIII-C-2 (Type 2), and EDIII (Type 3). All plasmids were manufactured, and expression for P6 and P7M have been confirmed in EBY100 cells via c-myc confirmations and mock agglutinations. P7M was found to not bind to its respective antibody. The remaining cells have yet to undergo testing in the secondary immunofluorescence assay.

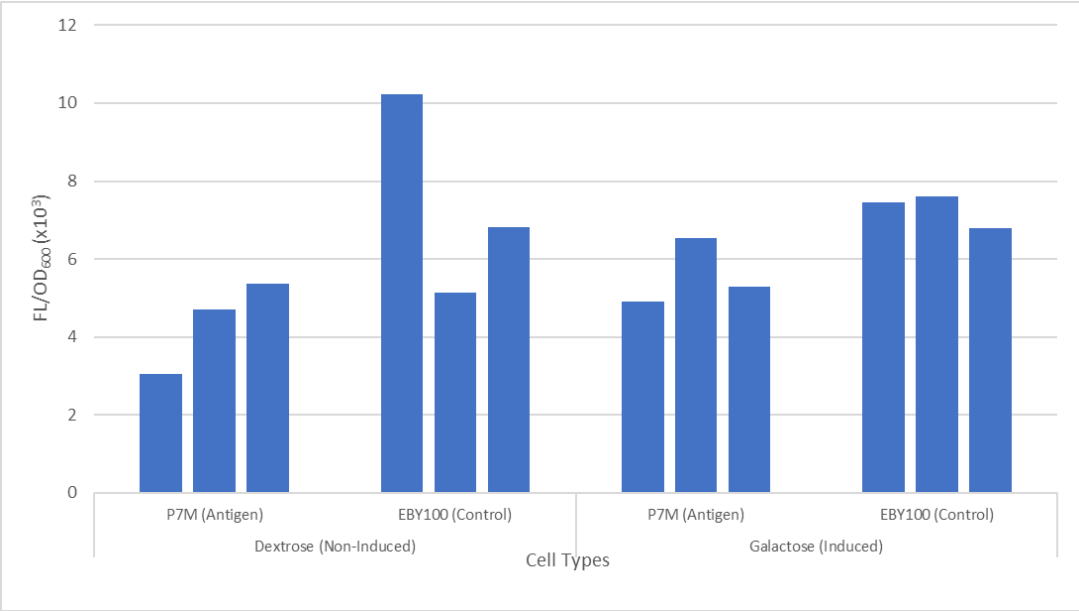


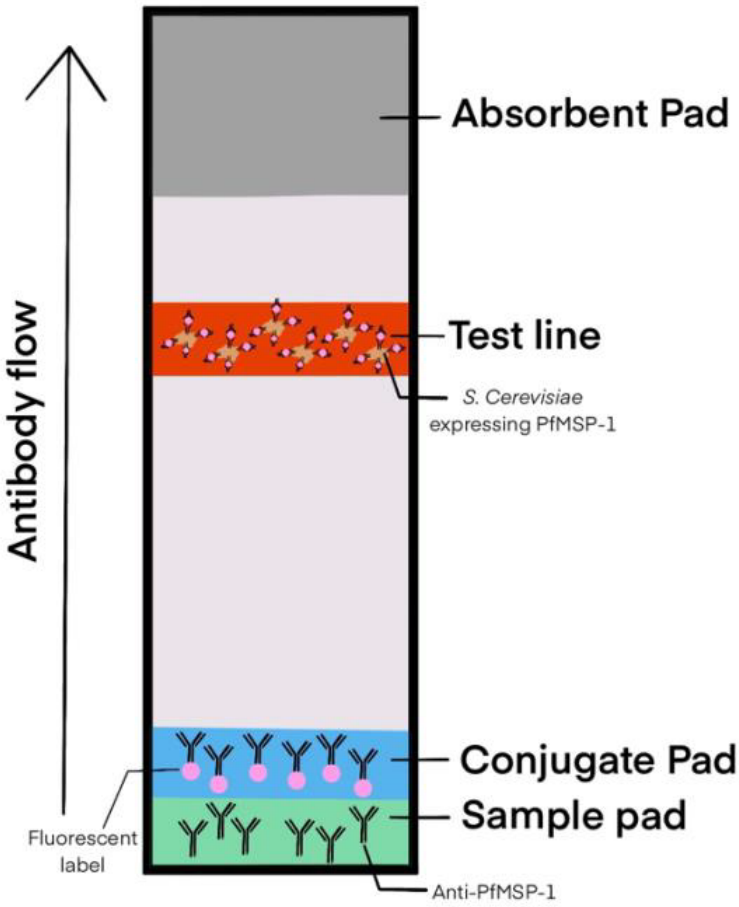
Figure 1. Bar graph of secondary immunofluorescence test on P7M. It shows the fluorescence per OD600 data for three replicates of induced and non-induced: P7M (the antigen-presenting cell), and EBY100 (the control). Test was done with the 1o antibody – Rabbit-Anti-DENV for the NS1 antigen and 2o antibody – Anti-Rabbit-FITC.

Ahmad Kubbar
David McMillen

9:45am

Lateral Flow Assay Development for Malaria Using S. *cerevisiae* as the Capture Element

Malaria is a parasitic infection characterized by the transmission of the Plasmodium falciparum parasite into the blood from a female mosquito of the Anopheles genus. Current malaria diagnostic techniques involve the use of a microscope to observe a blood smear, thus requiring expertise, or the use of a rapid diagnostic test involving antibodies, which are typically expensive. Malaria is rampant in countries of low socio-economic status, and as a result, we want to develop a diagnostic test for malaria that is simple and affordable. By expressing the malaria antigen Plasmodium falciparum merozoite surface protein-1 (PfMSP-1) on the surface of a genetically modified strain of Saccharomyces cerevisiae, we believe malaria antibodies from the subjects will bind and accumulate on the surface of these modified S. cerevisiae cells. We are developing a lateral flow assay that uses the PfMSP-1 antigen from the S. cerevisiae as the capture element on the nitrocellulose membrane. Upon running the subject's sample through the membrane, we expect the antibodies to bind specifically to the PfMSP-1 antigen. This binding should induce a visible colour change yielding a positive result for malaria, thus making this device simple and affordable. Spotting the cells directly onto the membrane surface did not yield the desired results, as the cells seem to be too large to penetrate into the pores of the membrane. Moving forward, we will be lysing the cells and isolating the functional fragments containing the PfMSP-1 antigen to lay on the membrane.



Sona Tissington
David McMillen

10:00am

Saccharomyces cerevisiae Kit Deployment for Local Testing of Viral Disease

Researchers in the McMillen laboratory at the University of Toronto Mississauga successfully created a novel agglutination assay in 2022, utilizing yeast (*S. cerevisiae*) as a capture element for antimalarial antibody detection. The agglutination test for viral disease provides easily visualizable qualitative results, where a pellet indicates a negative result (no antibodies present), and a sheet indicates a positive result (antibodies present). Questions remained regarding the effect of long-term storage on agglutination results. We developed protocols and methods directing the growth, drying, and long-term storage of transformed yeast cells (pGAL-S1a) for maximum surface protein intactness, vital for the capture of antibodies in the agglutination assay. We focused on cost-effectiveness along with suitable growth and drying methods for local implementation. This research emphasizes a practical implementation of affordable testing in low-income countries for viral diseases including Malaria, Dengue fever, and SARS-CoV-2. We utilized the primary antibody (cMyc) confirmation assay and the agglutination assay to examine the effects of rehydration of pGAL-S1a cells at various drying times and storage conditions. Preliminary results indicated negative relationship between the optical density and fluorescence of rehydrated cells over time. Agglutination results demonstrate that we successfully captured SARS-CoV-2 antibodies from dried yeast cells stored for approximately one month. These results support the hypothesis that the surface proteins displayed on yeast can remain intact in several long-term dried storage conditions. As a result, viral disease testing utilizing the agglutination assay may be possible for wide-scale deployment in an affordable kit for practical use.

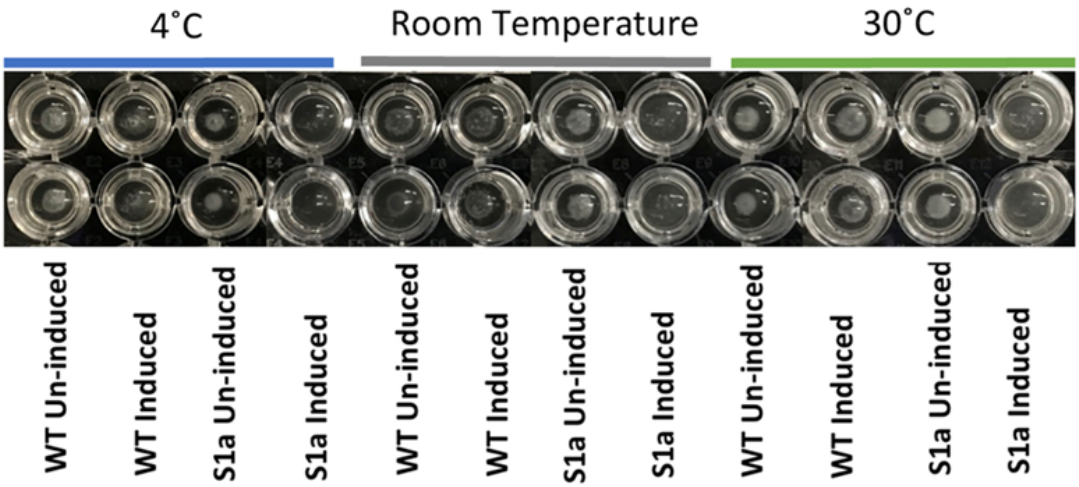


Figure 1. Agglutination results of pGAL-S1a transformed cells induced in Galactose and un-induced. Dried S. cerevisiae cells were rehydrated and tested after one month storage in varying temperature conditions. Results demonstrate visible pellets (negative) and sheets (positive) in the induced cells, indicating successful antibody capture at a concentration of 25ug/mL.

Daniela Cobo 10:15am
Patrick Gunning & Elvin de Araujo

Targeting protein degradation of α-tubulin and β-tubulin by utilizing small molecule inhibitors

Microtubules are one of the most essential macromolecules found within a cell, responsible for structural rigidity, internal transport, extracellular motility, and adhesion. Specifically, α- and β-tubulin subunits assemble to form a hollow microtubule composed of 13 protofilaments. We have developed a class of molecules which leverage 'electrophilic warheads' to prevent tubulin polymerization through targeted covalent inhibition, leading to arrest of important cellular processes and apoptosis. Dosing with increasing concentrations of these compounds in HeLa cells demonstrated a reduction in total cellular protein concentrations. Further specificity was examined using western blotting and immunofluorescence, in which increasing concentrations of compound 1456 caused the targeted degradation of α- and β-tubulin subunits. The potency of compound 1456 was evaluated via cytotoxicity assays in K562 (CML) and MV4-11 (AML) cells, demonstrating an increase in apoptotic features with increasing dosages of the inhibitor. For further comparison, a library of similar small molecule inhibitors was evaluated via cytotoxicity assay, with the most potent molecules demonstrating nM efficacy and Western blotting demonstrating on-target engagement.
us, our class of small molecule inhibitors are potent covalent inhibitors of tubulin polymerization that can induce cellular apoptosis in cancer cells.

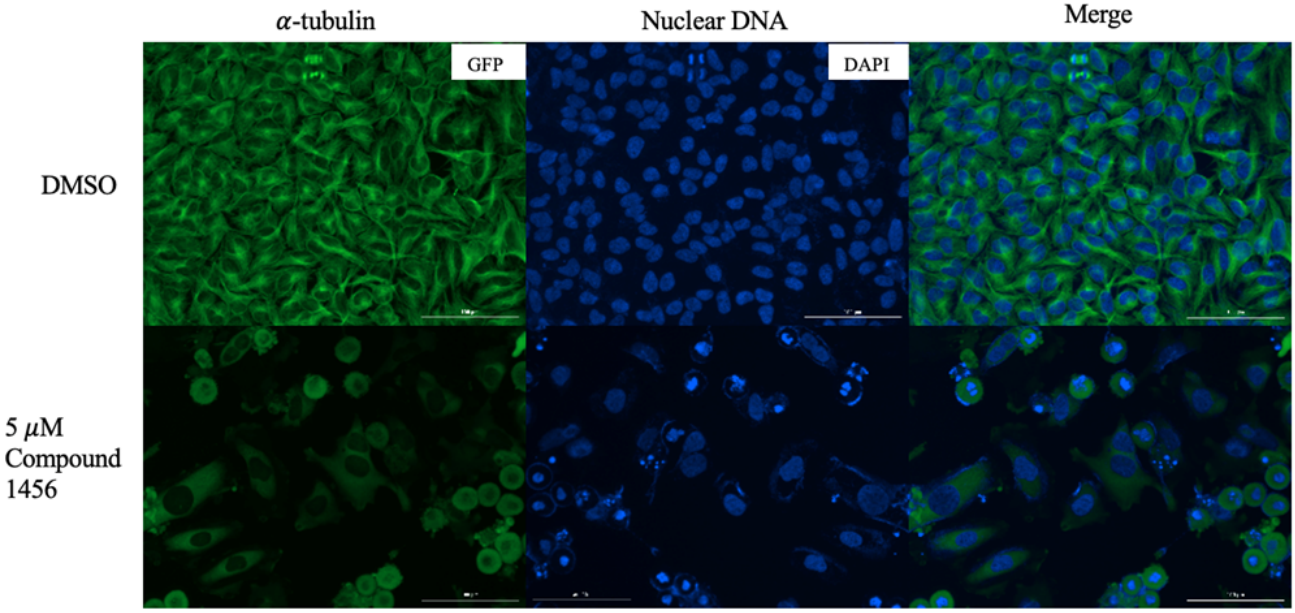


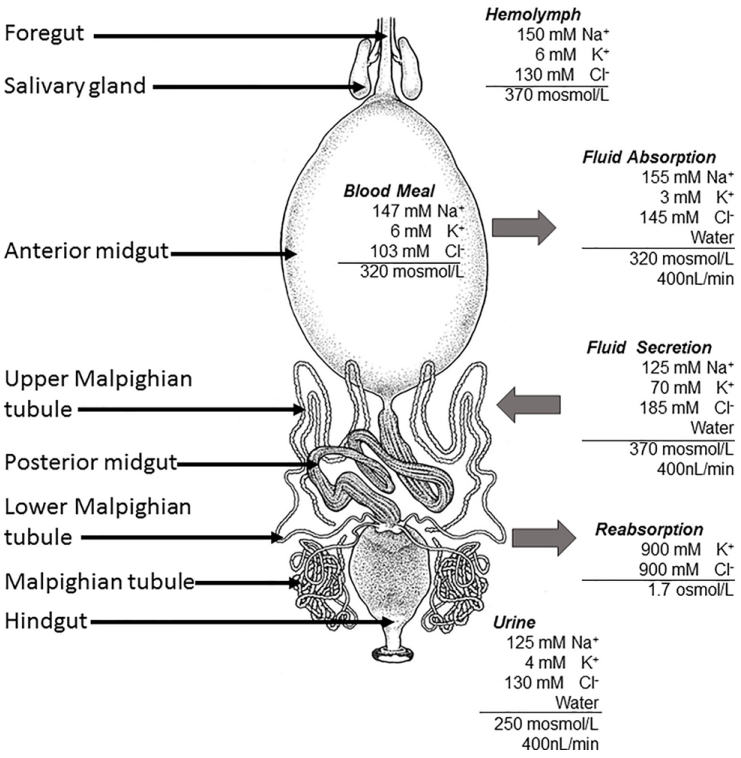
Figure 1. Immunofluorescence assay of α-tubulin using green fluorescent protein conjugated to anti-mouse/α-tubulin antibody. Nuclear DNA was labelled with DAPI and both images were merged. Images were taken over varying concentrations of compound 1456, with DMSO as a control.

Melvin Chelvanayagam
Angela Lange & Ian Orchard

10:45am

CRF's role in diuresis (5th instar) in *Rhodnius prolixus*, a vector of Chagas disease

Rhodnius prolixus is a blood-feeding insect that is a vector of *Trypanosoma cruzi*, a parasite that causes Chagas disease, affecting roughly 18 million people within Central and South America. Development in *R. prolixus* is controlled by blood-meals. After gorging on blood, rapid physiological and endocrinological changes modulate diuresis, a process in which there is an increased production of urine. Corticotrophin-releasing factor (CRF) is a hormone that is released at feeding into the hemolymph from neurosecretory cells located in the mesothoracic ganglionic mass and plays a role in short term diuresis; however, the involvement of its receptor in diuresis has been less studied. Interestingly, two genes for the *R. prolixus* CRF receptor (CRFR) have been identified in the genome of *R. prolixus*. Here, we studied the effects of one of the CRF receptors, namely CRFR2b, on the process of diuresis in R. prolixus. Using RT-qPCR, I identified that the highest CRFR2b transcript expression in the Malpighian Tubules (MT). RNA interference (RNAi) was used to knock down CRFR2b transcript expression. Following a blood meal, I measured weight loss over the course of 4 hours post-blood meal as well as, 24 hours post-blood meal, to quantify the effect of the knockdown on diuresis. The double stranded CRFR2b (dsCRFR2b) insects displayed a decrease in the amount of weight loss compared to insects with double stranded ampicillin resistance gene, dsARG. Thus, this study confirms that CRF signaling is involved in regulating diuresis and provides information for further studies in controlling the spread of Chagas disease.



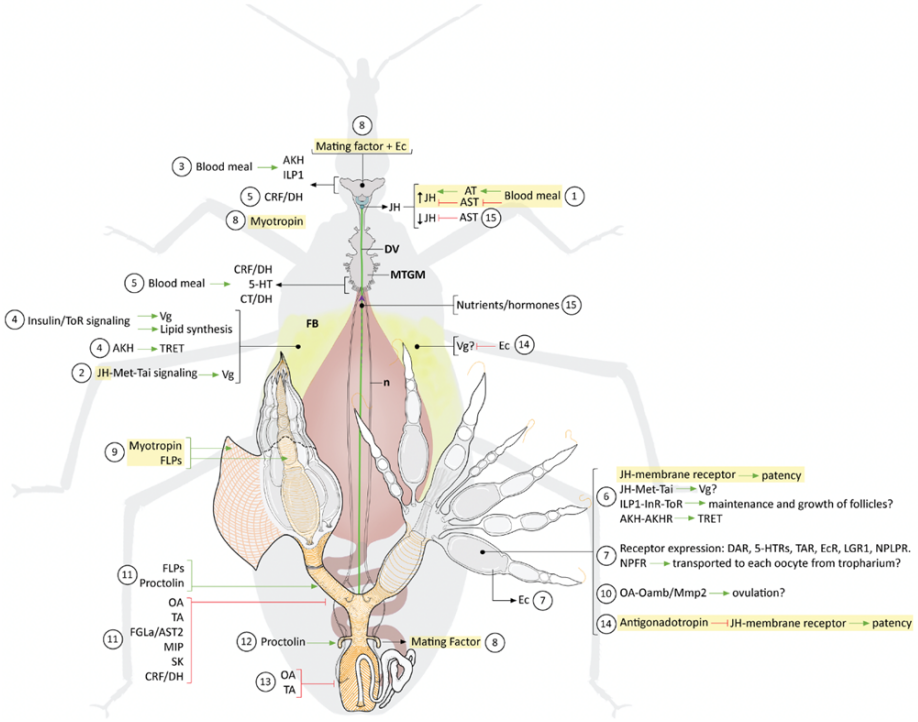
Nawar Faisal

Angela Lange, Ian Orchard & Luca Finetti

11:00am

Investigating the Role of Octopamine in Reproduction in *Rhodnius prolixus*

The biogenic amine octopamine is a monohydroxylic analog of norepinephrine and was first discovered in the posterior salivary glands of the octopus. Over the past decades, research on Octopamine has increased significantly. Octopamine is found in both vertebrate and invertebrate nervous systems, and it is involved in several functions in insects, acting as a neurotransmitter, neuromodulator and neurohormone. Here, we focus on the roles of the octopamine Alpha 1 receptor (Octα1-R) and its effect on egg laying and reproduction in *Rhodnius prolixus*, the blood-gorging kissing bug. This research topic is also medically significant, as *R. prolixus* is a principal vector of Trypanosoma cruzi, the causative agent of Chagas disease in humans. Through molecular biology techniques, it was determined that the transcript for Octα1-R is expressed in the greatest amounts in the reproductive organs of the female *R. prolixus* and mainly localized in the region of the calyx and the ovaries. To evaluate the involvement of octopamine signalling in female reproduction, the transcript expression of Octα1-R was knocked out in adult females using double-stranded RNA, the subsequent effect on egg quality and egg-laying was then assessed. It was found that a decrease in octopamine signalling resulted in a decreased number of eggs laid and also altered the appearance of the eggs in knockdown females. The study of the physiological processes involved in reproduction in R. prolixus can have significant implications for controlling the insect population and the transmission of Chagas disease. By understanding such reproduction, biopesticides and other strategies for controlling insect populations can be established.



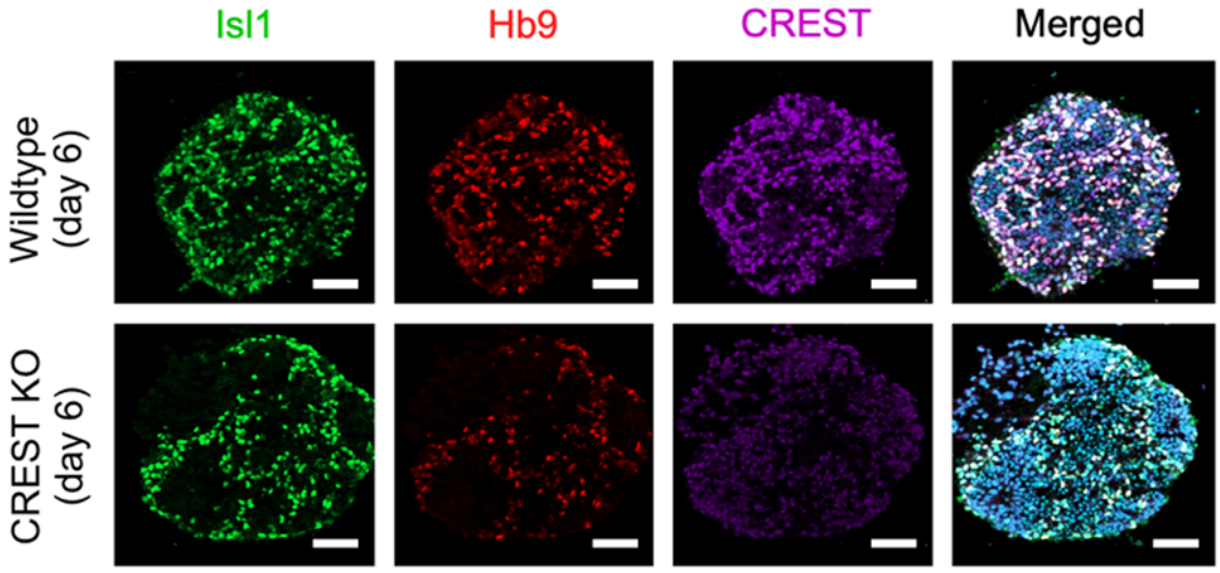
Lange, A. B.; Leyria, J.; Orchard, I. The Hormonal and Neural Control of Egg Production in the Historically Important Model Insect, Rhodnius Prolixus: A Review, with New Insights in This Post-Genomic Era. General and Comparative Endocrinology 2022, 321-322, 114030.

Andy Lu
Ho Sung Rhee

11:15am

Examining the role of CREST on Gene Regulatory Proteins During Motor Neuron Differentiation

Neurons are the fundamental cell types of the nervous system, and their development is a complex process that is tightly regulated by gene regulatory networks. The neuronal brg1-associated factor (nBAF) complex, a cell-specific chromatin remodelling complex, plays an essential role in this process by altering the accessibility of DNA and subsequently regulating gene expression. The nBAF subunit, CREST, is crucial for proper motor neuron function, and mutations in the CREST gene have been linked to amyotrophic lateral sclerosis (ALS). This study aimed to investigate the impact of CREST loss on key regulatory proteins, including Brg1 ATPase and CBP histone acetyltransferase, at various stages of motor neuron development. Brg1 catalyzes ATP hydrolysis to remodel the chromatin and make DNA regions accessible, while CBP is a transcriptional coactivator that acetylates histone tails and activates transcription. Wildtype and CREST knockout spinal motor neurons were differentiated from mouse embryonic stem cells in vitro. Immunostaining for motor neuron specific markers, including Hb9 and Islet-1, was employed to confirm the identity of the differentiated cells as motor neurons. The western blot analysis showed that Brg1 is ubiquitously expressed in primitive ectoderms, post-mitotic maturing neurons, and CREST knockout mutants. The downregulation of Brg1 expression in CRESTKO motor neurons suggests that CREST may impact Brg1 expression. Furthermore, the western blot analysis also revealed that loss of CREST might also impact the expression of CBP, as CBP is downregulated in day 6 CRESTKO compared to day 6 WT. These findings suggest that CREST may be involved in regulating the expression of critical gene regulatory proteins such as Brg1 and CBP during motor neuron development. Further studies using co-immunoprecipitation may help elucidate how CREST interacts with Brg1 and CBP to carry out the nBAF function.



Fatima Nagi
Ingo Ensminger

11:30am

Resilience of trees to hotter and drier climate determined by anatomical and mechanical properties of the vascular tissue

The Canadian boreal region is home to 28% of the world's boreal forests and thus has great ecological and economic significance. This region is expected to experience a temperature increase of approximately 2-4 °C by the year 2060, as well as droughts of increasing frequency and severity. Drought stress is already impacting the Canadian boreal forest and is the leading cause of tree mortality. According to various studies, this lethal effect of drought stress on conifers is also exacerbated by heat stress. Xylem anatomy determines hydraulic and mechanical properties and hence the ability of trees to resist drought and heat stress. In this study, we sought to characterize the relationship between growth, xylem anatomy, and hydraulic function in five-year-old white spruce (*Picea glauca*) seedlings from two different genotypic origins (a slow growing northern genotype from Sundridge, Ontario [BM1] and a fast-growing southern genotype from Petersborough, Ontario). Seedlings were exposed to four different treatments (control, warming, drought, and warming combined with drought). We examined four traits that are involved in resistance to heat and drought stress, including mean hydraulic diameter (Dh), xylem-specific hydraulic function (Ks(t)), and mean cell wall reinforcement ([t/b]2) in order to assess whether a functional trade-off exists between xylem efficiency and xylem safety in Picea glauca of the aforementioned treatments and genotypic origins, as this could have implications on the heat and drought tolerance and survival of *Picea glauca* and other evergreen coniferous species in the future.

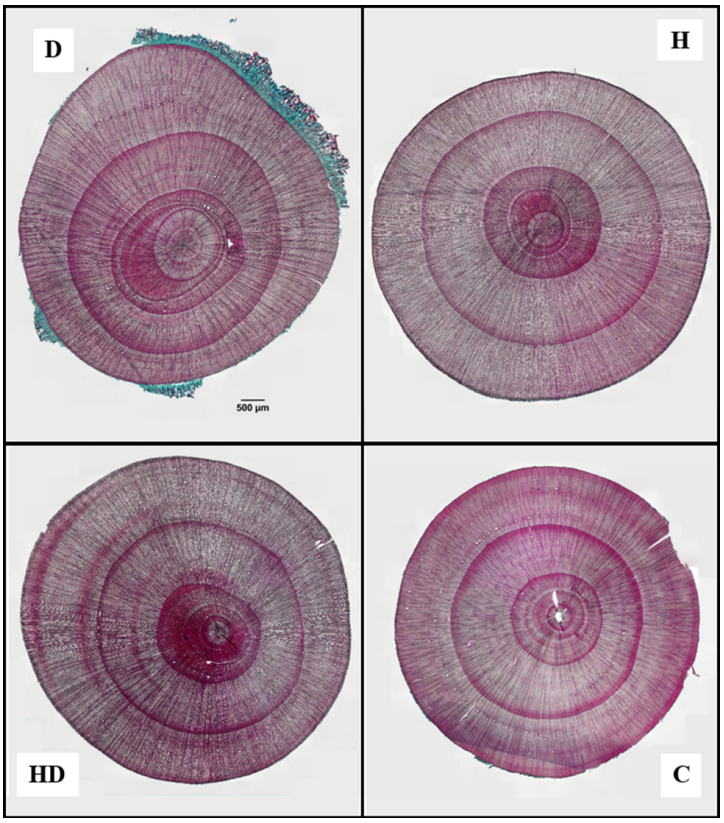


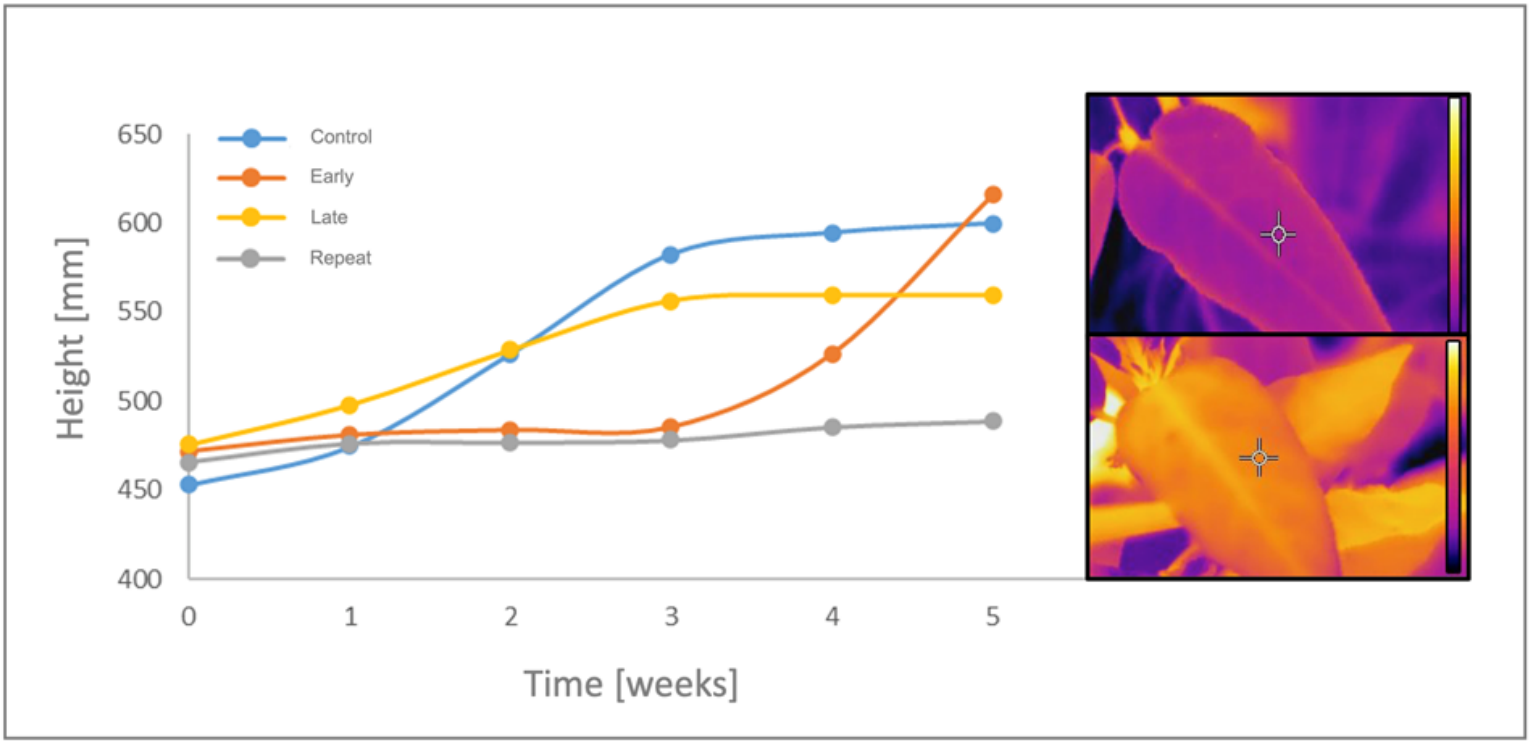
Figure 1: Microscopy images of cross-sections from five-year-old *Picea glauca* seedling stems exposed to drought (D), warming (H), drought combined with warming (HD), and control (C) treatments. Cross sections were stained with Alcan blue and safranin, and tracheid cells are shown in red. All images are shown to a common scale; scale bar = 500 µm.

Andrew Yu
Katharina Braeutigam

11:45am

Responses to heat waves in a deciduous tree

Extreme weather events such as heat waves have been linked to anthropogenic climate change and are projected to increase in frequency and severity. Heat waves can lead to plant mortality, increased fire risks, and result in rapidly declining forest health. It is, thus, critical to understand tree responses to high temperature extremes events in detail. Here, we investigate responses of the native balsam poplar (*Populus balsamifera*), a tree common to Canadian forests. Plant performance under individual and repeated heat waves was assessed by growth documentation, thermal imaging, and chlorophyll fluorometry to study photosynthetic energy conversion. Growth was not only affected by the treatment itself but also by the timing of the heat wave event. Thermal imaging detected effective evaporative cooling under all treatments with leaf temperatures not exceeding 35°C. Along with data on the quantum yield of Photosystem II (ΦPSII), thermal imaging data further highlight the interplay between timing of the heat wave, plant age, and repeated occurrence of the stress event. In addition to high temperatures, heat waves in temperate regions can be accompanied by soil moisture deficits. Our data offer insights into the roles of increased temperature and drought in shaping plants responses during heat wave events. Studies into molecular responses during such stress events are currently underway.



Samantha Bestavros
Alex Nguyen Ba

1:00pm

Variant effect mapping through protein folding and stability

Variants of uncertain significance (VUS) make up the largest proportion of clinically reported amino acid substitutions, and because the biological consequences of these allelic variants are unknown, their impact on informing patient prognosis and treatment options is limited. Variant effect maps score all possible missense variants in specific proteins for impact on protein function, and interpreting these can provide clinically relevant information regarding patient disease prognosis and treatment plans, though they are only available for <1% of disease-associated genes. Deep-mutational scanning combined with multiplexed-phenotyping as a measure of protein function is a revolutionary approach for building these maps, though this strategy is quite costly and relies on the presence of in vivo phenotypes in model systems, which are not always detectable. Protein conformation, however, is a universal property of proteins that can be assessed in vitro, and disease-causing mutations are known to impact protein structure. Here, we aim to develop a thermal shift assay that can assess protein misfolding propensity and stability of allelic variants to create variant effect maps for the purpose of assessing disease risk. We have established a framework to test the denaturation midpoint of mutations in NTHL1, paving the way for in vitro functional determination. Future work with Tiled Region Exchange Mutagenesis (T-REX) and protein barcoding will allow for the production of variant effect maps, which will enhance the ability to test for genetic diseases, aid in informing human disease risk and treatment, and contribute to the development of equitable methods of genetic counselling.

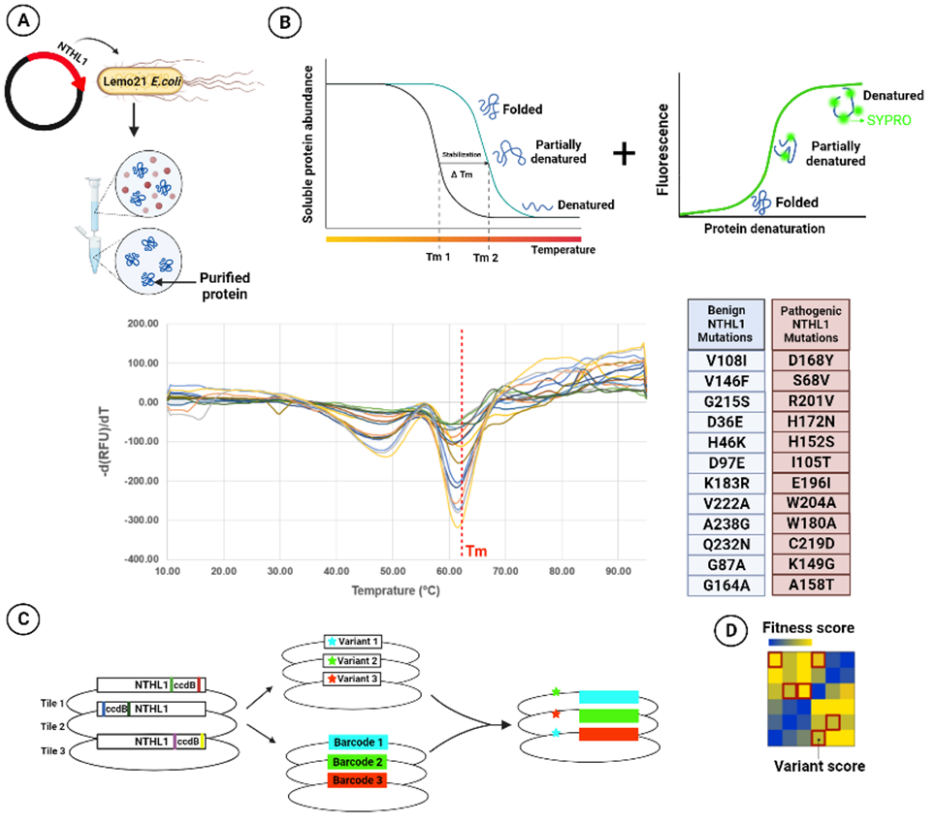


Fig. 1. Schematic of the experiment. (A) Protein purification. Expression of NTHL1 in Lemo21 E.coli strain followed by protein production and purification. (B) In vitro assay and thermal shift assay. In vitro assay using FRET oligos (FAM/TAMRA) and qPCR. Thermal shift assay using SYPRO stain as a test. The amount of fluorescence detected is positively correlated with the proteins' denaturation. (C) Protein barcoding and full variant mutagenesis. Using Tiled Region Exchange Mutagenesis (T-REX), tile the proteins with ccdB, add protein barcodes, and systematically perform pooled mutagenesis. (D) Full pooled thermal shift assay. Perform pooled thermal shift assay, analyze the data in the context of protein structure, and contribute to the variant effect map construction for NTHL1.

Kortni Kindree
Alex Nguyen Ba

1:15pm

Uncovering the pathogenic variants of ATP7a leading to Menkes' Disease

Identifying the pathogenicity of variants of unknown significance (VUS) in disease-causing genes is extremely beneficial in genetic screening and healthcare, as these missense mutations will target one quarter of all individuals who are unknowingly carriers of non-mild disease genes. The ATP7a gene, located on the X-chromosome, was analyzed using a budding yeast surrogate model system with its homologous gene, CCC2, to establish the functionality and phenotype of benign and pathogenic mutations. Various mutations in the ATP7a gene are currently of unknown significance, yet are known to contribute to Menkes' Disease, while other ATP7a variants are less pathogenic, forming Occipital Horn Syndrome. The pathogenic mutations of this gene are indicative of disrupted copper and iron transport systems in the cell, which causes death in early childhood. Throughout this project, the difference in phenotypic function between pathogenic and benign variants are tested, where each variant is grown under iron starvation environments and compared to the wildtype strain for difference in growth ability and function. This project is leading towards evidence that certain missense mutations will have a greater impact on the phenotype when all possible missense mutations of the protein are tested. Contributing to the variant effect map through identifying pathogenicity of variants of unknown significance will lead to the discovery of disease-causing mutations in genes such as ATP7a, while also further informing genetic counselling to reduce burdens on health care and families suffering from these rare diseases.

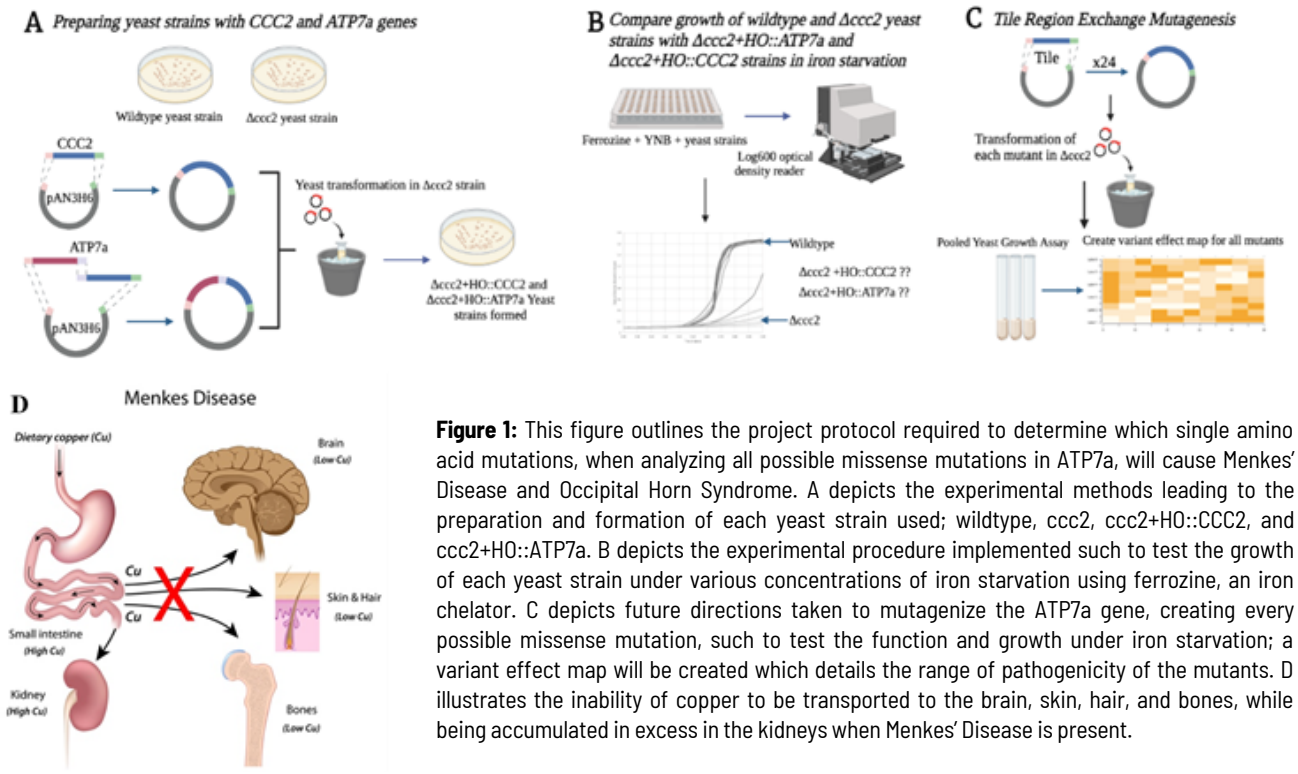


Figure 1: This figure outlines the project protocol required to determine which single amino acid mutations, when analyzing all possible missense mutations in ATP7a, will cause Menkes' Disease and Occipital Horn Syndrome. A depicts the experimental methods leading to the preparation and formation of each yeast strain used; wildtype, ccc2, ccc2+HO::CCC2, and ccc2+HO::ATP7a. B depicts the experimental procedure implemented such to test the growth of each yeast strain under various concentrations of iron starvation using ferrozine, an iron chelator. C depicts future directions taken to mutagenize the ATP7a gene, creating every possible missense mutation, such to test the function and growth under iron starvation; a variant effect map will be created which details the range of pathogenicity of the mutants. D illustrates the inability of copper to be transported to the brain, skin, hair, and bones, while being accumulated in excess in the kidneys when Menkes' Disease is present.

Simran Rakhra
Steven Short

1:30pm

The Diversity of Haptophytes within Freshwater Environments

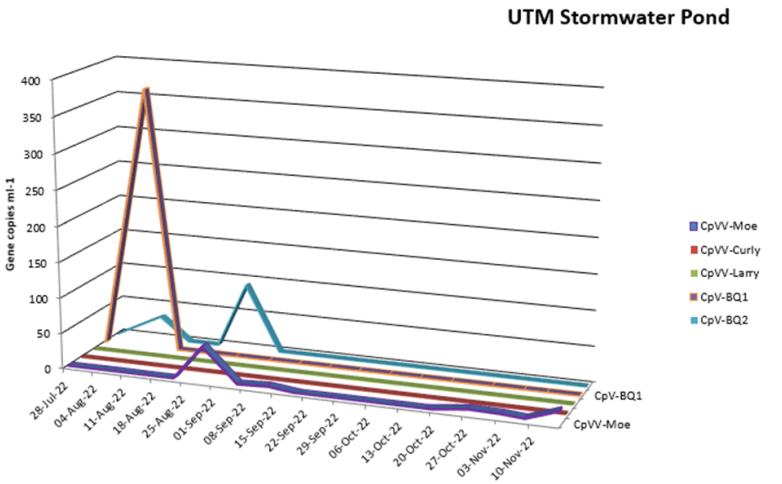
To determine the haptophytes that are infected by algal viruses, the diversity of haptophytes was studied in two Lake Ontario water samples. Promega Maxwell automated DNA extraction instrument was used to extract DNA from organisms present on the 0.45 µm pore-size membrane filter, which was used to filter the water samples. Eukaryote and haptophyte gene fragments were PCR-amplified from eDNA (environmental DNA) using five universal primer sets. One primer set targeted 18S rRNA genes from most eukaryotes, the second primer set targeted 18S rRNA genes from algae, the third primer set targeted 18S rRNA genes from Prymnesiophyceae (a class of Haptophytes), the fourth primer set targeted 18S rRNA genes from Pavlovophyceae (a class of Haptophytes), and a fifth primer set targeted viral genes. The PCR reactions confirmed the presence of amplifiable eukaryote and algal DNA in the nucleic acids extracted from Lake Ontario's water samples. However, a single PCR reaction failed to amplify Prymnesiophyte gene fragments, which is why a nested PCR reaction was conducted using two primer sets. The first primer set targeted algae's 18S rRNA gene and the PCR products from this reaction were targeted using the Prymnesiophyceae primers. This determined that there are Prymnesiophytes present in both water samples. The nested PCR's reaction products were cloned into a plasmid, and E.coli was used to uptake a plasmid. A total of 35 cloned PCR fragments were sent for Sanger sequencing, and BLAST was used to classify the sequenced haptophytes.

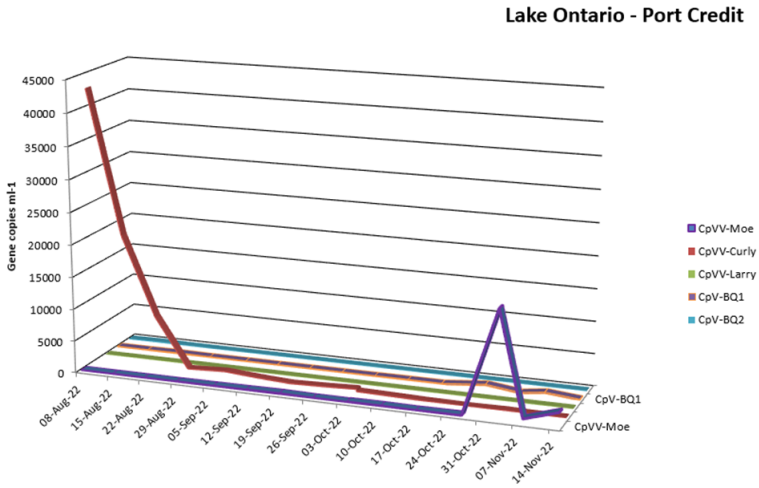
Daphne A. Arguelles
Steven Short

1:45pm

Monitoring the environmental abundances of multiple algal viruses using multiplex quantitative PCR

Lake Ontario contains many viruses, including the virophages CpVV-Larry, Curly, and Moe which presumably parasitize the viruses CpV-BQ1, and BQ2 of the freshwater phytoplankton *Chrysochromulina parva*. To determine if these viruses' abundances varied over time and between two distinct environments, water samples were collected weekly from two sites, Lake Ontario at the mouth of the Port Credit River (LO-PC), and a stormwater pond at the University of Toronto Mississauga. Samples were filtered and concentrated, and multiplex quantitative PCR was used to track virus abundances. In the UTM pond, BQ1, BQ2 and Moe were present at low abundances, yet, Larry, and Curly were not detectable. In comparison, in LO-PC, Moe was abundant in all samples, Larry was never detected, and the others' abundances were highly variable. This study demonstrated the highly dynamic nature of these viruses infecting a single host and highlights the distinct microbial ecology of nearby freshwater environments.





Eunhye Lee
Shannon McCauley

2:15pm

Effects of increasing road salt conditions on survival and behaviour in water boatmen

An important aim in urban freshwater ecology is to understand how human activities impact aquatic organisms living in urban ponds. Road salt is used to increase winter driving safety, making research on the effects of salinization on aquatic organisms necessary in order to develop appropriate strategies to mitigate negative effects. I conducted a lab experiment in microcosms to examine activity level and survival rate of semi-aquatic water boatmen species, Hesperocorixa vulgaris, under different road salt concentrations. I measured survival and behaviour of water boatmen exposed to 4 levels of salt concentration treatment (control, less than chronic, mid, and greater than acute). Water boatmen not exposed to road salt had increased survival and that in the road salt treatments, the survival rate of water boatmen was reduced. There also tended to be differences in activity level when exposed to increasing road salt; water boatmen in the control group and L. chronic treatment spent the most time actively swimming whereas individuals in the mid and G. acute treatment tended to swim less. My results suggest that increasing road salt may impact the survival and movement behaviour of Hesperocorixa vulgaris. The results of this study suggest that road salt inputs into freshwater negatively affect survival and behaviour of some freshwater organisms. These changes can have trophic impacts on other freshwater organisms in urban stormwater pond systems. Lastly, this research improves our understanding of the impacts of road salt on freshwater insects and may help to efficiently protect freshwater biodiversity in Canadian cities using road salt.

Krista Kueviakoe
Shannon McCauley

2:30pm

Community Structure of Trichoptera in the Credit River Watershed between 1950 and 2019

This study demonstrates how the sex ratios and diversity of the primarily aquatic insect order of Trichoptera (Caddisfly) varies over space and time. The community being examined is the Credit River Watershed, between the 1950s and 2019. Diversity will be assessed on the alpha, beta and gamma scales: using raw richness, as well as the Shannon-Wiener index for species richness and evenness. Due to the varying levels of urbanization that exist between sites, and an increase of urbanization (and pollution) within the last seven decades, it will be possible to determine how caddisfly communities have changed: whether they show resilience or are negatively affected by these changes. Caddisflies are an ideal study organism because they have been shown to have varying sensitivities to pollution. The ethanol-preserved caddisfly samples for this study were taken from the Royal Ontario Museum Entomology collection, collected by Glen B. Wiggins in the 1950s, and the samples from 2019 were collected by PhD candidate Kelly Murray-Stoker. This paper will help develop the knowledge we have about long-term community change in Trichoptera of the Credit River Watershed.

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	Site 15	Site 16	Site 17
Family, Genus	Alton	Belfountain	Caledon	Cataract	Cheltenham	Churchville	Forks of the	Erindale	Glen William	Hillburgh	Meadowvale	Melville	Norval	Orangeville	Port Credit	Snelgrove	Streetsville
Hydropsychidae Cheumatopsyche	*	*			*	*	*	*	*		*	*	*				*
Hydropsychidae Hydropsyche		*		*	*	*	*	*			*		*			*	*
Hydropsychidae Potamyia																	*
Hydroptilidae Agraylea			*									*					
Hydroptilidae Hydroptila		*	*		*	*		*	*			*	*		*		*
Hydroptilidae Ithytrichia						*											
Hydroptilidae Mayatrichia						*		*					*				*
Hydroptilidae Orthotrichia															*		
Lepidostomatidae Lepidostoma		*															
Leptoceridae Ceraclea		*	*			*	*		*						*		*
Leptoceridae Mystacides														*	*		*
Leptoceridae Oecetis		*			*	*	*	*	*		*		*		*	*	*
Leptoceridae Triaenodes																*	
Limnephilidae Anabolia		*															
Limnephilidae Asynarchus		*										*					
Limnephilidae Drusinus		*															
Limnephilidae Frenesia							*										
Limnephilidae Hesperophylax							*										
Limnephilidae Limnephilus		*				*	*					*				*	
Limnephilidae Onocosmoecus		*								*							
Limnephilidae Pycnopsyche		*					*										

Presence: *
Absence:

Tolerance levels to pollution:

Sensitive	Medium	Tolerant

Figure 1: Presence/Absence chart for Trichoptera genera across sites of the Credit River Watershed. The table also outlines the varying levels of tolerance to pollution of the different Trichoptera genera present.

Margaret Anderson
Mark Currie

2:45pm

Examining Heterochromatin Protein-Protein Interactions using a Yeast Two-Hybrid System

Not available online.
Printed copies will be provided at the event.

Alexis Konopny
John Ratcliffe

3:00pm

Habitat-specific differences in relative bat species' abundance on UTM campus and beyond

In Southern Ontario, there are eight species of bat, three of which are migratory. Most use a variety of foraging habitats, but each species prefers a subset of habitats. Furthermore, with increased urbanization, some species appear to do better (or worse) in urban environments. UTM campus is composed of wild, parkland, and entirely human-made habitats. Over the months of June, July, and August, I sought to document which species are found in each of four habitat types – open fields, forest trails, ponds, and parking lots – and to document the relative abundance of these eight species in each of these habitat types. I found that overall bat activity was highest in July, and that bat species exhibited expected differences in habitat type use. Most of these habitat-use differences can be explained by differences in echolocation behaviour, body size, and wing shape between species. However, big brown bats, *Eptesicus fuscus*, were by far the most common species in and around UTM campus, including Erindale Park and Riverwood Conservation Area, an observation consistent with this species' known heavy use of urban spaces for foraging and roosting. Overall, while Ontario's bat populations face a variety of human-induced hurdles, from white-nose syndrome, wind turbines, to human encroachment, I found that even with the use of a conservative auto-ID program for species-species echolocation call behaviour, that in and around our campus we can find all eight species of bats.

THANK YOU

to our amazing judges!

Dr. Jimena Leyria
(Postdoctoral Fellow, Lange/Orchard)

Dr. Brandon Walters
(Assistant Professor, UTM Biology)

Dr. Hind Emad
(Postdoctoral Fellow, Johnson)

Dr. Nagham Abdalahad
(Assistant Professor, Teaching Stream, UTM Biology)