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Abstract
The Richard E. Peter 16th Annual Biology Conference is organized by the Biology Graduate Student's Association at the University of Alberta. This event is a multi-day, interdisciplinary, student run conference that showcases research conducted by graduate and senior undergraduate students in the biological sciences. In alignment with the conference theme, "Sustainability," presentations explore innovative research that addresses environmental challenges and conservation, as well as fosters sustainable practices. This conference serves as a platform for students to present their findings in various fields, including Molecular Biology and Genetics, Paleontology, Microbiology, Plant Biology, Physiology and Development, Marine Biology, Immunology and Infection, Entomology, and Health Sciences. For more information, visit the conference website https://repeterconference.weebly.com/.

Keywords: biology; ecology and evolution; microbiology cells and systems; sustainability; genetics; health sciences; immunology and infection; plant biology; entomology; paleontology

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Conference Abstracts

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Oral Presentations in Ecology and Evolution

Soil greenhouse gas dynamics on pastures subjected to legume sod-seeding in central Alberta

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Grassland management is crucial for reducing greenhouse gas (GHG) emissions such as methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O). Cattle overgrazing negatively impacts grassland plant communities, leading to a decline in legumes that enhance pasture productivity, and soil carbon sequestration, and alter GHG fluxes due to changes in soil processes. Rejuvenating pastures through direct legume sod-seeding (LSS) may aid in rehabilitating degraded grasslands. LSS improves nitrogen-fixing plant abundance and diversity and increases nitrogen availability and forage production, which could impact soil GHG dynamics. This study quantifies soil GHG dynamics at three LSS pastures in Central Alberta as part of the Alberta Agrisystems Living Lab. In May and June 2023, producers seeded alfalfa (*Medicago sativa* L.), cicer milkvetch (*Astragalus Cicer* L.), and sainfoin (*Onobrychis vicifolia* Scop. L) into the study pastures. Treatments were implemented using a randomized complete block design, with low-disturbance commercial direct seeding equipment. Each site consisted of four blocks, with two treatments; control (non-seeded) and seeded (legume sod seeding) within each block (N=8 plots). Twenty-four treatments were subsampled with three, georeferenced and randomly 1 x 1-meter quadrats used to assess vegetation cover. Adjacent to each quadrat, a PVC tube (soil collar) with a 21.43 cm diameter was permanently inserted 7.5 cm into the soil, located approximately 1 meter from the subplot designated for regular GHG sampling. We used the Li-Cor Smart Chamber and two gas analyzers (LI-COR Biosciences, Lincoln, NE, USA) to measure soil gas fluxes. Data collection occurred at three to four-week intervals during the growing seasons of 2023 (from July to October) and 2024 (from May to October). Preliminary results showed that soil disturbances significantly increased CO_2 flux in all seasons. LSS pastures demonstrate a greater potential for carbon sequestration, contributing to a reduced climate footprint of grasslands while simultaneously improving livestock productivity.

Swimming ecology of polar bears (*Ursus maritimus*) in the western Hudson Bay

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Movement ecology is complex, influenced by behaviour, phenology, habitat, and resource availability. For this reason, it is difficult to understand the patterns and processes of movement; yet investigating animal movement is crucial for understanding niche, life history, individual traits, and population dynamics. The gap in knowledge is particularly large for marine animals, for which collecting relevant data is challenging while the animal swims. Polar bears (*Ursus maritimus*) are one of such species. In this study, we use satellite telemetry data from 15 adult female polar bears in the western Hudson Bay (WH) subpopulation, Canada to study swimming ecology. Telemetry location and activity data are used to study spatiotemporal patterns of swimming behaviour, including individual variation. Preliminary results via statistical analysis and GIS mapping used to analyze the data indicate variation in swimming phenology, location, and tendency between individuals. These analyses also suggest that, overall, these bears tend to swim more frequently during summer months, in comparison to winter months. Further analysis is expected to provide insight into potential correlations between swimming behaviour and sea ice availability in WH; as well as the location of swimming activity and distances of seasonal migration. The results of this study contribute to our understanding of polar bear ecology, particularly in seasonal ice ecosystems. Additionally, this description of swimming ecology contributes to the extensive literature regarding WH polar bears, yet provides new insights into their movement and behaviour. Understanding how animals interact with their environment provides context for future climatic and ecological shifts, especially in a system characterized by seasonal change.

Factors in the distribution and composition of biological soil crusts in northern grasslands

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Biological soil crusts (BSC's) are a complex mixture of lichens, green algae, cyanobacteria, moss, and fungi that occupy the first few centimeters of soil in a grassland. They play a role in soil stabilization thought to aid in the establishment of vascular plants as well as roles in nitrogen fixation, photosynthesis, and soil moisture retention. Previous studies have shown that small-scale, bottom-up (i.e., soil) processes primarily influence biocrust distributions and composition. However, little research has been done in understanding how crust communities shift along a gradient of environmental variation. Especially in more northern, vascular-plant dominated grasslands where biocrusts are still present and vascular-plant competition may play a stronger role. We use field data collected from the Mattheis (dry-grassland) and Roy Berg Kinsella (mesic mixed-grassland) UofA research ranches to describe the similarities and differences between contrasting crust communities. NMDS ordinations were used to visualize and describe soil moisture, pH, broad vascular-plant cover (i.e., grass, forb, and shrub) and biomass in relation to the crust morphology groups in our two study sites. This research aims to address gaps in the understanding of biocrust community composition in a mesic-grassland, as well as the environmental factors associated with various crust morphology groups. It also aims to expand the potential of crusts as indicators of certain environmental variables.

Inoculation of developing honey bees with black queen cell virus shows complex infection dynamics and mortality trends

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Speciation in the spruce budworm species complex (*Choristoneura spp.*) driven by faster evolution in the sex chromosomes

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Speciation involves divergence but it remains unclear by what mechanisms these divergences develop. Comparisons of genomes now allow tests of mechanisms like the faster X effect, which posits that the X chromosome evolves faster than autosomes. This theory is based on the X chromosome's smaller effective population size and hemizygosity in males, promoting disproportionate involvement of sex chromosomes in reproductive isolation and speciation. In Lepidoptera, females are the heterogametic sex, with the ZZ/ZW sex determination. Previous work has detected the faster Z(X) effect between some sister species, but has not investigated the effect at increasing phylogenetic distances, and hence different degrees of introgression. A test is of this effect is feasible in the spruce budworm species complex (Tortricidae: *Choristoneura spp.*) which includes 7 species, including major forest defoliators in North America, which vary substantially in divergence distances between species, with some pairs showing incomplete speciation. These species maintain their genomic integrity despite introgression, with disproportionate sex linkage in the traits that delimit species, such as pheromone composition, host adaptation, larval developmental rate and photoperiodism. We will assemble de-novo genomes for each species, using both shotgun sequences and long reads where possible, and conduct pairwise comparisons between genomes at increasing phylogenetic distance. By calculating neutral and adaptive divergence rates, we will test the faster Z effect in the spruce budworm species complex, and detect loci with positive selection at specific genes. We expect to find a faster Z effect between closely related species, while more distant species as well as populations within species will have more homogeneous rates of evolution throughout the genome.

Bodies, bodies, bodies: *Drosophila nigrospiracula* avoidance of infected conspecific carcasses

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The landscape of disgust describes a topography of infection risk and avoidance that can influence an organism's behavior. Increased presence of parasite cues can amplify avoidance behaviors; however, being within conspecific groups has also been shown to mediate the perception of risk. *Drosophila nigrospiracula* show avoidance behaviours in the presence of a facultative ectoparasite *Macrocheles subbadius*. Other systems respond to carcasses as parasite hotspots, eliciting avoidance. *D. nigrospiracula* has not been well characterized for its interactions with carcasses, particularly the ability to discriminate between food contaminated with either an infected carcass (IFC) or frozen carcasses (FC). We hypothesized that the presence of parasite cues on carcasses amplifies carcass avoidance due to a greater perception of environmental risk. We also tested the capacity for group-mediated risk perception. We predicted that lone flies and groups of 10 flies will avoid both carcass types; however, IFC are expected to elicit a greater magnitude of avoidance. Groups of flies are also expected to have a lower degree of avoidance in all conditions, relative to lone flies. Behavioural responses were observed in modified Petri dishes with variations in carcass type and fly density. Preliminary results showed that lone flies responded to FC and IFC carcasses with the same degree of avoidance in the first 20 minutes. At 30 minutes, treatment groups for IFC had a lower level of avoidance than that observed in FC treatments. Lone flies showed avoidance of carcasses, but not to a greater extent when exposed to infected carcasses. Groups of flies responded to FC and IFC carcasses with a similar degree of carcass attraction across all time points. Density-dependent differences were observed, with groups of flies engaging in riskier localization that placed them in closer proximity to parasite cues. The social aspect of flies influences the risk perception of carcasses.

An experimental test of the effects of food availability on daytime body temperature in black-capped chickadees

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For species that overwinter in cold climates, when ambient temperature is low and food resources are limited, the ability to efficiently manage daily energy budgets is critical for survival. As such, species often exhibit several strategies related to increasing energy acquisition and limiting energy expenditure. For example, there is evidence that black-capped chickadees (*Poecile atricapillus*) use reductions in daytime body temperature as an overwintering strategy, likely to reduce energy expenditure. Currently, this strategy is not well understood due to limited empirical studies. However, food availability is predicted to be a proximate driver of controlled reductions in metabolic rate and body temperature (often termed torpor). We investigated how food availability shapes daytime body temperature in over-wintering black-capped chickadees and whether individuals differed in their response to food availability. If diurnal reductions in body temperature are used as an energetic strategy when environmental energy availability is limited, we expected that once chickadees gain reliable access to a food resource their daytime body temperature would increase. During a single winter, we monitored the daytime body temperature of 23 chickadees during visits to a bird feeder using unique temperature-sensing passive integrated transponder (PIT) tags. To assess the effect of food availability we experimentally manipulated the presence of food at the feeder, such that chickadees experienced alternating 6-day periods where black-oil sunflower seeds were available at the feeder or not. Here, we present preliminary results from our experiment, highlighting whether chickadees exhibit adjustments in their daytime body temperature in response to the availability of supplemental food. To our knowledge, this study is one of few experimental tests assessing the role of food availability in shaping daytime body temperature in small birds in the winter. As such, this study will provide insights as to how daytime reductions in body temperature are used as an overwintering strategy.

Feed or flee: How different predator cues influence foraging behaviour in black-capped chickadees

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The ability to accurately assess and appropriately respond to the threat of predation is essential to the survival of prey animals. Prey animals can gather information about the level of predation risk in their environment in two, non-mutually exclusive ways. Some individuals might opt to personally survey their environment for predators, for example, by listening for predator calls. This 'personal information' may be more accurate and reliable but could come at the high cost of encountering a predator and being eaten. Other individuals may opt to watch and respond to the behaviour of group members, such as listening for group alarm calls. This 'social information' allows individuals to reduce the likelihood that they encounter predators personally, however, it may quickly become outdated. We asked whether individuals in a population of black-capped chickadees (*Poecile atricapillus*) differed in the strength of response to social or personal information about a predator while making foraging decisions. Chickadees were exposed to personal predator cues, social predator cues, and a mix of both, and we recorded the time it took a chickadee to visit a feeder after the cue exposure. While we did not find evidence that individuals consistently valued one information source over the other, we did find that a combination of social and personal predator cues elicited different responses among chickadees. Individuals varied in how they integrated and responded to simultaneous personal and social information, which suggests that information gathered about predators in the environment is used in different ways by members of a population.

Seeds on the move: Comparing the biodiversity of human-dispersed vs. natural seed rain

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The dispersal of invasive species is a significant concern to biodiversity conservation efforts in National Parks. However, these parks also attract large numbers of tourists, which can inadvertently facilitate the introduction of non-native species, especially around popular visitor areas. This study examines the types of species introduced to such areas through human dispersal and compares them to those dispersed via natural seed rain. To investigate this, we grew seeds collected from a boot collection tray in Elk Island National Park and compared the species composition to that of species present in natural seed rain. We found that the most abundant species were shared between both boot and seed rain samples. Additionally, raw seedling counts in boot brush samples were much higher, pointing to higher contributed propagule pressure. While boot samples were consistent in their composition, the natural seed rain exhibited significant variation depending on the sample location. The high number of non-native species in the natural seed rain, coupled with the substantial propagule pressure from hikers, suggests that Elk Island may already be heavily impacted by non-native species.

Characterizing plant communities in grassland ecosystems

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Grasslands are a critically threatened ecosystem that provides important ecosystem services, including biodiversity maintenance and forage provisioning for livestock. Plant communities are vital components of grasslands, serving as forage and habitat for cattle and other organisms. Understanding the plant community is fundamental for disentangling complex plant interactions in these ecosystems. This study focuses on quantifying which plant species commonly occur together, and if these communities can be predicted based on environmental or spatial factors. Plant community composition was measured monthly at the University of Alberta's Kinsella and Mattheis research ranches. These research ranches represent two distinct ecoregions: the former, in parkland dominated by non-native species, and the latter in the mixed-grass ecoregion dominated

by native grassland species. Analyses will identify clustered community types and the environmental factors that impact these plant community dynamics. Understanding the broad-scale ecological effects of different environmental conditions on plant community structure can inform management practices. Improved management of grassland ecosystems will boost livestock production while maintaining ecosystem integrity, further supporting sustainable food production goals.

Seasonal and social influences on caching in black capped chickadees (*Poecile atricapillus*)

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Caching (i.e. storing food for later) acts as an external energy reserve, increasing the probability of winter survival in small birds. For many species, high intensity caching occurs in fall when food is comparatively abundant. Theoretical models predict that reduced foraging predictability favours increased caching. Higher-ranked individuals often experience greater predictability in their access to food and are therefore expected to need less caching. We used RFID-enabled feeder to quantify the use of cacheable and non-cacheable food in a free-living, marked population of 91 black-capped chickadees (*Poecile atricapillus*), to quantify the daily and seasonal caching patterns for individuals of different dominance ranks. We used two separate proxies for dominance. First, sex (male or female) because in chickadees, males are dominant over females. Second, we used off-territory feeder use (yes or no), based on both theoretical and empirical work suggesting that subordinates have a higher probability of foraging of territory, presumably due to their lower ability to monopolize resources. Contrary to our predictions, both measures of dominance suggest that dominants invest more in caching than subordinates. We found that males invested more in caching than females, and within sexes, individuals using only the feeder on their territory cached more than individuals using multiple feeders. We also found a decline in caching over the course of the experiments (mid-October to mid-November), which was steeper for dominants compared to subordinates. We suggest that our finding that dominants cache more than subordinates may be because the food provided was perceived as being limited, leading to dominants outcompeting subordinates, and preventing subordinates from realising their caching needs, even with non-core territories use. Future work manipulating food abundance during the caching period is needed to test this hypothesis.

Identification in a snap: Accuracy of mobile apps for vascular plants and lichens in vegetation plots

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Species identification in the field requires substantial botanical expertise, making it inaccessible to most nature enthusiasts. Automated species identification holds great potential for alleviating the burden of routine identification tasks. Widely available mobile applications offer users, regardless of their expertise, the potential for simple and rapid automated species identification. The increasing accuracy of these mobile apps is well established. However, it is currently unclear under what circumstances these apps are accurate for species identification in vegetation plots, where image acquisition is limited to a small pool of individual plants. In this study, we evaluated two free plant identification apps, Flora Incognita and iNaturalist using 8262 curated field images of vascular plants and lichens from 54, 0.8 m^2 plots to examine the ID accuracy and their robustness to different image covariates; image perspective (reproductive vs. not reproductive), image quality (low, average, high), and image background (single vs. multiple species focused). We did Chi-square tests to see if ID success varies across growth forms and image covariates. We also used a score fusion method based on the sum rule to test whether using multiple images of the same individual (perspective combination) improved ID accuracy. Overall ID accuracy was 76.9% in Flora Incognita and 67.8% in iNaturalist. Herbs had the highest ID accuracy, while graminoids had significantly lower ID accuracy in both apps. Images featuring reproductive structures and high-quality images significantly improved the ID accuracy for vascular plants and lichens in both apps. Moreover, single species-focused images significantly improved the ID accuracy of lichens. Image perspective combination improved the overall ID accuracy of vascular plants by 5% in Flora Incognita and 7.1% in iNaturalist with significant improvement for graminoids in both apps. Understanding the factors affecting ID success in the field can guide app users to optimize image acquisition for species identification in surveying and monitoring efforts.

Why do male American red squirrels (*Tamiasciurus hudsonicus*) cache more cones? Testing hypotheses from a long-term dataset

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Animal survival depends on balancing energy intake and expenditure, with food hoarding helping species navigate seasonal scarcity. American red squirrels (*Tamiasciurus hudsonicus*) are central-place foragers that cache white spruce (*Picea glauca*) cones each autumn to fuel survival and reproduction the following year. Using a 16-year dataset from Kluane, Yukon, which includes annual cone cache measurements and detailed trapping data, we found that adult male caches are, on average, 1.68 times larger than female caches. Given the species' sexual monomorphism and similar annual energetic budgets, this difference is unlikely due to greater male energetic demands. To attempt to explain these differences, I tested three non-mutually exclusive hypotheses. First, males' peak energy demands occur 4-6 weeks earlier during the snow-covered mating season, when income and scattered foods are inaccessible, while females' demands peak later during snowmelt. Preliminary results indicate that females may rely on recovering scattered cones during this period, potentially decreasing their need for larger central caches. Second, I investigated whether males cache more to compensate for pilferage during the scramble-mating season, when they spend most time off territory. However, pilfering pressure does not appear to significantly influence cache sizes for either sex. Lastly, I tested whether extended parental care reduces females' caching ability during the fall caching period. Results show no significant impact of breeding status on female cache size, suggesting other factors, such as feeding strategies, drive the observed differences. These findings suggest that male and female squirrels employ distinct caching and feeding strategies to balance resource availability and energetic needs. This research provides insight into how territorial species manage resources across life history stages and environmental conditions.

Growth and dietary variation in British Columbia's grizzly bears (*Ursus arctos*)

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Body size varies considerably within species, driven by resource availability and quality, selective pressures, and individual ability to acquire and allocate energy for growth, maintenance, and reproduction. Larger size generally confers fitness gains through increased reproduction, survival, and resource access. However, it is physiologically demanding and constrained by genetic, physiological and resource limitations, as well as environmental factors and competition. In large, long-lived mammals, growth is shaped by these factors over many years, leading to local and population-level differences. Grizzly bears (*Ursus arctos*) are globally widespread, omnivorous generalists with substantial dietary, spatial, and morphological variation. To better understand this variation, we examined how diet, population density, and spatial factors influence grizzly bear size and growth across British Columbia. Using stable isotope analysis of guard hairs, we determined dietary proportions of salmon, kokanee, terrestrial meat, and vegetation. First, we analyzed over 900 skull lengths from hunter compulsory inspections to assess the effects of isotope-derived diet estimates and other covariates on growth. Second, we assigned dietary proportions from predictive dietary maps to over 8,000 skulls to evaluate how diet and other factors influence growth. We then compared patterns across methods to assess consistency in dietary effects on size and evaluate predictive map modeling. We found bears with higher dietary proportions of salmon and terrestrial meat reached larger adult sizes than those with more vegetation in both analyses. Unexpectedly, population density and mean annual temperature also had positive effects on size. While provincial density estimates are coarse, the positive temperature effect suggests climate-driven shifts in resource availability or other temporal trends may be influencing growth.

Evaluating the efficacy of sweep net sampling as a method for measuring grasshopper species abundance

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Grasshoppers are key proponents of grassland ecosystems, serving as primary consumers that support nutrient cycling. However, Grassland ecosystems are among the most endangered ecoregions in Canada, frequently being converted into ranchlands for agricultural purposes. Grasshoppers are significant pests that damage both cultivated crops and rangeland grasses, with serious infestations resulting in significant economic losses to Alberta crops. However, despite their ecological and economic significance, grasshoppers remain largely understudied. The invertebrates are highly mobile, making them incredibly difficult to measure across a large scale. For these reasons, it is critical to use valid sampling methods when studying grasshoppers. The aim of this study is to evaluate the efficacy of localized sweep net sampling as a method for measuring species abundance in comparison to traditional count methods. We hypothesize that, when scaled appropriately, sweep net sampling will yield abundance data comparable to that obtained using traditional count methods. To investigate this hypothesis, we randomly selected sixty sweep net samples from various ten-meter transects, and sixty samples from fifty-meter transects using the traditional count method. Half of all samples were collected from a ranch located in the Central Parkland Natural Subregion of Alberta, while the other half were taken from the Dry Mixed Grass Natural Subregion. If our hypothesis holds true, a linear regression should reveal a positive correlation between grasshopper abundance measured by sweep net sampling and traditional count methods. A key implication of this study is that if our results are accurate, sweep net sampling serves as a cost-effective method for gathering abundance data while also evaluating species diversity. Accurate data collection is essential for both pest management in ranchlands, and the conservation of grasshoppers within grassland ecosystems.

When will the food return? Individual differences in information-gathering strategies in black-capped chickadees

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To make the best decisions about essential resources such as food, mates and territories, animals must gather information from their environment. This information can be gathered in two, non-mutually exclusive ways. For example, animals may gather information by personally surveying their environment by searching for food patches. Searching expends time and energy; however, this 'personal information' will provide the animal with reliable information. Alternatively, animals may gather information by observing the behaviour of group mates such as seeing their group mates return hungry from a food patch. This social information can save an individual time and energy; however, it may be less reliable. Individuals within a population are expected to gather different amounts of personal and social information because the costs and benefits of gathering information differ depending on individual state such as energetic needs and priority access to feeders. We asked whether a black-capped chickadee (*Poecile atricapillus*) population exhibited differences in information gathering among individuals in a foraging context. Eight feeders on our study site went through simultaneous treatments of being filled with black-oil sunflower seeds, and treatments of being emptied. We measured how often chickadees visited empty feeders that they previously experienced as full to assess differences in gathering personal information. In my presentation, I will discuss preliminary results and implications for understanding among-individual differences in information gathering strategies.

Molecular insights into the conservation of northeastern Arctic Grayling populations

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Molecular ecology can provide powerful tools to understand genetic diversity and inform conservation efforts for declining species. In North America, the freshwater fish species, Arctic Grayling (*Thymallus arcticus*), have faced significant population declines in the eastern and southwestern parts of their distribution. Previous research has used mitochondrial DNA (mtDNA) and microsatellite variation to investigate the amount of genetic divergence within these populations and found at least two distinct lineages. However, populations in the northeastern range of their distribution have yet to be sampled, leaving critical gaps in understanding the genetic diversity of Arctic Grayling. This research uses molecular biology

techniques to extract and sequence a region of Arctic Grayling mtDNA in historical samples taken 37 years ago from Saskatchewan and contemporary samples from populations in Yukon and the Northwest Territories. Comparison of these newly generated sequences to prior samples will allow us to gain insights into how Arctic Grayling populations have evolved and gain a more comprehensive understanding of their genetic diversity, particularly in previously underrepresented populations. Thus far, successful extraction of DNA from all of the samples has demonstrated the viability of historical samples for genetic analysis, which is critical for understanding the evolutionary history and genetic diversity within populations. With sequencing and subsequent analyses underway, further results will give insight into the genetic variation within North American Arctic Grayling populations. Understanding this genetic diversity is essential for conservation as it provides insights into a population's adaptive potential and resilience. By establishing genetic baselines, this research provides critical information for the conservation of Arctic Grayling, contributing to broader efforts to preserve biodiversity amid the ongoing biodiversity crisis. Furthermore, it demonstrates the value of historical DNA analysis in molecular ecology and future conservation efforts for at-risk species.

Risky business: Do chickadees alter the timing and intensity of foraging in response to the starvation and predation risk?

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Across taxa, the timing and intensity of foraging by an individual is shaped by its relative investment into mitigating both starvation and predation risk. Theory predicts that when starvation risk is high, such as when ambient temperatures are low, individuals will forage more intensely earlier in the day. Conversely, the presence of predators is predicted to cause individuals to delay intense foraging to later in the day. If both starvation and predation risk are high, individuals are predicted to display two peaks of intense foraging; one early and one late in the day, with reduced foraging midday. We observed a population of more than 150 individually marked black-capped chickadees (*Poecile atricapillus*) over 48 winter days (January through February 2025). We used radio frequency identification systems to automatically record all visits to feeders in the study area, and obtained daily average ambient temperatures from a nearby weather station. Perceived predation risk was manipulated by repeatedly alternating between 6 days of predation risk playbacks with predator calls (northern shrike, *Lanius borealis)* and chickadee mobbing calls, and 6 days of control playbacks from non-threatening and non-competitor congeners (downy woodpeckers, *Dryobates pubescens*, and redpolls, *Acanthis flammea*). We will use these data to test the predictions that individuals shift to later peaks of intense foraging during the predation risk treatments compared to control treatments, but only when ambient temperatures are mild. If ambient temperatures are low, individuals are expected to display a bimodal foraging activity curve, with morning and end of day peaks in foraging activity. These results will provide a direct empirical test of the predictions from earlier theoretical models. By analyzing results at an individual-level resolution, we will also gain insight into how plastic foraging activity patterns can be, potentially improving our understanding of small-bird-in-winter survival strategies.

Form and function of unusual sternal rib expansions in pelicans

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In birds, each rib has two sections, namely a vertebral rib articulating with a vertebra and a sternal rib articulating with the sternum. The sternal ribs are typically relatively uniform in morphology, being slightly curved, elongate, and mostly unvarying in width. In pelicans, the rearmost sternal ribs bear a backwardly projecting flange at the upper end. The flange's function is unknown, and the only documented analogous structure among avians is in the Cretaceous bird *Jeholornis*, which also possesses anomalously expanded rearmost sternal ribs. This study aims to describe these expanded sternal ribs and determine their function - through dissections, and osteological comparisons between pelicans and other avian taxa. The obliquus internus abdominis muscle of birds consistently attaches to the last vertebral and sternal rib, therefore the flange may increase the mechanical advantage of this muscle about the joint between the vertebral and sternal rib sections. This would enhance the mechanical advantage of this muscle about the joint between the vertebral and sternal rib sections, increasing the muscle's ability to contribute to forceful ventilatory movements of the ribcage. The same mechanism may explain the expanded sternal ribs in *Jeholornis*.

Comparative analysis of common home range estimation techniques: A case study using Canadian polar bears (*Ursus maritimus*)

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Quantifying an animal's home range has been a core focus in movement ecology literature, integrating landscape patterns and a species' inherent biological needs into its definition. Understanding aspects of the home range, such as spatial and temporal variability, provides valuable information on foraging opportunities, community interactions, and population dynamics. Most estimation tools utilize location points derived from GPS tracking devices; common techniques include Brownian bridge movement models, kernel density estimators, local convex hulls, and 95% minimum convex polygons. Each method presents unique strengths and weaknesses but should be thoroughly examined for their suitability in home range analyses at both species-specific and broader contexts. This study aims to compare these common estimation techniques using data from adult female polar bears (*Ursus maritimus*) in the Western Hudson Bay, Canada. Polar bears have large home ranges compared to other terrestrial carnivores, and the locations of these ranges may vary due to sea-ice conditions and prey availability. Using GPS points between 2004-2020 from individuals with at least two concurrent years of location data, annual home range estimates will be constructed using each technique. Percent overlap between an individual's two annual home ranges will be calculated and then modelled using general linear mixed models to determine whether overlap is influenced by the estimation method used or environmental and biological variables. Should results indicate that annual overlap is largely driven by the estimation method, one can infer which tools may be more suited to identifying home ranges in polar bears and species with similar movement ecologies. Additionally, findings from this case study could have broader conservation applications by providing resource managers with more accurate assessments of home ranges in target species, which may assist in critical habitat protections.

When more isn't better: Fragmentation limits the benefit of habitat amount for an at-risk old-forest specialist

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Understanding how species respond to habitat loss and fragmentation is critical for effective conservation. We examined how habitat amount and fragmentation independently and interactively influence Black-throated Green Warbler (*Setophaga virens*; hereafter BTNW) occurrence in Alberta's boreal forest. We compared models using habitat amount only, fragmentation only, their additive effects, and their interactive effects across three spatial scales (150m, 500m, 1000m). For each scale, we tested three increasingly comprehensive definitions of what fragments habitat from this species' perspective: large disturbances only, large disturbances with wide linear features (roads and pipelines), and all disturbances including narrow linear features (seismic lines). We found strong evidence that fragmentation matters beyond habitat amount, with significant negative effects across all scales. Importantly, fragmentation effects intensified when habitat amount was low, supporting the nonlinear fragmentation hypothesis. The most comprehensive fragmentation definition consistently provided the best model fit, suggesting BTNWs perceive even narrow disturbances as functional barriers. Effects were strongest at scales relevant to territory selection (150m) and home range (500m). Our findings demonstrate that conserving adequate habitat area alone is insufficient for this old-forest specialist; habitat configuration matters too. This research advances our understanding of species-specific responses to landscape modification and highlights the importance of maintaining unfragmented forest patches, particularly as overall habitat diminishes.

Oral Presentations in Paleontology

Fossil leaves reveal the climate of northern Alberta's prehistoric forests

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As sessile organisms, plants are often useful indicators of the environmental conditions present in an ecosystem. Temperature, light, humidity, and precipitation are all environmental variables that exert powerful selective pressures on the

evolution of plants. In particular, the leaves of angiosperm (flowering) plants are highly plastic, and respond rapidly and dramatically to these environmental variables. As a result, angiosperm leaf morphology is highly convergent; entirely unrelated groups of plants that inhabit similar environments can and often do possess leaves that appear identical to the untrained eye. The tendency of angiosperm leaves to converge towards specific morphologies in response to specific environmental conditions has enabled the creation of modern technologies like CLAMP (Climate Leaf Analysis Multivariate Program) and DiLP (digital leaf physiognomy). These statistical methods allow researchers to robustly estimate over 20 different climate variables (e.g. mean annual temperature, relative humidity, mean annual precipitation, and length of growing season) based solely on diverse collections of angiosperm leaves, including fossils. Our study details a novel angiosperm-dominated fossil flora from northern Alberta believed to be Late Maastrichtian-Early Paleocene (68mya-64mya) in age. It is made up of over 300 fossil leaves, the majority of which are larger than any others previously identified from ancient Alberta. The fossil assemblage is reflective of multiple depositional environments including both high-energy fast-flowing rivers, and stagnant anoxic waters (though floral compositions remain generally equivalent throughout both). In addition to the detailed description of this fossil flora, a CLAMP analysis will be used to provide new insights into the climate of ancient northern Alberta. Importantly, the overall large and broad shape of the leaves suggest a very wet climate with a dense canopy structure.

A regurgitalite from the Late Cretaceous Dinosaur Park Formation, Alberta, Canada

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Bromalites are fossilized remains from an organism's digestive system. They include coprolites (feces), consumulites (stomach contents), and regurgitalites (regurgitated material). Among these, regurgitalites have the most limited fossil record. A newly discovered regurgitalite from the Late Cretaceous Dinosaur Park Formation, Alberta, Canada, adds to this limited record, and is the first occurrence of a regurgitalite in the formation. Discovered in an upper marine unit from the Dinosaur Park Formation, the specimen preserves the indigestible remains of a prehistoric organism's meal along the ancient coast of the Bearpaw Sea. Unlike coprolites from the same formation, the regurgitalite lacks a calcium phosphate matrix and shows no signs of extensive acid etching, suggesting it was ejected before complete digestion. The regurgitalite measures 22 mm long and 11 mm wide and contains hundreds of fish bones held together in a compact iron-rich pellet. A high-resolution micro-CT scan reveals the diversity of elements preserved inside, including acanthomorph spines, centra, and numerous toothed elements. The elements within the regurgitalite are exceptionally well preserved. Many vertebrae retain all their delicate protruding processes, a preservation atypical for the microfossils of Dinosaur Park. Preliminary identification of the elements indicates the presence of one or more acanthomorph fishes (spines), while the vertebrae and other elements may belong to other taxa. The presence of at least two distinct sizes of centra indicates there are more than one individual preserved within the regurgitalite. Further CT segmentation is expected to improve the identification of the fish elements. With continued morphological analysis, it may be possible to suggest the trace maker who expelled the pellet. This unique discovery provides insight into the fishes of the Dinosaur Park Formation, and the trophic interactions that shaped Alberta's Late Cretaceous ecosystem.

A fossil insect renaissance at the E.H. Strickland Entomological Museum, University of Alberta

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Insects, often described as "the little things that run the world," play a fundamental role in sustaining ecosystems, and their fossil records offer insights into long-extinct ecological communities. Despite their significance, fossil insect collections remain underutilized in paleontology. The E.H. Strickland Entomological Museum houses over 3,000 fossil insect compression specimens alongside a modern insect collection of approximately one million specimens. While specialists outside the University of Alberta have published on specific taxa, no comprehensive effort has been made to organize or categorize the collection as a whole. This project revitalizes the E.H. Strickland Museum's fossil insect collection by enhancing its visibility and accessibility through inventory while reassessing its stratigraphical contexts and depositional environments to document its diversity and significance for paleontological and taxonomic research. A new inventory

highlights the collection's remarkable diversity and preservation, with specimens primarily from the Paleocene and Eocene epochs of the Paleogene, alongside limited material from the Late Cretaceous. The fossils originate from 60 localities, including riverbank sites in central Alberta such as Genesee and Burbank. The collection includes numerous holotypes and paratypes, with most specimens belonging to the insect orders Coleoptera and Diptera. Additionally, ongoing research on coleopteran larval taxonomy from the Paskapoo Formation in Alberta will be presented.

Survey of Tyrannosaur rake marks on fossil bones from the Cretaceous period: A research study

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Bite marks in fossilized bones provide evidence of trophic interactions between organisms across the geological time scale, intraspecific competition, and cause of death. In this project, one type of tooth mark found in fossilized Tyrannosaur prey, termed "rake marks", will be surveyed and categorized according to the recently published Category-Modifier (CM) tooth mark classification system. The goal is to identify patterns or unusual trends in the tooth marks that could help distinguish between scavenging and predation events and provide insight into the morphology of the dinosaur responsible for creating the marks. Larger rakes with a greater distance between the grooves are indicative of marks made by a larger organism and can differentiate between types of potential actors, and the type of curvature in the marks can discern scavenging from other feeding behaviours. Bone specimens were collected from paleontological institutions across Alberta and the western United States, then cast in silicone. They were photographed and analyzed using ImageJ. Each rake mark was classified and described according to the CM system. On average, the rake marks were 17mm long and 5mm wide, with 12 grooves per mark. From the rake measurements, 45% of the rake marks were determined to be non-elongate (large width relative to length) while the remainder were elongate (large length relative to width). Out of the non-elongate tooth marks, all were classified as shallow pits (small depth relative to width) except one, and all elongate tooth marks were considered shallow scores. Overall, raked tooth marks on bones, whether they come from predation, scavenging, or a combination of the two, are crucial to fill gaps in the fossil record and further the understanding of dinosaur evolution and ecology.

Surface collected fish fossils (Osteichthyes and Chondrichthyes) from a marine unit in the Dinosaur Park Formation, Alberta

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The Dinosaur Park Formation (DPF) is a unit of rock in Southern Alberta which has excellent preservation of Late Cretaceous fossils. The formation was deposited in flood plains along the coast of the Western Interior Seaway (WIS), which occupied much of the interior of North America during the Cretaceous. By the time of deposition of the latest DPF, terrestrial ecosystems were replaced by marine, as the seaway transgressed over portions of the floodplain. In this presentation, we describe vertebrate microfossils from a unit of marine rock lying east of Dinosaur Provincial Park in the uppermost DPF, which we name here as the Johnson Unit. Our sample was collected via surface collection, whereas previous work in these same sediments focused on screen washing. We collected 1432 vertebrate microfossils representing a minimum of 40 taxa. Our collection yielded 8 new osteichthyan taxa from the Johnson Unit: *Belonostomus longirostris*, Amiidae gen. indet., Elopiformes gen. indet., *Horseshoeichthys* sp., *Enchodus gladiolus*, *Dercetis magnificus*, Acanthomorpha gen. indet., and teleost indet. type O. The acanthomorph spines and centra differ from freshwater acanthomorphs from the Belly River Group. This is the first record of *Enchodus gladiolus* in the province, and the northernmost record of Teleost indet. type O. Additionally, we identify previously indeterminate holocephalian tooth-plates as *Elasmodus* sp., and *Ischyodus bifurcatus*. We use a Principal component analysis to compare our sample to previous screen washing samples from the Johnson Unit. Initial results indicate that differences between the samples are due to the collection method. Larger, intact specimens are better represented from surface collections, and have increased the specificity of our identifications. This impacts our conclusions about the ecology and biogeography of several of our study taxa. Thus, when performed together, surface collection and screenwashing give a more complete understanding of an assemblage than either does on its own.

Micro-CT scans of *Belonostomus* (Aspidorhynchiformes) skulls from the Dinosaur Park Formation

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Belonostomus belongs within Aspidorhynchiformes, an order of extinct Mesozoic neopterygian fishes found in all continents except Asia. Their morphology superficially resemble garfishes because of their long, slender bodies, and a posteriorly placed dorsal fin that is opposite the anal fin. However, aspidorhynchiforms are unique in that they have an extended rostrum formed by the rostral and premaxillary bones, and elongate ganoid scales. Their fossil record begins in the Middle Jurassic and ends in the Late Cretaceous, possibly due to the environmental change occurring at that time with the regression of the Western Interior Seaway. With *Belonostomus tenuirostris* as the type specimen, many species of *Belonostomus* have been discovered around the world such as Canada, Lebanon, Argentina, Germany, and most recently the USA. Regarding their taxonomic relationships, *Belonostomus* sits comfortably within Aspidorhynchiformes as a monophyletic group along with *Aspidorhynchus*, *Vinctifer*, *Richmondichthys*, *Pseudovinctifer* and *Jonoichthys*. They are considered to be the sister-group to Pachychormiformes. However, their relationship to teleosts still remains unclear. Some researchers place them as the sister-taxon to teleosts while others place them within teleosts. To help resolve this question, we here describe two almost complete skulls of *Belonostomus* from the Dinosaur Park Formation of Dinosaur Provincial Park in southern Alberta, Canada. By using micro-CT scanning, we were able to isolate the specimen from the infilled matrix and observe the interior structure of the cranial elements for the first time for *Belonostomus*. By identifying the individual cranial elements of these two specimens, we aim to gain a better understanding of aspidorhynchiforms and their taxonomic relationship to true teleosts.

Oral Presentations in Marine Biology

Dynamics and drivers of coral predation on restored Caribbean reefs: A research study

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The degradation of coral reefs worldwide has prompted a proliferation of active restoration efforts in which nursery-propagated corals are re-planted onto reef sites. However, coral predation (i.e. corallivory), which represents a chronic source of mortality for many restored coral species, is a major challenge hindering project success. Despite the prevalence of corallivory, we lack understanding around the environmental and ecological factors that influence its intensity on degraded reefs. We investigated functional relationships between corallivory pressure, fish and benthic community composition, and habitat structure, which are hypothesized to influence patterns of corallivory via altering resource availability and predation risk to corallivores. To do so, we measured fish community diversity and biomass alongside habitat structure and benthic composition metrics derived from SfM photogrammetry across restoration sites in the Florida Keys, USA. We then quantified corallivory pressure over time using visible predation scarring on live wild and restored coral colonies. We evaluated relationships between corallivory pressure and species community composition across sites with differing levels of habitat complexity using generalized linear mixed effects models and multivariate analysis. Insights gained from this study will inform spatial restoration planning to mitigate negative feedbacks from coral predation, thus facilitating the functional recovery of restored reefs.

Investigating primary production across The North Water Polynya, Pikialasorsuaq, in Baffin Bay

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The North Water Polynya (NOW), also known as Pikialasorsuaq, is a very productive area in the Arctic. Driven by upwelling in Baffin Bay, it brings warm Atlantic waters to the surface, creating an area of open water and thin sea ice surrounded by pack ice that encourages phytoplankton communities - which serve as the foundation for the rich biodiversity in the area. It creates a warm refugium for marine mammals and has been a prominent fishing and hunting region for thousands of years, and in the modern day, a region of conservation and research. The NOW is the largest Arctic polynya and lies downstream of

Nares Strait in the north of Baffin Bay. It spans two major currents: the West Greenland Current, which carries warm Atlantic water into Baffin Bay northward via Davis Strait on the West Greenland Shelf, and the Baffin Island Current, which carries cool Arctic water into Baffin Bay via the Canadian Arctic Archipelago, it then exists into the Atlantic Ocean south through Davis Strait. How primary productivity is affected by these dynamics in the NOW though, is an ongoing question. In this study, we analyze a number of CTD casts from a cruise in 2014, and synthesize those findings with current literature surrounding Baffin Bay water dynamics and the NOW. Investigation into the spacing of productive areas across latitude opens the door to further research into how well oceanographic data can predict biological dynamics in Baffin Bay. This research could bring further insight across different trophic levels, exhibited in fisheries, marine mammal diversity, and seabird diversity, which then help inform environmental policy in this protected area.

Oral Presentations in Plant Biology

Integrating compost-based biostimulants for sustainable crop production

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The heavy reliance on synthetic fertilizers in modern agriculture has increased crop yields but at a cost of significant environmental degradation, including nutrient runoff, soil health decline, and greenhouse gas pollution. Compost-based biostimulants offer a sustainable alternative by enhancing crop production while promoting soil health and ecological stability. These organic inputs improve soil structure, support microbial diversity, and increase plant available nutrients, reducing the need for full-rate synthetic fertilizers. H-start, a compost-based biostimulant, combines proprietary blends of beneficial bacteria and fungi with matured compost, using mild processing techniques to preserve microbial diversity. This innovative bioinoculant replenishes soil microbial communities and fosters sustainable nutrient cycling. This study investigated the effectiveness of H-start in conjunction with two levels of nitrogen-phosphorus-potassium (NPK) fertilizer (75% and 100% of the recommended rate) on crop yield in wheat, canola, and barley rotations across two agroecological regions with distinct soil types: the Battle River Research Group (BRRG) site near Forestburg (dark brown soil) and the Gateway Research Organization (GRO) site in Westlock (dark gray soil) in Alberta, Canada. Overall, applying H-start alongside 75% of the recommended NPK fertilizer produced yields comparable to the 100% NPK rate for all three crops at both sites. The seed protein and oil content of wheat, barley and canola under the 75% NPK with H-start were equivalent to those under 100% NPK treatment. Total available soil nitrogen under the 75% NPK along with H-start was also found to be comparable to the 100% recommended rate of fertilizer for all three crops at the BRRG site, while H-start treatments showed higher available soil nitrogen as compared to the recommended fertilizer rates at the GRO site. These findings highlight the potential of H-start to reduce synthetic fertilizer inputs without compromising crop productivity and quality. Furthermore, the soil microbial community structure under different treatments will be evaluated to understand their role in increasing nutrient availability in the soil and enhancing crop yield.

Clocking in for better nutrition: The role of diel biology in determining healthy habits in plants

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The plant circadian clock is a regulatory mechanism evolved to anticipate and respond to environmental cues by adjusting developmental and metabolic functions. It helps sessile organisms–such as plants–adapt to changing environments, better enabling them to survive in volatile conditions. The plant clock is governed by a series of interconnected transcription factor feedback loops that are expressed at different times of the day, facilitating the oscillatory production of key proteins. Previous research indicates a connection between the plant circadian clock and abiotic stress responses. Beyond sunlight and water, macronutrients are integral to plant survival as they play key roles in energy metabolism and protein synthesis. Here, I investigate how the circadian clock intersects with nutrient responses in plants. To do this, we use diverse genetics to better understand plant growth and development on a range of growth media. The results gathered will enable us to determine how diel biology impacts plant nutrient requirements and responses.

Effect of arbuscular mycorrhizal fungi on the performance of wheat in canola-wheat rotation system

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Arbuscular mycorrhizal fungi (AMF) are a group of fungi that form mutualistic associations with higher plants, including wheat. Canola, a non-mycorrhizal crop, is often grown in rotation with wheat due to its benefits, such as boosting yield and decreasing diseases in subsequent crops. However, reduced AMF colonization is commonly observed in wheat following canola. AMF is critical in improving plant nutrient and water uptake, particularly under stress conditions. Limited research has explored the impact of AMF on wheat performance in a canola-wheat rotation system, especially on Canada Western Red Spring (CWRS) cultivars under Alberta field conditions. This study hypothesizes that genotypic variability exists among CWRS cultivars for AMF mutualistic interactions. Thus, when grown in fields where canola was the preceding crop, these interactions would differentially influence plant growth, nutrient uptake, water use efficiency, yield, and grain quality parameters in different wheat cultivars. This study evaluates six CWRS cultivars with and without AMF inoculants at two field sites in central Alberta over two growing seasons. We expect to identify cultivars with enhanced AMF colonization, water use efficiency, nutrient uptake, grain yield, and protein content. Preliminary results show that AMF inoculation significantly improved plant growth, nitrogen and phosphorus uptake, and grain yield compared to the AMF uninoculated cultivars. Cultivar response to AMF varied, and site-specific conditions affected AMF effectiveness. Importantly, AMF inoculation enhanced grain yield without compromising grain protein in AMF-responsive cultivars. These findings suggest that AMF inoculation could be a valuable tool for improving wheat productivity, but its effectiveness depends on cultivar compatibility and environmental conditions. This study highlights the potential of AMF as a sustainable strategy to enhance wheat performance and provides crucial information for Alberta's crop producers seeking environmentally friendly approaches to boost yield and improve soil health.

Balancing growth and defense: Investigating morphological traits in progeny of lodgepole pine attacked by mountain pine beetle

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Forests play a critical role in biodiversity and climate regulation, yet they face significant threats from pests and environmental change. The lodgepole pine (*Pinus contorta* var. *latifolia*) - mountain pine beetle (*Dendroctonus ponderosae*; MPB) co-evolved system exemplifies these challenges, with recent MPB outbreaks devastating over 27 million hectares of lodgepole pine forests in western North America. To survive, plants allocate limited energy between growth (cell proliferation and enlargement) and differentiation (developing physical and chemical defences), as described by the Growth-Differentiation Balance (GDB) theory. This concept predicts that plants prioritize defence traits like resin production overgrowth when resources are constrained. Regeneration of forests affected by MPB highlights the need to identify mechanisms that promote lodgepole pine resilience to MPB, and determine whether these defences are offset by productivity costs. In our recent studies, we identified genetic fingerprints distinguishing progeny of lodgepole pines that survived (F1MPB-survivor) or died (F1MPB-killed) MPB outbreaks. The goal of this study is to determine whether resin-associated traits are correlated with survivorship in these progeny, and whether these traits are negatively correlated with productivity traits. We hypothesize that F1MPB-survivor trees from at least some families produce more resin ducts, a key defence mechanism in confers, while showing reduced productivity, consistent with the GDB concept. Tree height, diameter at root collar, and both standard and microcores were collected from the same population of trees at ages 11-12. Fluorescence microscopy is being used to quantify axial resin ducts, and tree ring analyses are underway. The findings could inform tree improvement programs, particularly by providing empirical evidence for whether resilience traits correlated with MPB survivorship are balanced with reduced productivity.

Assessing the role of volatile tree emissions on lodgepole and jack pine host quality for mountain pine beetle

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Forests provide critical ecosystem services, including the regulation of climate change and preservation of biodiversity but are significantly threatened by pests and altered environmental conditions. The mountain pine beetle (*Dendroctonus ponderosae*; MPB) epidemic in western North America has wreaked havoc on pine forests. Lodgepole pine (Pinus contorta var. latifolia), an historic host, has been the main species affected by this bark beetle epidemic. MPB underwent considerable range expansion over the course of the epidemic, including spread into forests containing a new host, jack pine (*Pinus banksiana*). However, even when faced with intense outbreaks of these insects, some trees survive on the landscape. Our research indicates that progeny of lodgepole pines that were killed during MPB outbreaks can be genetically distinguished from those that survived. We hypothesize that surviving lodgepole pine exhibit traits that contribute to lower host quality. Similarly, the limited progression of the MPB epidemic into jack pine forests suggests that jack pine exhibits lower host quality than lodgepole pine. I am testing the hypothesis that MPB attraction to host trees is one trait contributing to host quality. Specifically, I am testing the hypotheses that volatile emissions contribute to lower host quality (1) of progeny of lodgepole pine families that exhibited greater versus lower MPB survivorship (resistance) and (2) of jack pine relative to lodgepole pine. To test these hypotheses, I am conducting olfactometer assays, in which I document adult beetle choice between pine seedlings. In tandem, we are quantifying volatile monoterpene profiles and exploring gene expression of the terpene synthases that are putatively involved in synthesizing these monoterpenes. The outcomes of this research will shed new light on whether pine traits that influence MPB host selection contribute to the host quality of pines for this devastating forest insect pest.

Effect of humalite on plant growth, root nodulation, and symbiotic nitrogen fixation in red clover (*Trifolium pratense*)

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Legumes play a vital role in sustainable forage systems by fixing atmospheric nitrogen (N) through a symbiotic relationship with *Rhizobium* bacteria. Establishing a functional symbiosis involves several stages, beginning with signal recognition between Rhizobia and the plant, followed by root nodule formation. Incorporating legumes into cropping systems enhances soil fertility, increases crop yields, and reduces reliance on N fertilizers by improving soil N availability. However, sub-optimum nodulation and symbiotic nitrogen fixation (SNF) can limit these benefits, highlighting the necessity for strategies to optimize these processes. Humalite, a granular product rich in humic acid sourced from a large natural deposit in Southern Alberta, Canada, possesses plant biostimulant properties that have been shown to enhance growth and SNF in grain legumes. However, its effects on forage legumes remain largely unexplored. This study evaluates the impact of varying Humalite application rates on plant growth, root nodulation, and SNF in red clover. Red clover plants were grown in pots under greenhouse conditions with five Humalite application rates (0, 200, 400, 800, and 1600 kg/ha). After ten weeks, plant growth traits, including shoot and root biomass, root length, surface area, volume, and nodulation, were measured. The results show that Humalite application at 400 and 800 kg/ha significantly increased plant dry biomass, with increases of 69% and 75% in shoots, 139% and 77% in roots, and 81% and 75% in total plant biomass, respectively, compared to the untreated control. Humalite application at 800 kg/ha rate also significantly increased root length (43%) and root volume (64%). However, Humalite treatments did not significantly affect the total or average nodule dry weight. Symbiotic nitrogen fixation (SNF) will be evaluated using the ^{15}N-based isotope dilution method. These initial findings highlight the positive impact of Humalite on red clover growth.

Characterizing the role of a novel family of phosphatases in *Arabidopsis thaliana*
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As the site of photosynthesis, the chloroplast has a critical role in plants. The chloroplast relies on light to carry out photosynthetic processes and as a result, many signaling pathways within the chloroplast are tied to the circadian rhythm. The chloroplast can respond to changes in light, and other stresses using key signaling mechanisms linked to post-translational modifications, such as phosphorylation. Both kinases and phosphatases respond to stimuli; kinases catalyze phosphorylation, while phosphatases remove phosphate groups. The addition and removal of these phosphate groups act like a switch to activate and inhibit various processes. For example, in response to light, a phosphatase may dephosphorylate its target protein and cause changes to a photosynthetic process in order to make optimal adjustments for photosynthesis. This study investigates the role of a family of chloroplast-localized proteins in *Arabidopsis thaliana*, which are putatively identified as phosphatases. Many of them show rhythmic circadian expression and responses to a variety of different stressors, however, little previous functional characterization has been performed to define their role or to confirm their phosphatase activity. Using Gateway cloning, I have generated expression vectors for each of the phosphatases for expression in *E. coli*. Here, I will present efforts to purify the proteins and test their phosphatase activity using phosphatase assays. Overall, the result will further our understanding of the role of these phosphatases in plants.

Oral Presentations in Entomology

Performance of MPB, bark beetle competitors, and associated fungi in jack pine in a warming climate
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After an unprecedented outbreak, mountain pine beetle (MPB), *Dendroctonus ponderosae*, populations in Alberta have recently crashed. However, the vulnerability of jack pine to future threats from MPB in the boreal forest is uncertain, especially in the context of a warming climate. At low population densities, MPB and its associated blue stain fungi interact with a community of facilitating and competing insects and their fungal associates under the bark of weakened trees, which impacts MPB establishment and population growth. However, it is unclear how these relationships may shift in a novel host species and climate. In a laboratory bioassay, we analyzed how the patterns of interaction between MPB-associated fungi (*Grosmannia clavigera*) and *Ips pini*-associated fungi (*Ophiostoma ips*) vary with temperature and jack pine chemical defenses. Fungal symbionts were placed on artificial media amended with one of three host chemical defense profile treatments (control, constitutive, induced) in one of three fungal arrangements: alone, concurrently, and after the prior establishment of the other respective fungal species. Fungal growth was measured after two days at one of three temperatures (15°C, 20°C, 28°C). Results suggest that temperature and host chemical defense profiles independently and interactively influence the relationships between fungal symbionts. At elevated temperatures, prior establishment of *O. ips* had a greater facilitatory effect on the growth of *G. clavigera* compared to the concurrent establishment of *O. ips* in induced environments. As temperatures increased, the effect of prior establishment of *O. ips* on the growth of *G. clavigera* shifted from inhibitory to neutral in constitutive environments. The shift from an inhibitory to a neutral relationship in warm environments may impact the role that *I. pini* and its associated fungus will have in the establishment and persistence of MPB in its expanded jack pine range

Exploration of host plant volatile to attract specialist insect *Brassica* feeders for population monitoring
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Striped (*Phyllotreta striolata*) and crucifer (*P. cruciferae*) flea beetles (Coleoptera: Chrysomellidae) are the most significant insect pests of canola, *Brassica napus*, on the Canadian Prairies. The crucifer and striped flea beetles attack canola when it is most vulnerable, as seedlings and can cause extensive yield loss in Alberta, Saskatchewan, and Manitoba. Current monitoring for flea beetles involves in-field scouting from canola emergence through the third true-leaf stage, after which canola can

tolerate feeding damage. This research examines the use of synthetic copies of a *Brassica*-produced volatile signal (allyl isothiocyanate (AITC)) as an attractant to monitor and manage flea beetles on the Canadian Prairies. Field experiments compare commercially available lures and traps to attract and retain both spring and fall populations of striped and crucifer flea beetles. In the 2024 field season, we tested three trap types and two commercially available AITC lures in eight canola fields in central Alberta. Captured beetles were counted and identified. The efficacy of the trap-lure combinations will be discussed for both species of flea beetle and *Delia* (Diptera: Anthomyiidae) flies that specialize on *Brassica*. Developing a semiochemical-based tool for monitoring flea beetles can positively impact integrated pest management of flea beetles in canola fields.

They mite not be eating them: *Macrocheles subbadius* interactions with *Drosophila nigrospiracula* pupae

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Macrocheles subbadius mites are a facultative ectoparasite of *Drosophila nigrospiracula* flies, known to feed on their eggs, larvae and adult flies. However, our understanding of its interactions with pupae is limited. These answers may lie in the mite's feeding capabilities and the development of the pupae. We examined the ability of *M. subbadius* to feed on *D. nigrospiracula* pupae by looking at mite longevity when provided early-stage or pharate pupae as food sources. We also observed residual impacts of direct mite exposure on pupal emergence and adult fly weight. Our findings indicate that mites given pupae showed no significant increase in longevity compared to starved individuals, suggesting that pupae are not a viable food source f*or M. subbadius.* However, early-stage pupae exhibited significantly lower emergence rates than pharate pupae, implying non-consumptive effects from direct mite exposure. These results contribute to a more nuanced understanding of the host-parasite relationship within this system, prompting exploration of the underlying mechanisms and examination of other host-parasite dynamics.

Precocious honey bee (*Apis mellifera*) foragers exhibit transcriptional similarity to nurses and reduced stress tolerance

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The western honey bee (*Apis mellifera*) often encounters chemical stressors in the form of phytochemicals, or pesticide residues. The first line of defense against these agrochemicals is constitutively expressed detoxification enzymes such as cytochrome P450s. Honey bees with lower levels of constitutively expressed enzymes experience greater immediate stress, which can lead to death, sub-lethal effects, and precocious foraging, if nursing. Precocious foragers are young, less developed honey bees that are recruited into the foraging population, whereas normal-aged foragers are much older. These precocious foragers are less capable flyers, and are more likely to die during their flights, which can in turn further stress the colony due to reduced food stores. In our study, we hypothesize that chemical stress sensitivity is directly linked to the onset of precocious foraging. Additionally, we believe that precocious foragers are less suited for foraging due to poorer expression of certain stress tolerance genes like detoxification enzymes, and are likely more transcriptionally similar to nurses instead of older foragers. We measured the survival of honey bees taken from 26 different colonies when exposed to a chemical stressor over 48 hours. Honey bees from each of these colonies were also all added to the same colony, and we measured the age and number of foragers from each colony over a 2-week period. Lastly, we performed transcriptomics of young and old nurses and foragers. Our results suggest that chemical stress tolerance was linked to the honey bee's origin colony. Moreover, we found no significant correlation of the honey bees' chemical tolerance to the age at which they began foraging. Rather, we found that bees from more tolerant colonies exhibited lower volumes of foraging by two weeks. We also show that older foragers exhibit higher expression of P450s and other stress resistance enzymes than younger foragers, suggesting their increased tolerance to stressors over precocious foragers. Lastly, we report that the age of the honey bee is the predominant factor in determining its transcriptomic profile.

Effects of mountain pine beetle (*Dendroctonous ponderosae*) disturbance on pine engraver (*Ips pini*) abundance in northern Alberta pine stands

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The expansion of the mountain pine beetle's (MPB) range east of Rocky Mountains has created large scale disturbance in the lodgepole pine forests of northern Alberta, altering pine stands through mass tree death and opening of the forest canopy. Disturbance events have complex impacts on forest arthropod communities, the impacts of disturbance by mountain pine beetle on insect community succession in pine stands remains unclear. Understanding how large scale MPB outbreaks impact arthropod communities can provide insights into the post-disturbance trajectory of insect community recovery, assisting in forest and pest management. The objective of this study was to assess the long term impacts of MPB disturbance on the abundance of the secondary bark beetle *Ips pini* in lodgepole pine stands of Northern Alberta. 0.25 ha plots were established at 8 sites in the upper peace region of northern Alberta. 4 sites located in undisturbed lodgepole pine stands and 4 located sites in pine stands that experienced disturbance from MPB outbreak 12-18 years prior. Semiochemical baited funnel traps targeting *I. pini* were placed randomly along two linear transects at each plot. Trap samples were collected on a biweekly basis between June and August 2024. Results from count data of *I. pini* from two sampling periods showed no difference in mean *I. pini* abundance between disturbed and undisturbed sites. A difference in *I. pini* count between collection periods was observed but no interaction between site type and sampling period was observed. These results indicate that secondary bark beetle populations in disturbed pine stands return to levels similar to those in undisturbed stands despite alterations to the canopy and stand characteristics.

Immune defenses of *Apis mellifera* queens against Israeli acute paralysis virus

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The honey bee queen is the central hub of the colony, yet a plethora of viruses compromise her health and reproductive vigor. Since the understanding of viral pathogenicity in queens remains limited, we investigated the effect of IAPV infection on the transcriptome of queen pupae, 2-day-old virgin, and 2-week-old queens exposed to CO_2 to mimic the reproductive stage. Queens were injected with either an IAPV inoculum or phosphate-buffered saline as a sham control, and samples were collected 20 hours, 20-24 hours, and 40-44 hours post-injection from pupae, virgin, and reproductive stages respectively. Comparison of transcriptome profiles between IAPV-inoculated and sham-treated queens revealed 1078, 771, and 426 significant DEGs for pupae, virgin, and reproductive stages respectively. GO and KEGG pathway enrichment analysis revealed that the affected biological functions result from a combination of adaptive host responses to combat the virus and viral strategies to manipulate the host. RT-qPCR analysis of the differential expression of 20 immune genes, representing key immune pathways in bees aligned with the transcriptome analysis. The study shows that viral infection significantly impacts the pupal stage, supporting a trade-off between immunity and growth, making pupae most susceptible. In contrast, the reduced effect on the transcriptome of reproductive queens may reflect a viral strategy to minimize harm to the queen, facilitating viral transmission despite the queen's stronger immune defenses. Our findings suggest that IAPV infection alters transcriptomes across all developmental stages of queens, activating antiviral immune responses through key pathways including Toll, Imd, JAK-STAT, and RNAi.

Effect of hydration on courtship song in field crickets: A research study

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Condition dependence of sexually selected traits is a growing area of study, as research is ongoing to understand the development of these characteristics according to the handicap hypothesis of sexual selection. Many avenues of condition dependence remain unknown despite their importance to mating systems, which vary greatly among different organisms.

Current research in cricket courtship song has found conflicting evidence for condition dependence. I tested how and to what extent hydration affects courtship song by applying a hydration stress to sexually mature males of the species *Gryllus ovisopis* and *Gryllodes sigillatus* for 24 hours and comparing the courtship songs they produce with those produced by males provided water *ad libitum*. Differences in mass, proportion of males that produce song, and proportion of males that mated were also evaluated. It was found that dehydration influenced health and may influence willingness to produce songs. However, it was not possible to evaluate effects on song quality. Hydration may influence the mating systems of these species, and it is possible that dehydration has differential effects on species living in different habitats, increasing or decreasing motivation to mate depending on context.

Dine-in or delivery? The host-seeking strategies of the parasite *Macrocheles subbadius* and its host *Drosophila nigrospiracula*

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An organism can change its behaviour depending on the context of its internal state and the external environment, termed phenotypic plasticity. Having plastic behaviour for different circumstances allows the selection of strategies that maximize energy gained and minimize energy expenditure when an animal forages for prey. Foraging strategies have been extensively studied in predator-prey studies, but rarely in parasite-host relationships. The objective of my research is to observe how a free-living parasite's host-seeking strategy would be affected by parasite starvation and host density. Specifically, would the parasite have an active search (cruising) or a sit-and-wait strategy (ambush) when seeking its host? I will test this on the facultative parasitic mite *Macrocheles subbadius* and its host fly, *Drosophila nigrospiracula*. I hypothesize that *M. subbadius* determines its host-seeking strategy based on the interaction between parasite starvation and host density, whereby I predict non-starved mites in high host density will cruise, starved mites in low host density will ambush, and starved mites in high density and non-starved mites in low density will have a mixed strategy. The experiment was done in Petri dishes, where I added mites with a selection of pupae or adult fly hosts in Petri dishes with different starvation levels and the number of adult flies. A mite on the pupae suggests an ambushing strategy, waiting for the pupae's emergence to parasitize. A mite wandering around suggests a cruising strategy to seek an adult fly. Understanding the effects of internal energy levels and external resource availability will help determine in what context feeding strategies are plastic.

Sex and parasites: Impact of parasite exposure (*sans* infection) on the mating behaviour of *Drosophila nigrospiracula*

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Predators and parasites can impact the behaviour and physiology of prey and host species, respectively, through non-consumptive effects such as avoidance behaviours and increased stress hormones. Previous work on this ecological interaction, referred to as the ecology of fear, has shown non-consumptive effects within the *Drosophila nigrospiracula*-*Macrocheles subbadius* model system. Host species show heightened behavioural defense from the mere presence of parasites, and this may trade off (e.g., time and energy) with other fitness-related traits such as reproduction. I used this system to investigate the impact of parasite exposure (*sans* infection) on the mating behaviour of adult flies at 7- or 10-days post-eclosion. Direct exposure to mites occurred in Petri dish arenas to observe mating behaviour and copulation duration. I recorded frequency and duration of grooming, kicking, fleeing, pursuance by males, latency to copulate, and copulation. I found that younger flies exposed to mites displayed shorter copulation latency compared to unexposed flies, while the opposite effect was observed in older flies. Younger flies may be increasing mating effort (i.e., shorter copulation latency) in the presence of mites in anticipation of future infection costs. Also, copulation duration was significantly shorter in the presence of mites, regardless of age, and flies displayed more defensive behaviours compared to unexposed flies. These results suggest that flies exposed to mites decrease copulation duration to allocate more time and/or energy to defensive behaviours in order to reduce infection risk. Generally, copulation duration was shorter in younger flies, regardless of exposure treatment. Relative to older flies, younger flies likely have more opportunities to mate again later in life. These non-consumptive effects of parasites on host mating behaviour have important implications for host mating behaviour, reproductive success, and the ecology of fear.

Oral Presentations in Molecular Biology and Genetics

Functional conservation of *unc-119* in *Caenorhabditis elegans* and *Danio rerio*

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Unc-119 in *Caenorhabditis elegans* displays lipid binding activity and plays an important role in trafficking proteins in cilia and the nervous system. Mistrafficking of proteins by mutant *unc-119* protein causes nervous system errors and paralysis. *Danio rerio* has four *unc-119* paralogues due to a teleost-specific genome duplication. *Unc-119a1* and *unc-119a2* are orthologous to *C. elegans unc-119.* These paralogues do not display evidence of neofunctionalization, but do exhibit spatial subfunctionalization. These two model organisms provide a great opportunity for the exploration of gene evolution and divergence. Here, we investigate if *C. elegans unc-119* is functionally conserved with *Danio rerio unc-119a1* and *unc-119a2*. To do so, vectors with GFP-tagged *unc-119a1* and *unc-119a2* are currently being generated to perform *C. elegans unc-119* knockout assays. Results will be obtained by observing the genes' abilities to rescue as well as their distribution patterns. We believe both *unc-119a1* and *unc-119a2* will be able to rescue *unc-119* mutants and we do not predict a large difference between the phenotypes and expression of *unc-119* in rescued worms. This research is funded by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada.

Sepsis alters fat transport protein expression in the kidney

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Sepsis is a life-threatening condition caused by widespread inflammation and metabolic changes. Few studies have investigated the metabolic changes that occur in the liver and kidney during sepsis, or how they change over time. Here, we sought to determine how sepsis affects fatty acid metabolism in these organs as a way to identify potential treatments, as the kidney and the liver use fats more than other sources of fuel to generate ATP. Sepsis was induced in mice by injecting fecal slurry, while controls received vehicle. Analgesics were administered 4 hours post-injection for pain control. Fluids and antibiotics were administered 12 hours post-sepsis. At 4h, 12h, and 24h post-injection, mice were euthanized under anesthesia, and livers and kidneys were collected for assessment of gene expression profiles of inflammatory cytokines. Protein expression profiles of mediators of fatty acid transport were also assessed by Western blotting 24h post-sepsis. We found that the gene expression profiles of IL1β, IL6, and TNFα were increased in the liver and kidneys at all time points. Western blots revealed no differences in fat transport proteins in the liver by 24h. However, PPARα protein expression was reduced in the kidney, and CPT1β expression was increased in the kidney by 24h. To conclude, sepsis results in increased cytokine expression in both the liver and kidney as early as 4h post-sepsis. Sepsis also alters fatty acid transport within the kidney, but not the liver by 24h. These results suggest that there may be impaired fatty acid metabolism in the kidney, which could result in reduced ATP synthesis. Increased renal inflammation may be responsible for this phenomenon.

Deciphering distinct signaling networks that regulate dopamine-mediated learned and innate behaviours

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A critical function of the brain is to elicit optimal and adaptive behavioural responses needed to survive. An animal's response adapts based on past events (i.e. memory) or internal state (i.e. starvation-altered innate behaviour). Interestingly, in *Drosophila melanogaster*, dopaminergic (DA) signalling through a receptor, Dop1R1, regulates both learned and state driven behavioural plasticity. Dop1R1is highly expressed in the memory center and is critical for encoding memories through adenylyl cyclase driven synaptic depression. In contrast, signaling through a separate adenylyl cyclase strengthens synapses and drives state dependent changes to innate odour preference. It's unclear how these Dop1R1 pathways bi-directionally

regulate memory synapses. To understand Dop1R1 signaling environments, we utilized TurboID proximity labelling proteomics and RNAi screening to identify interactors that help regulate learned and innate behaviour. *In vivo* Proximity labelling using Turbo-V5 fused to endogenous Dop1R1 identified candidate proteins significantly enriched around the Dop1R1 receptor compared to another memory regulator, Dop1R2. Disruption of candidate proteins in memory circuits lead to significantly altered behavioural plasticity in starved odour preference and associative learning contexts. Interestingly, many candidate interactors affect either internal state or learned behavioural plasticity but not both, indicating distinct pathways. *Ex vivo* imaging experiments will be conducted to reveal if these candidates alter Dop1R1 receptor mediated effects on downstream cAMP signalling in active neurons. Follow up *in vivo* functional imaging will be used to confirm disrupted neuron signalling during stimuli. Altogether, our study will identify and characterize novel pathways regulating dopaminergic signalling to illicit two distinct behaviours and illuminate how the brain fine-tunes these behaviours through one receptor.

The case of missing synaptic vesicles: Did *Nep1* do it?

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Learning and memory are fundamental processes essential for survival that are conserved across the animal kingdom. Synaptic vesicles, which store and release neurotransmitters at synapses, are key regulators of neuronal function and synaptic plasticity. Although it has long been believed that synaptic vesicles numbers are determined by other aspects of synaptic architecture, it was recently discovered that Stromalin, a core subunit of the cohesin complex, acts as a learning suppressor in *Drosophila melanogaster* by limiting synaptic vesicle numbers without altering their morphology. We hypothesized that Stromalin does so due to the cohesin complex's role in regulating gene expression. Using RNA-sequencing and RNAi screening, we identified *Nep1* as a promising candidate to mediate Stromalin's effects on synaptic vesicle pool size and learning. We observed that pan-neuronal knockdown of Stromalin or SMC1, another cohesin complex subunit, significantly reduced *Nep1* mRNA levels. Furthermore, reducing *Nep1* in the brain using RNA interference replicated the effects of Stromalin on both learning and synaptic vesicle numbers in dopaminergic neurons, as well as pan-neuronally. Overexpressing *Nep1* in *Stromalin* knockdown flies restored normal learning and synaptic vesicle protein levels. We expect it to do the same in *SMC1* knockdown animals. These findings suggest that *Nep1* functions downstream of the cohesin complex to suppress learning and reduce synaptic vesicle numbers. The mechanism by which Nep1, a metallopeptidase, regulates synaptic vesicle numbers without affecting other aspects of vesicle morphology or synaptic architecture remains to be explored.

Uncovering neural systems implicated in isolation associated learning and memory changes

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Public health studies highlight social isolation as a major risk factor for poor mental health and mortality. However, little is known regarding its mechanism of action and impacts on neurophysiology. Non-invasive neurotransmitter activity assays in rodents and humans indicate isolation alters activity in various systems of the brain. This has been implicated in the development of many psychiatric and behavioural disorders. Particularly, changes in monoaminergic activity have shown to alter both social and non-social behaviours in the model organism *Drosophila melanogaster*. Results from learning and memory behaviour experiments in the Phan Lab have concluded that isolation reduces the ability of a fly to form aversive memories. Data from concurrent single-cell RNA sequencing experiments have shown isolation also alters transcriptional activity in dopaminergic and serotonergic neurons which control these behaviours. This indicates reduced neural activity in these circuits as the cause for alterations in learning and memory. Using a fluorescent reporter tagged to a transcriptional cofactor which translocates to the nucleus in a calcium-dependent manner associated with neural activity, we can directly quantify the activity of these specific neural clusters to confirm this theory. Preliminary results indicate dopaminergic circuits which modulate learning and memory behaviours are not involved in effectuating the effects of social isolation. However, these results must be verified in larger sample sizes to affirm their significance. Other neural systems might also be involved in bringing about changes in learning and

memory associated with social isolation. Understanding how social isolation impacts neural activity in monoaminergic systems is vital for delineating the mechanisms of action for the effects of isolation. Delineating these mechanisms will help suggest potential treatments for rescuing the effects of isolation and related psychiatric disorders as well as contribute to our knowledge regarding the causality between isolation and poor mental health as well as mortality.

Defining the development of larval zebrafish rods and short wavelength cones via transgenic Nrl expression and lineage tracing

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Disentangling the link between neuroinflammation and seizures post-traumatic brain injury: Implications for tauopathy and dementia prevention

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Inducing beta-cell transdifferentiation using CRISPR-on technology in type 1 diabetes: A research study

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The role of memory suppressors in cognitive flexibility and associative accuracy

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Learning and memory are crucial to everyday life, shaping how we acquire and retain information. While research has extensively explored molecular pathways that enhance learning and memory, far less is known about the mechanisms that limit these processes. Memory suppressor genes are thought to fine-tune cognitive function, yet their precise roles remain unclear. To investigate their function, we examined sec22, a vesicle-associated SNARE protein, and stromalin, a subunit of the cohesin complex, in *Drosophila melanogaster*. Using RNA interference (RNAi), we knocked down these genes in mushroom body neurons (MBn), which mediate associative learning, and dopaminergic neurons (DAn), which modulate reinforcement signaling. Flies were tested in two behavioral paradigms: reversal learning, which assessed cognitive flexibility by requiring flies to update a learned odor-shock association when reinforcement contingencies reversed, and latent facilitation, which measured associative accuracy by examining how prior neutral odor exposure influenced later learning. Knockdown of sec22 in DAn increased learning in latent facilitation, suggesting it normally limits the influence of prior exposure on new learning. In reversal learning, sec22 knockdown in MBn enhanced acquisition of the reversed association without affecting initial learning, indicating a role in restricting cognitive flexibility. In contrast, stromalin knockdown in both neuronal populations impaired both initial and reversal learning, suggesting a broader role in supporting flexible learning rather than restricting it. These findings suggest that memory suppressor genes act in a cell-specific manner to balance learning precision and adaptability. Rather than serving solely as inhibitory factors, these genes regulate cognitive processes to ensure optimal learning efficiency. Understanding these mechanisms could provide insight into cognitive rigidity seen in neurodevelopmental and neurodegenerative disorders, where disruptions in learning flexibility contribute to behavioral deficits.

Characterization of novel plant genes associated with seed oil content using a yeast heterologous system

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There is an increasing demand for vegetable oil due to the growing global population, whereas arable land remains limited. Therefore, it is important to increase seed oil production per acre. Triacylglycerol (TAG) is the major component of vegetable oil, and much research has been focused on increasing its content in seeds via metabolic engineering. The rapid development of omics has also generated a rich source of genetic information from plants, where we can identify promising genes for genetic engineering. In this study, we identified nine candidate genes potentially involved in TAG production via mining available transcriptomic, QTL, and co-expression datasets, and then individually expressed them in baker's yeast *Saccharomyces cerevisiae*, for initial characterization. Although the growth of all transgenic strains exhibited a similar sigmoid pattern and growth rate compared with the wild-type control, lipid analysis revealed that three candidate genes belonging to the *Peroxidase*, *GDSL-esterase*, and *MYB* families, respectively, significantly enhanced cellular oil accumulation. Moreover, the expression of a *Lipase* gene exhibited a negative regulatory role in oil accumulation, probably due to its involvement in lipid degradation. In addition, several candidate genes resulted in changes in fatty acid content, though they did not cause changes in total oil content. Together with baker's yeast, we rapidly tested and verified those candidate genes, and the results provided solid preliminary results for further investigation in plants.

Oral Presentations in Microbiology

Identification of novel bacteriophages for climate change mitigation

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Ammonia-oxidizing bacteria (AOB) play a pivotal role in the rate-limiting step of nitrification, where ammonia is oxidized to nitrite, consequently leading to nitrous oxide emissions. Nitrous oxide is the most potent of the three major greenhouse gases, 300 times the strength of CO_2, in global warming potential. Agriculture and wastewater treatment are the largest sources of human-derived nitrous oxide emissions. To develop a directed means of controlling nitrification in these systems, bacteriophages are garnering growing interest for their specificity and potency. However, there is little literature investigating the presence of AOB phages. Here, we demonstrate the discovery of a robust set of virulent phages infecting AOB. We found that these phages possess both narrow and broad host range potential against a collection of 4 AOB isolates. This study establishes a robust understanding of diverse phages infecting AOB, with a potent means of controlling nitrification holding the potential to reduce global GHG emissions by 3 $GtCO_2eq$.

Increasing the arsenal of bacteriophages targeting the clinical and agricultural pathogen *Burkholderia gladioli*

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Starting in 1928 with the discovery of penicillin by Alexander Fleming, the antibiotic revolution was responsible for increasing human life expectancy by approximately 20 years. A century later, the rise of antimicrobial resistance threatens our control of infectious disease. *Burkholderia gladioli* is an emerging, broad-host-range pathogenic bacterium that infects several plant species including onions, carnations, gladioli, and rice. *B. gladioli* also causes serious respiratory infections in individuals living with cystic fibrosis or chronic granulomatous disease. Novel treatment strategies for clinical and agricultural infections are urgently needed, as *B. gladioli* is highly resistant to antibiotics. Abundant in the environment, bacteriophages (phages) provide strong pathogen specificity without significant side effects, making them a promising treatment option for antibiotic resistant infections. Numerous phages active against members of the closely related *Burkholderia cepacia* complex (Bcc) have been characterized; however, few *B. gladioli* specific phages have been isolated.

Many Bcc phages are lysogenic, wherein they integrate into the bacterial host genome during infection and become dormant prophages. My work aims to harness prophage-containing strains of *B. gladioli* as a source for *B. gladioli* specific phages, which may target environmental and clinical pathogens. Six prophages (AB1-AB6) were induced from *B. gladioli* strains using the DNA-damaging agent, Mitomycin C. Prophages AB1 and AB4 have undergone characterization via whole genome sequencing, transmission electron microscopy (TEM), extended host-range analysis, pH stability testing, and virulence assays. AB1 and AB4 have narrow host ranges specific to *B. gladioli* strains. TEM identified AB1 and AB4 as myoviruses. Comparison of AB1, AB2, and AB4 genomes with 3 published *B. gladioli* phages show that *B. gladioli* phage genomes are diverse. Future work will investigate receptors, and the impact of AB4 tail fibre and integrase gene mutations on host range and lifestyle. These conclusions bring new understanding to *B. gladioli* phages, and their therapeutic potential.

Incorporating SLIMEr reactions into the genome-scale model of *Methylomicrobium album* BG8 for enhanced lipid metabolism modelling

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Genome-scale metabolic models (GSMs) are a computational framework, providing a systems biology approach to understanding an organism's metabolism through gene-protein-reaction associations and predicting the associated metabolic flux that can be derived under differing conditions while maintaining optimization of biomass production. For methanotrophic bacteria like *Methylomicrobium album* BG8, accurately modeling lipid metabolism is crucial for optimizing the flow of carbon toward valuable products, such as biofuels. Lipids play a fundamental role in cellular processes, influencing membrane composition, energy storage, and metabolic efficiency, however, current GSMs often oversimplify lipid biosynthesis through restrictive or permissive modeling approaches. The restrictive model assumes that all lipid classes share the same acyl chain distribution, failing to capture the diversity of lipid species found in nature. In contrast, the permissive model allows for diverse acyl chains but introduces biases by prioritizing the most energy-efficient lipids, disregarding many biologically relevant species. These simplifications do not accurately reflect real lipid metabolism, limiting the predictive power of existing models. This project integrates SLIMEr reactions into the *M. album* BG8 GSM to address these shortcomings, balancing lipid backbone and acyl chain composition using measured lipid abundance data. The enhanced model will be analyzed using flux balance analysis (FBA) under various growth conditions, including differing carbon sources (methane vs. methanol), nitrogen sources (ammonium vs. nitrate), and temperatures (20°C, 30°C, and 37°C). Experimental validation techniques, such as Fatty Acid Methyl Ester (FAME) analysis, will be used to compare predicted and observed lipid profiles, ensuring accuracy of the model and enabling adjustments if necessary. This research advances the metabolic engineering of *M. album* BG8, providing a more realistic framework for methane-derived lipid production and enabling more sustainable biotechnological applications.

Opening The X Files: Understanding the link between high-risk A1972T/G1764A mutations in hepatitis b virus' x protein and liver cancer

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Hepatitis B Virus (HBV) infects over 300 million people worldwide and is responsible for more than 50% of hepatocellular carcinoma (HCC) cases. However, the exact mechanisms by which HBV leads to HCC remain incompletely understood. Two mutations in the pre-core promoter (PCP) region of HBV, A1762T/G1764A, are associated with an increased risk of HCC. HBV produces the Hepatitis B X protein (HBx), which plays a role in carcinogenesis by stimulating various oncogenic pathways. The tail of HBx overlaps with the PCP region, leading to K130M and V131I mutations in the protein. Whether these high-risk mutations exert their strongest effect through promoter activity or by creating a more oncogenic HBx protein remains unclear, and this study aims to investigate both possibilities. To explore this, a plasmid containing luciferase under

the control of the HBV PCP was mutated using site-directed mutagenesis (SDM) to introduce the high-risk mutations. Wild-type (WT) and mutant plasmids were transfected into HepG2 cells, and luciferase activity was measured. Altering combinations of WT and mutated PCP and WT and mutated HBx will be done to also determine luciferase activity. Additionally, a Flag-tagged plasmid expressing wild-type HBx (HBx-wt) was designed and purchased. The high-risk mutations will be introduced using SDM, and transfected HepG2 cells will undergo pulldown assays with Flag-tag antibody beads. Mass spectrometry will be used to identify proteins interacting with wild-type and mutant HBx. Preliminary results show that the high-risk mutations increase HBV PCP activity. Mutant HBx production is in progress, and HBx-wt transfections are being optimized for pulldown experiments. Our findings suggest that these mutations enhance promoter activity, and ongoing work aims to determine how they alter HBx protein interactions. Identifying differentially binding proteins may provide insights into HBV-driven HCC pathogenesis and the oncogenic role of mutant HBx.

Understanding the coculturing between a methanotroph and a recently discovered methylotroph

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It is common in nature to find methanotrophs and non-methanotrophic methylotrophs together in communities where methane fuels the food web. To date, the nature of this association, especially when methane is the sole carbon and energy source, remains underexplored. It is unclear whether their relationship is competitive, commensalistic, or mutualistic, particularly regarding the presence of a growth factor that could enhance methanotrophic growth. Additionally, the changes in gene expression within methanotrophs and the metabolic exchanges between the two groups have yet to be extensively studied. One potential application of cultivating methanotrophs in coculture is enhanced methane oxidation, which could be utilized in bioreactors designed for methane removal. Recently, our research group discovered a methylotroph in a methanotroph stock. Successive transfers in a non-methanotrophic methylotrophic medium were performed to obtain an axenic culture of the methylotroph. To understand the coculture system, experiments will be performed to track the growth of the two bacteria separately, as well as to measure methane consumption in the axenic cultures and in coculture. Additionally, transcriptomic and metabolomic analyses will be conducted to elucidate the changes in gene expression and metabolic profiles when a methanotroph grows alone versus in coculture.

Optimizing methane bioconversion: Harnessing *Methylomicrobium album* BG8 for sustainable biotechnological applications

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As the specter of climate change looms large, methane—a potent greenhouse gas that traps heat 80 times more efficiently than carbon dioxide—poses a significant challenge. Yet within this environmental dilemma, *Methylomicrobium album* BG8 (MABG8), a promising bacterium known for its methane consumption, emerges as a dual-purpose solution, mitigating methane's impact while transforming it into valuable bioproducts. Our project harnesses the potential of MABG8 to convert this environmental threat into an industrial reality and a sustainable asset for our future. However, effectively harnessing methane presents challenges due to the low metabolic activity of methanotrophs and the high energy demands of methane bioconversion. To overcome these barriers, we delve into the inner workings of these microorganisms through the synergy of metabolic engineering and bioinformatics. Genome-scale metabolic modeling provides a comprehensive view of MABG8's metabolism, allowing for precise manipulation of its internal processes. By leveraging advanced techniques such as Flux Balance Analysis and multiomics integration, we aim to engineer the microorganism's behavior toward more efficient methane conversion while exploring symbiotic co-cultures with photosynthetic and methylotrophic organisms. This approach offers a groundbreaking and economically feasible strategy for methane biocatalysis, paving the way for innovative solutions in methane fixation.

The use of TLC direct bioautography to examine the antibacterial properties of plant extracts against *Bacillus subtilis*

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Antibiotic resistance is becoming a major global threat as more bacteria are becoming resistant to the first-choice antibiotics used against them. Plants are promising sources of new antibacterial compounds that could be used to treat such infections. This study focuses on noxious plants found in Alberta that have not been previously researched. Standard antibiotic susceptibility tests like disc diffusion can help identify antimicrobial activity in the whole extract. However, they require repeated fractionation and activity testing on each fraction to identify the compound responsible for the activity, which can be difficult and time-consuming. An alternative method is direct bioautography. Direct bioautography can reduce time and effort by combining chromatography techniques like TLC with activity assays. This study aims to test the antibacterial properties of noxious plant extracts using TLC direct bioautography. We identified two plant extracts with antimicrobial activity using disc diffusion assay: Himalayan balsam ethyl acetate extract against *B. subtilis* and vetch extract against *E. coli*. Growth conditions for both bacteria were optimized for TLC direct bioautography. TLC plates were then spotted using the plant extract and developed using different solvent systems. Developed plates were either stained for visualization or dipped in *bacterial* suspension, incubated, and sprayed with MTT for visualization. Clear zones of growth inhibition against a purple background indicated regions with antibacterial activity. The preliminary results from this study suggest that Himalayan balsam ethyl acetate extract contains at least one compound with antimicrobial activity against *B. subtilis*. The fraction containing this compound will be separated, and further analyses will be performed to identify the compound(s) responsible for this activity. Overall, this study demonstrates the potential of noxious plant extracts as antimicrobial agents for treating bacterial infections and combating the problem of antibiotic resistance.

Characterizing lab-adapted and wild reovirus strains in vivo

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Reoviruses are non-pathogenic enteric viruses that infect most mammalian species and have been extensively used as a model system to study host-virus interactions in the gastrointestinal niche. However, current knowledge in the field is based on virus strains that have been grown and adapted to non-enteric conditions. This is a common theme with enteric virus studies; however, how non-enteric adaptations may affect virus interactions with host factors in the natural gastrointestinal environment remains understudied. To address this question, "wild" reoviruses have been previously isolated from the sewage, and provide a system to study the effects of cell-culture selective pressures on gastrointestinal viruses. To examine how selective pressures influence virus interactions with the gastrointestinal tract, mice were orally inoculated with wild and lab-adapted reoviruses. Intestinal samples were collected 1- and 4-days post inoculation while fecal samples were collected daily. To determine infection duration and burden of different reovirus variants, fecal and intestinal samples were analyzed by plaque assay for viral titers. Preliminary data suggests that wild reovirus variants are shed at greater levels than lab-adapted strains, however, similar titers are observed in intestinal tissues. This project provides insight into evaluating the effects of using cell culture-adapted strains to study virus behavior in the gastrointestinal niche and will provide insight into how research into treatments of gastrointestinal viruses should proceed in the future.

Investigation into the effects of carbon dioxide on type II methanotrophs

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Methane emissions are a crucial topic of concern within the scope of the climate crisis, and one that requires innovative mitigation strategies. One popular strategy is utilizing methanotrophs, a diverse group of bacteria that utilize single carbon compounds like methane and methanol as their sole carbon source. Methanotrophs typically fall within two groups depending on their metabolic pathway, Gammaproteobacteria and Alphaproteobacteria, the former possessing the ribulose

monophosphate cycle and the latter the serine cycle. While both groups contain the ability to assimilate methane and methanol, Alphaproteobacteria exhibit poorer growth on methanol compared to Gammaproteobacteria, despite methanol being a natural metabolite. We hypothesize that the cause of this growth inhibition is due to the accumulation of formaldehyde naturally produced by the cells. In addition, we hypothesize that the incorporation of carbon dioxide in the growth environment will reduce the accumulation of the formaldehyde, due to carbon dioxide being utilized in the serine cycle. To investigate this we cultured three different strains of Alphaproteobacteria under methane and methanol, and at the end of their growth we assessed the extracellular concentrations of formaldehyde through a developed assay by measuring their absorbance. These strains were then cultured with carbon dioxide added to the growth conditions, and assessed for the presence of formaldehyde. Observations reveal that all strains tested exhibited increased levels of formaldehyde when cultured on methanol, and strains exhibited different responses in formaldehyde levels to carbon dioxide amendment. For the strain *Methylosinus trichosporium* OB3b all formaldehyde was eliminated with the addition of carbon dioxide, whereas the other two strains had no observed significant effect. Overall, this study seeks to address crucial bottlenecks in the large-scale industrialization of methanotrophs as a sustainable solution to mitigate methane emissions.

Adaptive evolution of acid-tolerant *Methylobacterium extorquens* AM1 for improved industrial affinity

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Bioprocessing is a sustainable manufacturing method in which living organisms are used to produce various products of commercial interest, such as biofuels and enzymes. One bacterium being eyed for use in these systems is *Methylobacterium extorquens* AM1, due to its ability to utilize single carbon compounds such as methanol – a major industrial waste product. However, upscale of this microbe for commercial use is challenged by harsh industrial conditions such as low pH, which leads to decreased growth and product yield. Thus, the aim of this research is to utilize adaptive lab evolution to create a strain of *M. extorquens* AM1 which performs better in response to acid stress. Starting with a phosphate buffered media of pH 6.8, *M. extorquens* AM1 was grown and sequentially passaged into increasingly acidic conditions by decreasing the pH of the buffer by 0.2 pH points after every 5 transfers. When pH 6.2 was reached, bioaggregates began to form within the cultures and death phase was observed. To address this, 1mm glass beads were added to culture flasks. This restored growth and allowed cultures to reach pH 4.8 before growth was diminished. When the adapted culture was grown back in media buffered at pH 6.8, growth was restored. Further, when non-adapted cultures were grown at pH 4.8, little to no growth was observed. Taken together, this suggests that the strain adapted to have an expanded acid tolerance range. To confirm whether these changes are occurring at a genome level, Illumina HiSeq genome sequencing is being pursued. Additionally, RNA-seq analysis is also being conducted to better understand how the genes function in the adapted strain. Overall, this research aims to elucidate aspects of how *M. extorquens* AM1 adapts to handle acid stress. Further, it seeks to ameliorate this microorganism's propensity for industrial use.

Exploring the cyanide insensitive oxidase from *Pseudomonas aeruginosa* and its role in pathogenesis

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Pseudomonas aeruginosa (PA) is a highly antibiotic-resistant opportunistic bacterium. It is especially prevalent among hospital patients with burn wounds or organ transplants, and individuals who have cystic fibrosis or injection-drug use-related infections. Understanding how PA colonizes the human body is essential for developing more effective therapeutics to counter its mechanisms for resistance. To promote infection, PA relies on quorum-sensing, which allows cell-cell communication for rapid adaptation, and the formation of biofilms, which are communities of bacteria that live in a protective polymeric matrix. One quorum-sensing regulator, LasR, is frequently mutated in clinical isolates. These spontaneous LasR mutants are referred to as "social cheaters" which exploit their bacterial community by growing rapidly and exploiting public goods produced by "cooperators." Public goods are regulated by quorum-sensing and include hydrogen cyanide, pyocyanin, and proteases. Importantly, this quorum-sensing pathway is dependent on the electron transport chain (ETC) of this bacterium. The cyanide insensitive oxidase (CIO) is an ETC enzyme produced only by cooperators that allows PA to police cheaters and grow in the presence of the cyanide it secretes to kill surrounding cells. It has been found that cheater levels stabilize at twenty percent of the population, but the advantage of maintaining this equilibrium is unknown. We

have successfully purified the CIO and used homologous recombination to create several strains of PA involving a CioA/B:deletion, a CioB:3xFlag insertion, and fluorescently labeled strains using mCherry and eYFP. We aim to develop antibiotics that can exploit this population equilibrium to cause a social collapse of PA populations. We will test these antibiotics using high-throughput fluorescence assays to measure growth rates, and oxygen consumption assays to measure CIO function. Using cryogenic electron microscopy, we will also determine a high-resolution structure of the CIO and optimize grid production and sample preparation methods.

A novel salmonella-specific lipoprotein activates the antimicrobial peptide response of the PhoPQ two-component system

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Salmonella enterica is an infectious pathogen of mammalian hosts that poses a major public health burden worldwide in foodborne illnesses. *S. enterica* serovar Typhi (*S. Typhi*) and related typhoidal serovars are the causative agent of typhoid fever in humans. A unique aspect of Salmonella pathogenicity is that it establishes an intracellular niche within infected cells known as the Salmonella-containing vacuole (SCV), where the bacterium's virulence programme is activated. PhoPQ is a two-component transcriptional regulatory system within Salmonella that is a major regulator of the virulence factors induced within the SCV. PhoPQ responds to low extracellular concentrations of cations, low pH, and cationic antimicrobial peptides (CAMPs) by transcriptionally regulating genes involved in virulence, resistance, outer membrane remodelling, and nutrient starvation. This project aims to contribute to unpacking the mechanisms of PhoPQ regulation of Salmonella virulence and does so by highlighting the essential role of the novel lipoprotein PalA. palA was first identified via genetic screening as a small lipoprotein that activates PhoP-regulated genes. We demonstrate that PalA is essential to PhoPQ's enhanced response to CAMPs by initiating a positive feed-forward loop. Intriguingly, PalA's ability to activate PhoPQ does not functionally translate with the PhoPQ orthologue of the closely related bacterial species *E. coli*, providing insight into the diversification of two-component system regulation in response to varying lifestyles and environmental challenges. PhoPQ is a widely shared two-component system across many Gram-negative bacteria. Within Salmonella, PhoPQ has adopted an expanded role as a major regulator of intracellular-specific virulence factors including the typhoid toxin. PalA represents an addition to the repertoire of Salmonella-specific adaptations for virulence within its unique intracellular niche. Ultimately, this project will contribute to the growing body of knowledge on how the well-studied PhoPQ system has evolved to be able to regulate a large network of intracellular pathogenicity within Salmonella.

Identifying lactobacilli-yeast interactions in kefir fermentations

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Fermentation is among the most ancient methods of food preparation; this process can both preserve food and increase the digestibility of certain nutrients contained therein. Kefir is a fermented dairy beverage with many health benefits associated with its consumption. Many different organisms, including multiple species of lactobacilli and yeast, contribute to the production of kefir from milk. Recent work has shown that the removal of either the lactobacilli or yeast populations from a model kefir fermentation results in the loss of the associated health benefits, such as cholesterol lowering activity, suggesting that interkingdom interactions between the yeast and the lactobacilli may facilitate the observed health benefits. To detect whether yeast-lactobacilli interactions are a determining factor in kefir community dynamics, we examined the lactobacilli-yeast interactions between 2 species of lactobacilli and 4 species of yeast in a reconstituted kefir community. We performed this analysis in a pairwise manner, using monocultures as control groups and cocultures as experimental groups. We examined both liquid and solid environments to recreate both the stagnant/spatially limited condition of the kefir grain and the planktonic/less spatially restricted conditions of milk. We performed growth performance (CFU), pH and colony morphology (pinning) assays during our investigation. The preliminary results indicate a nearly twofold increase in lactobacilli growth when cocultured with certain kefir dwelling yeast species. Such results evidence lactobacilli-yeast interactions in kefir fermentations and therefore support our hypothesis that interkingdom interactions are an important factor in influencing kefir community dynamics. Although this study does not paint a detailed picture of these interactions, it does highlight specific lactobacilli-yeast interactions that merit further, more intensive, research. This study thus primarily serves

to direct future work. Such research will seek to elucidate the mechanistic action the health benefits associated with kefir consumption and could contribute to the development of new antihypertensive drugs.

Establishing a methane-driven consortium in a co-culture of *Methylomicrobium album* BG8 and *Methylobacterium extorquens* AM1

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Methylotrophs, a diverse group of microorganisms, play a critical role in the global carbon cycle by oxidizing reduced single-carbon (C1) compounds like methane and methanol. These organisms are essential for methane consumption, mitigating greenhouse gas emissions, and supporting plant growth through their ability to consume single carbon waste emissions and repurpose them into useful organic materials. This study investigates the potential for a metabolic consortium between *Methylobacterium extorquens* AM1, a methylotrophic species, and *Methylomicrobium album* BG8, a methanotroph. Gas chromatography, spectrophotometry, and visual observations were used to establish the feasibility of creating a consortium between *M. extorquens* AM1 and *M. album* BG8 in co-culture. The preliminary results show that despite *M. extorquens* AM1 being unable to utilize methane as a sole carbon source, it grows in the presence of *M. album* BG8, suggesting a metabolic interaction. Methane consumption was detected in co-cultures supplied with methane, along with visible growth of *M. extorquens* AM1, likely due to the utilization of fermentation products excreted by *M. album* BG8. This study provides evidence for a potential mutualistic relationship between these two species, where *M. extorquens* AM1 may utilize fermentation products like formate and acetate produced by *M. album* BG8 when supplied with methane. These preliminary results are followed by quantitative PCR (qPCR) for an accurate cell count of each species to reveal their relative fitness in co-culture over time. Understanding the dynamics of methylotrophic consortia in a laboratory setting offers insights into their roles in natural ecosystems, including their contributions to the methane cycle, carbon flux, and interactions with plants.

Oral Presentations in Physiology and Development

Aestivating and live *Oreohelix subridis*: Care, morphology, and novel parasite detection

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Oreohelix is a genus of terrestrial snails endemic to mountain ranges in North America and play critical roles as detritivores and sources of calcium for other organisms in these isolated ecosystems. Individuals in the genus display a remarkable ability to aestivate for extended periods when unfavourable conditions arise. Despite this adaptation, many *Oreohelix* spp., such as *O. cooperi* found in Alberta's Cypress Hills, face endangerment due to their limited geographical range, low dispersibility, sensitivity to climate disturbances, and parasitization. In order to aid in their conservation, we studied the care, morphology, and parasitization of aestivating and live *O. subridis* collected from Alberta's Cypress Hills. *Oreohelix* have never successfully reproduced in captivity; we conducted a literature review of established husbandry guides for species within the family Stylommatophora and have, with minimal mortalities, maintained a colony of *O. subridis* in a 16ºC growth chamber with controlled humidity and specific weekly feeding routine. Using a histological analysis to study the morphology of previously embedded aestivating and non-aestivating *O. subridis*, we detected infection of a possibly novel helminth species within renal structures. DNA barcoding is to be conducted on DNA extracted from the infected paraffin embedded specimens to determine parasite identity. We have begun developing a potential method to non-invasively detect parasite infection using mucosal secretions collected from live specimens and characterize secreted immune cells. Our research will contribute to being able to better maintain colonies of endangered *Oreohelix* species within captivity and further our understanding of ecological factors that contribute to their endangerment. Successfully developing a non-invasive technique to detect parasitic infection would improve monitoring of infection within wild and especially endangered mollusc populations.

Ontogenic analysis of a toll-like receptor in *Biomphalaria glabrata* snails

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Biomphalaria glabrata is a freshwater snail that acts as an intermediate host for *Schistosoma mansoni*, a trematode parasite that is infectious to humans particularly in tropical and subtropical regions. Toll-like receptor (TLRs) proteins have been shown to play an evolutionarily conserved role in pathogen recognition and resistance. TLRs in some invertebrate species have also been characterized as multifunctional and important in development as well as immunity. In *B. glabrata*, one TLR protein (BgTLR) is highly expressed during infection with *S. mansoni*, particularly in resistant snails (BS-90 strain) compared to susceptible snails (M-line strain). However, BgTLR expression has yet to be characterized in developing snails, nor is it clear which tissues use BgTLR signalling. This study utilizes wholemount juvenile *B. glabrata* snails to investigate BgTLR expression. Snails were fixed with 4% PFA and optically cleared using the DEEP-Clear method. Snails were then stained for anti-acetyl-alpha tubulin, BgTLR, and nuclei were stained with Hoescht 33342 for epifluorescent and confocal imaging. Preliminary results have shown that BgTLR staining is present in juvenile M-line snails but only after hatching, indicating BgTLR may not contribute to embryological or larval development. Additional experiments will compare the presence of BgTLR in M-lines and BS-90 snails following exposure to immunostimulatory agents. I expect that BS-90 snails will show higher BgTLR expression. This research contributes to understanding when functional differences in parasite resistance develop in the snail hosts. In addition, identifying the spatiotemporal patterns of TLR expression in a gastropod species is a first step to understanding the functional evolution of this protein family.

Impact of excessive hypercholesterolemia in pregnancy on the placentas of the male and female offspring

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Hypercholesterolemic preeclampsia (abbreviated as HC-PE) reduces placental efficiency in both fetal sexes, but the underlying mechanisms are not known. Placental dysfunction is central to the pathophysiology of preeclampsia, and may be linked to endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR). Furthermore, in conditions of prolonged ER stress, the UPR can activate the NLRP3 inflammasome, leading to inflammation and cell death. Outside of pregnancy, hypercholesterolemia induces ER stress, but whether HC-PE activates the placental UPR and NLRP3 inflammasome is not known. We hypothesized that HC-PE activates the UPR and NLRP3 inflammasome in the placentas of the male and female offspring. Sprague Dawley rats were fed a control diet or high cholesterol diet (to induce HC-PE) from gestational day (GD) 6 to 20 (term=22 days; n=7-8/group). On GD20, placentas of the male and female offspring were collected, and the protein expression of the UPR sensors (ATF6, eIF2α, IRE1α) and NLRP3 were assessed by Western blotting. Placental lipid and reactive oxygen species (ROS) levels were evaluated using Sudan IV and dihydroethidium, respectively. Data were analyzed by Student's t-test (significance: $p<0.05$) within each sex. Lipid levels were increased, while ROS levels were similar, in both the male and female HC-PE placentas compared to controls. However, HC-PE altered the UPR in placentas of both male (reduced phospho-IRE1α; sign of prolonged ER stress) and female (reduced phospho-eIF2α) offspring, and increased NLRP3 levels in only the male HC-PE placentas compared to controls. In conclusion, HC-PE impacted the placentas of both male and female offspring, but the underlying mechanisms were sex-specific. The activation of the NLRP3 pathway in only the male placentas may indicate that the male offspring are more susceptible to the impact of HC-PE.

Restoring vision in dim light: NRL gene converts cones to functional rods

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Identification, quantification and timing of hox gene expression during *Dermacentor andersoni* embryo development

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Clusters of orthologous HOX genes can be found in the genomes of most vertebrates and invertebrates. Since the sequences and functions of these genes are conserved across lots of different species, HOX genes are often used by EVO-DEVO researchers to elucidate phylogenetic relationships that exist between species. Recently the genome of the Rocky Mountain wood tick, *Dermacentor andersoni*, has been published on GenBank, and within the genome seven putative HOX genes have been identified. However, the expression of these genes has not yet been characterized. In this paper we characterize the timing and quantity of expression of three HOX genes, HoxB4, HoxB5, and HoxA5, during *D. andersoni* embryo development. Using gel electrophoresis, we have identified that there is continued expression of HoxB5 and HoxA5 throughout tick embryo development starting on day six, and that HoxB4 is expressed intermittently throughout development starting on day zero. Currently qPCR is being used to quantify this HOX expression. This information will hopefully contribute to a better understanding of developmental gene regulation in ticks, as well as the phylogenetic relationships that exist between ticks and other arthropods.

Glucagon-Like Peptide 1 and Glucagon-Like Peptide 2 promotes short bowel syndrome intestinal adaptation: A research study

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The human digestive tract is an advanced, complex system, designed to ensure nutrient absorption from food for survival and growth in childhood. Some babies are born with, or due to disease acquire, a short bowel causing intestinal failure (IF) and death without intravenous nutrition. The solution for these babies is to grow the bowel or make it work better (structural and functional adaptation, respectively). Fortunately, new hormone compounds are being developed that might help. Because babies with short bowel syndrome (SBS) are so sick, a neonatal piglet model of SBS is used to study the benefits and safety of new treatments. The objectives of this study were to investigate if two hormones Glucagon-Like Peptide-1 (GLP-1) and Glucagon-Like Peptide-2 (GLP-2) would act in synergy to improve structural and functional adaptation compared to either treatment alone or to control. Research ethics approval was provided, and each animal was obtained from the University of Alberta Swine Research and Technology Centre. Each piglet underwent surgery removing 75% of the small bowel with a distal jejunocolonic anastomosis. The animals received their respective treatment through subcutaneous injections. Measures of structural and functional adaptation were collected on day 0 during the initial procedure and on day 7. Outcome measures included change in animal weight, change in intestinal length (day 0 to day 7), small bowel total and mucosal scraped weight, villus length and crypt depth. This pilot study compared two doses of GLP-1 as this hormone has never previously been studied in piglets, compared to a dose of GLP-2 previously shown to be effective for adaptation in SBS piglets. The findings will contribute a critical first step towards a new biomedical pharmaceutical innovation by testing the validity of combining GLP treatments to improve the prognosis and quality of life of patients suffering from conditions such as SBS.

Behavioral fever as a segregation tool to improve *Tenacibaculum maritimum* outbreak management in aquaculture

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Tenacibaculum maritimum causes substantial economic losses in salmon farming and is the primary driver for antibiotic use in Canadian aquaculture. This study evaluates behavioral fever as a potential tool for early infection detection, segregation, and targeted treatment of salmonids. Using an annular temperature preference tank (ATPT) with a controlled 9–19°C gradient, the thermal preferences of fish exposed to *T. maritimum* extracellular products (ECPs) were monitored. High-resolution tracking revealed a distinct fever response in ECP-challenged fish, characterized by a discrete 2–3°C increase in temperature preference at the peak of fever. In contrast, thermal preference of control fish remained unchanged. Our experiments also revealed a discrete period of anorexia for ECP-challenged fish. Integration of thermal preference and reduced feeding behavioural datasets identified a clear period where challenged and control fish could be differentiated, which offered an opportunity to selectively treat "sick" fish. This segregation strategy could enhance targeted antibiotic delivery, improving treatment efficiency while reducing overall antibiotic use in aquaculture. Our results highlight behavioral fever as an early biomarker of *T. maritimum* infection and a novel tool for outbreak management at key stages of disease progression.

Mutations in primary cilia affect behaviour and influence motor and social function in mice

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Primary cilia are small non-motile organelles that are hair-like in shape and are microtubule based (not to be confused with motile cilia). A primary cilium is found to be protruding from the surface of many eukaryotic cells including neurons. These organelles serve as critical hubs for cellular signaling, playing essential roles in various physiological processes. Ciliopathies are a group of diseases that arise from mutations in genes regulating structure or function of primary cilia, and can lead to intellectual disability, developmental disorders, and brain malformations. The Arl13B (ADP ribosylation factor like GTPase 13B) gene is crucial for proper cilia function. The transgenic mouse model Arl13bFlox allows for the knockout of this gene which leads to impaired cilia function and various ciliopathies. Cre recombinase is injected directly into the mouse cortex via an AAV (Adeno-associated virus) vector, it excises the DNA, effectively knocking out the Arl13b gene in those cells. Microscopy and histology of the dissected tissue can verify the correct site of the injection and the expression of the Cre virus. I worked with mice that were injected via stereotaxic surgery into their M1&M2 regions of the motor cortex. The AAV9-CamKII-Cre-EGFP virus was used for the cilia mutants and AAV9-CamKII-EGFP for the controls. I conducted 3 gait assessment tests including rotarod, beam walk & paint walk to evaluate their motor function. Furthermore, mice injected in the mPFC have induced behavioral changes, which seem to be related to social hierarchy. I conducted the tube-test which is a behaviour assay that establishes a dominance hierarchy ranking for mice living in the same home cage. My aim is to establish further tools to measure hierarchy behaviour such as the urine marking assay, ultrasonic vocalization, and hot-cold spot test. I piloted these behaviour assays to verify the tube-test hierarchy.

Oral Presentations in Immunology and Infection

The role of GPR15 polysialylation in T-cell migration

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Migration of immune cells to the correct tissue at the correct time is a key feature of a properly functioning immune system. Polysialic acid (polySia) is a large anionic polysaccharide that modifies a small number of proteins in the nervous, immune, and reproductive systems and is well known to mediate cell migration. In the context of the immune system, polySia

mediates the migration of dendritic cells towards the chemokine CCL21. The Willis lab discovered that polySia is also present on T cells and modifies the G protein coupled receptor GPR15. GPR15 is a chemokine receptor that mediates the migration of T cells towards its ligand GPR15L, a highly basic protein that is expressed in inflamed skin and mucosal tissues. We hypothesized that GPR15L might interact with polySia, thereby increasing its activation of GPR15 and subsequent migration of T cells. To evaluate the potential interaction between polySia and GPR15L, we measured the ability of GPR15L to co-elute with an artificially polysialylated protein in size exclusion chromatography. We next generated recombinant GPR15L and confirmed its proper folding through transwell migration assays. We then demonstrated its ability to bind polysialylated cells by flow cytometry. Taken together, our data strongly suggest that GPR15L binds to polySia and that polySia plays a facilitatory role in the migration of GPR15+ T cells.

Exploring the functional output of the MAPK pathway activated by LITRs in goldfish (C*arassius auratus*)

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LITRs are a group of immunoregulatory receptors found in teleost fish that are homologous to mammalian FC immune receptors. Since its discovery, most of the research has focussed on LITRs found in lymphoid cells. The focus of this study was to study LITRs in myeloid cells and evaluate its immunoregulatory potential in these cells. The Goldfish was used as a model for this study due to the availability of a well established primary myeloid culture. This study delves into the mitogen-activated protein kinase (MAPK) pathway in goldfish immune response, specifically the activation of Jun N-terminal kinase (JNK) in Goldfish kidney neutrophils. These neutrophils have been shown to express these LITRs. Due to the lack of identified LITR ligands, we hypothesize that phosphorylated JNK will be detected when an antibody developed against a putative activating Goldfish LITR, CaLITR3 in neutrophils will lead to crosstalk and an eventual eliciting of an immune response. The proteins involved in the MAPK signaling pathway will get activated, with the expectation that JNK would be amongst the proteins that will end up being activated. To test this, kidney tissues from goldfish were extracted, and neutrophils were isolated, cultured, and sensitized with primary and secondary antibodies. Western blot analysis was performed to detect phosphorylated JNK using monoclonal P-SAPK/JNK primary antibody and a goat anti-mouse HRP secondary antibody. Results demonstrated the presence of phosphorylated JNK, confirming its activation. The study indicated an increasing intensity of JNK activation over 30 minutes, aligning with literature detailing JNK kinetics in immune signaling. The identification of activated JNK in goldfish neutrophils draws parallels in conserved immune mechanisms between fish and mammals and encourages the use of goldfish as a model for studying immuno-regulatory pathways. Understanding goldfish immunology has implications for disease management in aquaculture and offers insights into vertebrate immune evolution.

Investigating the trans-differentiation of CD71+ erythroid cells into B cells in the neonatal period

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The neonatal immune system is shaped by regulatory cells like CD71+ erythroid cells (CECs), linked to newborns' high susceptibility to infection and gut inflammation protection. A recent study suggests CEC-like cells can originate from malignant and non-neoplastic B lymphocytes under stress, but CEC plasticity in relation to B cells remains underexplored, with significant implications for understanding neonatal immunity. We aim to explore CEC trans-differentiation into B cells in neonatal mice under physiological and pathological conditions, such as systemic infection and tumour models. Baseline data will quantify the frequency of murine B cell marker-expressing CD71+ erythroid cells (B-CECs) in spleens, bone marrow, and livers of neonatal and adult BALB/c and C57BL/6 mice via flow cytometry, performed on different age groups (day 1 to day 28) to determine age-related changes in B-CEC frequency. Further studies will characterize their phenotype and effector functions. Additionally, neonatal CECs will be isolated through magnetic-activated cell sorting (MACS) and cultured in conditioning media for hematopoietic differentiation, supplemented with cytokines and growth factors known to promote B cell lineage commitment. This will be monitored for changes in surface marker expression of CECs to B cell markers. qPCR and RNA sequencing will assess the upregulation of key transcription factors associated with B cell differentiation, along with functional assays to confirm immunoglobulin production and B cell identity. Preliminary immunophenotyping data shows neonatal CECs in the spleen consist of 25% more B-CECs than in bone marrow and liver. Interestingly, mouse strain influences B-CEC frequency in the neonatal period. As neonates age, we expect B-CECs to mature, characterized by

the expression of mature B cell markers. Our observations provide novel insight into immune cell plasticity and trans-differentiation of erythroid progenitors to B cells. Understanding the mechanism underlying this immune cell plasticity has potential implications for enhancing neonatal immunity against pathogens.

Characterizing anti-reovirus antibodies (ARAs) binding sites in three different oncolytic reovirus variants used in novel breast cancer virotherapies: A research study

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Mammalian orthoreovirus (reovirus) selectively kills tumor cells without harming healthy cells and stimulates anti-tumoral immunity. Although reovirus-based cancer therapies are well-tolerated by patients in clinical trials, the clinical efficacy of reovirus is low and requires improvement. Repeated administration of the reovirus serotype 3 Dearing/PL lab strain (T3D-PL) in oncotherapy rapidly generates anti-reovirus antibodies (ARAs). Even though it remains unclear how ARAs impact the efficacy of the therapy, studies recognized that the σ1 cell attachment protein of reovirus, a homotrimer with two domains (head and tail), is the main target of ARAs. However, the immunodominant domain to which ARAs target is unknown. To characterize the σ1 domains recognized by ARAs, our lab generated three T3D-PL variants with antigenically distinct σ1 proteins (T1, T2, T3) that elicit antibodies that do not cross-inactivate. To create a model that discriminates between sigma 1 domains, we generated seven chimeric S1 gene constructs containing domains from two reovirus variants switched to different locations of the σ1 protein. We cloned each construct into a mammalian expression vector to verify the expression of the hybrid σ1-proteins to perform binding assays that will show which variant-specific antibody could bind, indicating the targeted immunogenic domain. Furthermore, neutralizing anti-reovirus antibodies (NARAs) is a specific subset of the ARAs capable of inhibiting viral entry. We transferred the constructs into a plasmid-based reovirus reverse genetics system to recover a set of recombinant reoviruses that can be tested in neutralization assays to identify the σ1 domain targeted by NARAs. Overall, this work contributes to the development of more effective oncolytic reovirus therapies by improving our understanding of NARA responses and their interactions with reovirus.

Investigating reovirus σ1 mutations mechanism for increased oncolytic potential

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Noncommunicable diseases are now responsible for the majority of deaths worldwide, with cancer being the largest barrier to increasing life expectancy. While traditional therapies show strong effectiveness in combating cancer, oncolytic viruses that preferentially target cancer cells have been recognized as a promising alternative treatment. A non-enveloped double-stranded RNA virus called reovirus is being explored as a cancer therapeutic. While well tolerated in cancer patients, T3Dwt (Type 3 Dearing) reovirus requires improvement in oncolytic potency. To address this, three reovirus mutations have been selected within binding protein sigma1 that produce larger plaques relative to T3Dwt on tumour cell monolayers, suggesting greater oncolytic potency. The sigma1 protein consists of two binding domains: the sialic acid binding “tail domain”, and the junction adhesion molecule-A (JAM-A) binding “head domain”. Reovirus mutations Q217H and R219Q (tail domain) and N312D (head domain) were found to have decreased binding at 4°C to L929 cells relative to T3Dwt, however, the mechanism by which the mutants cause greater oncolytic potency is still unknown. Previous reports studied reovirus mutant SV5 and its lowered binding to cells causing virus particles to spread farther from the initial site of production. To address this possibility, a consecutive infection assay was performed to study virus binding and infection at 4°C or 37°C through numerous transfers. Preliminary data suggests that reovirus mutations Q217H and R219Q, show increased binding at later transfers relative to T3Dwt, indicating viral particles retain the ability to bind at future opportunities causing increased spread through the cell monolayer. This demonstrates a potential mechanism behind Q217H and R219Q presenting greater oncolytic potency. These results show that mutations can improve adaptation toward the tumour environment and cancer cell killing. In the future, reovirus mutations could be combined to genetically engineer a virus with a further increasing ability to kill cancer cells.

Identification, distribution analysis, and qualitative assessments of putative stimulatory goldfish (*Carassius auratus*) leukocyte immune-type receptor CaLITR positive leukocytes in goldfish skin tissues

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Host immune defenses play a crucial role in protecting the body from harm and maintaining immune balance, helping to prevent and control infections and disease development. They do so by using intricate networks of immunoregulatory receptors both on their cell surfaces and intracellularly to distinguish between self and foreign target molecules. Leukocyte Immune Type Receptors (LITRs) represent a group of immunoregulatory receptors found in teleost fishes that bear resemblance to multiple families of mammalian immunoregulatory receptors. Goldfish LITRs (CaLITRs) have recently been characterized and shown to share common ancestry with several mammalian immunoregulatory receptors that control immune cell effector responses. While extensive research has expanded our understanding of immune cells and immunoreceptors in humans and other mammals, their counterparts in non-mammalian species such as fish are relatively unexplored. Using Goldfish as a model system, I established Immunocytochemistry (ICC) and In-situ hybridization (ISH) protocols for identifying and examining the distribution and relative amounts/types of putative stimulatory CaLITR positive leukocytes. Within goldish there are distinct populations of myeloid leukocytes (e.g., neutrophils and macrophages) that are CaLITR3 positive, CaLITR6.1 positive, as well as CaLITR3 / CaLITR6.1 double positive. At the site of an inflamed skin, there will be significant increases in the amounts and relative distributions of single as well as double (CaLITR3 and CaLITR6.1) positive CaLITR leukocytes. The dynamic distributions of these CaLITR positive leukocytes were tracked using the established ISH and ICC protocols. This was achieved by tracking the two putative stimulatory CaLITR-types using validated polyclonal antibodies against CaLITR3 and CaLITR6.1 for ICC and using fluorescently labeled RNA probes against the *calitr 3* mRNA transcripts for ISH protocols. This work has provided the platform/protocols for in vivo examination of leukocyte populations/subpopulations and for the identification and tracking of these subpopulations during homeostasis and inflammation.

Polysialic acid enhances GPR15-mediated T cell migration: Potential implications for inflammatory diseases

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T cell migration is a critical process in inflammatory diseases such as inflammatory bowel disease (IBD) and psoriasis. While integrins and chemokine receptors are well-established regulators of these processes, many aspects remain elusive. Post-translational modifications, particularly glycosylation, are emerging as key modulators of immune function and hold the key to advancing our understanding of immune cell migration. Polysialic acid (polySia) is a highly anionic glycan and post-translational modification previously shown to influence innate immune cell migration. However, its role in adaptive immunity remains largely unexplored. In our study, we found that a significant proportion of antigen-experienced T cells in human peripheral blood mononuclear cells (PBMCs) express polySia, while naïve T cells do not. Notably, smokers, who experience chronic inflammation and have an elevated risk for inflammatory diseases, exhibit a substantially higher proportion of $polySia^+$ T cells, especially in males. To investigate the functional significance of polySia in T cells, we employed a method developed in our lab to identify polysialylated proteins. We identified GPR15, a chemokine receptor implicated in smoking-associated inflammation and T cell migration, as a novel polysialylated protein in human T cells. GPR15 facilitates T cell migration toward its ligand, GPR15L, which is highly expressed in inflamed mucosal and skin tissues. Given that GPR15L contains basic amino acids that could interact with the negatively charged polySia chain, we hypothesized that polySia regulates GPR15-mediated T cell migration. Indeed, our findings demonstrate that polySia is crucial for this process. Flow cytometry analysis of human blood samples revealed a significant increase in $polySia^+GPR15^+$ T cells in smokers. These findings establish polySia as a key regulator of GPR15-mediated T cell migration and highlight a potential therapeutic target for modulating T cell trafficking in inflammatory diseases.

Poster Presentations in Ecology and Evolution

Evaluating fence permeability and seasonal mammalian movement in Elk Island National Park

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Habitat fragmentation caused by roads and fences is a key issue in conservation, as it can restrict wildlife movement, isolate populations, and reduce genetic diversity, ultimately posing significant challenges to both wildlife and habitat connectivity. Understanding how different species respond to such barriers is essential for maintaining a healthy ecosystem and preventing populations from becoming isolated. Elk Island National Park (EINP), located east of Edmonton, Alberta, is a fully fenced conservation area which helps keep bison populations contained and protected from disease. Over time, various factors such as animal digging, natural processes, and human activities have created gaps under the fence. These gaps allow some wildlife to move in and out of EINP while still keeping the bison enclosed but pose significant connectivity challenges on the landscape. Using 14 camera traps installed at fence gaps, I intend to analyze crossing events over a one-year period to identify trends in how different mammalian species navigate barrier permeability and how their movement patterns differ seasonally. Preliminary results have revealed that mammals crossed the fences more in winter and spring than in summer. These findings aim to inform fence management practices within EINP, offering valuable insights into the role of fence permeability in wildlife population control while maintaining ecological connectivity.

Exploring the loss of resistance in the *Drosophila-Macrocheles* system

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This experiment examines the previously-studied *Drosophila-Macrocheles* system in which *Drosophila nigrospiracula* flies are infected by ectoparasitic *Macrocheles subbadius* mites. Previous literature has demonstrated the presence of costly genes in these flies which confer increased resistance against mites; resistant flies had reduced longevity and fecundity. Studies have also indicated that over multiple years separated from their natural environment, costly resistance genes are selected against. In this study, we will test the hypothesis that when parasitic mites (i.e., selection pressure) are absent for several generations, costly resistance traits will be selected against. We will perform infection assays on wild-caught flies and lab-bred flies. The wild-caught flies were collected in November, 2024 from Phoenix, Arizona, USA. The lab-bred flies were collected from at the same location in February of 2020; no wild-caught flies have been incorporated since time of capture. We expect lab-bred flies to show decreased resistance to mites compared to wild-caught flies. We will measure the prevalence and intensity of infection (# mites per/infected fly). This information could further confirm the cost of resistance genes in insects, particularly *Drosophila*.

Bilateral organization of efferent projections of the visual wulst in barn owls (*Tyto alba*)

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Due to their forward facing eyes, owls have exceptionally large binocular fields in comparison to other birds. However in both owls and other birds there is a projection of the retinal ganglion cell to the contralateral opticus principalis thalami (OPT), which then extends bilaterally to the left and right visual wulst. This is thought to have a role in binocular vision. This study investigates Efferent projections of the visual wulst in barn owls (*Tyto alba*), focusing on terminal projections to various brain nuclei involved in visual processing. We confirmed previous findings by Karten et al. (1973), showing both ipsilateral and contralateral projections from the visual wulst to the optic tectum (TeO) and the geniculatus lateralis pars ventralis (GLv). Additionally, we discovered novel bilateral projections to the griseum tectale (GT), nucleus lentiformis mesencephali (LM), and nucleus of the basal optic root (nBOR), highlighting key differences between owls and other avians. Notably, owls exhibit stronger bilateral projections to LM and nBOR, potentially contributing to enhanced binocular visual processing and optic flow coordination. A distinct topographic arrangement of terminal clusters was observed, with ipsilateral terminals predominantly located medially and contralateral terminals laterally in both the TeO and GLv. The

presence of bilateral projections to LM, which is implicated in optic flow and optokinetic responses, suggests a unique aspect of owl vision, similar to primates. Furthermore, our findings challenge previous assumptions regarding the role of the nBOR in optokinetic behavior, suggesting alternative pathways for optic flow control. This study sheds light on the intricacies of the owl visual system and its potential parallels with mammalian systems, offering insights into the neural coordination of visual stimuli and behavioral responses in birds. Future investigations into the relationships between the visual wulst and these nuclei could deepen our understanding of visual processing and locomotion in avian species.

Enhancing soil carbon storage through restoration of native dry mixedgrass grasslands

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Building a lichen barcode library for species-specific identification in southern Alberta

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DNA barcoding has many applications including species identification and characterizing biodiversity. There is a global push to build DNA barcode libraries that encompass the breadth of species diversity. A well-curated, exhaustive, local reference DNA barcode library can increase species delimitation and assignment from environmental DNA such as soil samples. These genetic tools can address some of the limitations of conventional morphological surveys by revealing cryptic, dormant or juvenile species diversity that might otherwise remain undetected. At present, there are several gaps in species coverage for macro-lichens in Alberta grasslands, limiting the taxonomic assignment capabilities. There is also poor representation of species-level diversity from local collections in publicly available databases. Our goal is to build a local barcode library for macro-lichens found in Alberta's grasslands using published sequences augmented with expertly-identified vouchers to assess the utility of a regional species database for capturing species-level diversity in predetermined metabarcoded communities. We created a voucher table or custom database comprised of over 1000 sequences of nearly 150 species to determine the gaps in the public databases. We collected lichen vouchers from the Alberta grasslands and sequenced ~ 150 specimens using the ITS amplicon/region with Sanger sequencing. Finally, we created mock communities of 12 lichen specimens and sequenced these samples using high-throughput technologies. Forty-nine percent of species had under 5 available sequences in a public database. By increasing the available sequences and restricting our database to ecologically relevant species, we hypothesize we will improve the resolution of species identification. This highlights the importance of local barcode libraries to accelerate species identification, improving our ability to characterize communities of frequently overlooked species using genetic tools.

Evolving dissolved organic matter composition along the permafrost thaw affected Willow River: An FT-ICR mass spectroscopy analysis

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Arctic permafrost thaw due to climate change is significantly affecting the transport of carbon and nutrients from land to water. In topographically diverse regions with ice-rich permafrost, substantial amounts of organic matter are being mobilized into aquatic systems via retrogressive thaw slumps, altering biogeochemical cycles and potentially accelerating global warming. This is because the breakdown of this excess organic matter by heterotrophic microbes releases greenhouse gases, enabling a positive feedback cycle of global warming. This study investigated dissolved organic matter (DOM) composition along the permafrost-

thaw-affected Willow River in Canada's Northwest Territories. Using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) data, we analyzed samples from upstream (unimpacted), slump outflow, and main-channel (impacted) sites to characterize variations in DOM molecular properties. Upstream sites generally exhibited the highest aromaticity and structural complexity, while slump outflow sites generally had elevated amounts of heteroatom-rich (compounds containing nitrogen and/or sulphur) and aliphatic (compounds which have high hydrogen to carbon ratios) compounds. These "simpler" compounds were lost rapidly downstream, whereas the more complex, aromatic DOM persisted in the river. By isolating compounds that were unique to the slump outflow sites (when compared to unimpacted upstream reaches) and yet were found at the furthest downstream site of the main channel, we were able to conclude that the more aromatic permafrost-derived DOM being mobilized into the river are persisting downstream, contributing to changes in riverine carbon cycling. Similar to many other regions throughout the pan-Arctic, aliphatic permafrost-derived DOM appears to be more biolabile and can be consumed quickly by microorganisms, whereas the more complex and aromatic formulae appear to be more resistant to degradation. These findings highlight how permafrost-derived DOM could have significant effects on greenhouse gas emissions, the global carbon cycle, and microbial productivity as warming and wetting continue to increase.

Cause and composition of Arctic River microbial flocs: A research study

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The Arctic is disproportionately impacted by the effects of climate change, leading to some of the world's most dramatic climate change impacts to the landscape, water, and people. Intensification to the hydrological cycle and permafrost thaw has led to shifts in water regimes and biogeochemical cycling throughout Arctic rivers, posing concern for water quality. Since 2017, residents in the Gwich'in Settlement Area of the Northwest Territories (NWT) have observed the presence of rust-coloured flocs in the Peel, Arctic Red, and Mackenzie Rivers. Our research aims to understand the composition of these flocs along with the environmental constituents that drive their emergence. Here, we use 16S (for Bacteria and Archaea) and 18S (for Eukarya) rRNA gene sequencing to determine species composition of potential flocs collected at seven different sites of the Peel Channel, downstream of the Peel River in the Mackenzie Delta. Our microbial analyses will be paired with an analysis of flux trends for chemical constituents that we expect to be associated with the occurrence of flocs, and an assessment of trends in meteorological data to understand climate effects. We expect to see high concentrations of sulfate and iron oxides in the Peel River due to permafrost thaw, which releases iron and sulfuric acid into rivers. As a result, we also expect that flocs will be composed of sulfide and iron oxidizing bacteria, and photosynthetic algae. Since permafrost thaw increases with increasing temperature, we expect the flocs to be present in warmer years, with other meteorological parameters such as precipitation levels and wind speed likely also playing roles. Understanding the origin and composition of these flocs will provide insight into potential shifts in water quality throughout the Mackenzie and Peel Rivers, and answers to community members who are concerned by their appearance.

Permafrost thaw impacts on the Peel River and its potential effects on the carbon cycle: A research study

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Climate change has significantly impacted northern ecosystems, accelerating the thaw of ancient, carbon-rich permafrost. As this permafrost thaws, organic matter is increasingly mobilized from terrestrial ecosystems into nearby rivers. The Peel River, which flows through Yukon and the Northwest Territories, is undergoing extensive land deformation driven by this thaw, strengthening land-water connectivity, a process that ultimately leads to significant increases in erosional delivery of sediments and associated organic matter. As a result, microbial communities are exposed to novel sources of organic carbon,

with the potential for alteration of carbon cycling in this environment. This BIOL398 study is analyzing pre-existing datasets spanning the past 56 years to examine the riverine transport of five key constituents in the Peel River: dissolved organic carbon (DOC), particulate organic carbon (POC), total suspended and dissolved sediments, and turbidity. Riverine transport (flux) will be assessed using the EGRET (Exploration and Graphics for RivEr Trends) package in R, a tool designed for analyzing long-term water quality and flow trends using time series statistical methods. Trends in the transport of these constituents of interest will be assessed, and compared to meteorological data to assess how climate drivers are affecting land-water carbon transport. Findings from this study will contribute to understanding the ecological and biogeochemical consequences of changing land-water interactions in northern river systems.

Seasonal movement and habitat selection in an invasive wild pig population

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Animal movement is driven by physiological and environmental conditions, which change predictably across the year. Adjusting activity to align with these changes is critical for controlling energy balance, ensuring requirements are met for growth and reproduction. Wild animals generally exhibit seasonal patterns in reproduction, timing births with favourable conditions to meet the needs of both parent and offspring. Wild pigs (*Sus scrofa*) are prolific and invasive with spatial, dietary, and reproductive plasticity. Introduced in Canada in the 1980s, wild pigs are a concern because they pose risks to agriculture, livestock, public health, and the natural environment. They adapt to changing environmental conditions by altering movement rate and habitat selection and quickly establish across new landscapes due to an unusually high reproductive capacity. Although they can reproduce throughout the year, populations commonly display seasonal peaks in reproduction. Significant knowledge gaps remain in the fundamental ecological and biological requirements of wild pigs in Canada, including their spatial and temporal ecology and the effect of the environment on reproduction. Using a GPS-telemetry dataset of wild pigs collared in Saskatchewan from 2015 to 2017, we have studied seasonal movement patterns using Generalized Additive Mixed Modelling (GAMM) and have identified seasonal reproductive patterns by detecting abrupt changes in fine-scale movements, characteristic of ungulate reproduction. Preliminary results show significant drops in movement rate during winter months for both sexes, and a cluster of potential parturition dates have been identified in the spring.

Mammalian diversity and movement dynamics using automated cameras

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Wildlife fencing is a key issue in conservation, increasing habitat fragmentation and loss of habitat connectivity. Keeping wildlife isolated from surrounding areas while permitting wildlife to access the broader habitat is complex. To maintain connectivity, animal and human-made fence gaps allow wildlife to access outside landscapes to breed, establish new territories, and disperse. Using 14 camera traps over a 6-month period, I studied mammal movement dynamics across fence gaps in Elk Island National Park (EINP), a fully fenced conservation area. We identified 379 crossing events, which we used to evaluate interactions with gap size and animal body size. We found that coyotes (*Canis latrans*) crossed the most. Our results show that fence gaps support animal movement while containing bison populations. Fence gap size did not affect the number of crossings, but crossings were heavily skewed towards small individuals. These findings suggest that increasing fence permeability could enhance ecological connectivity, benefiting multiple species. We recommend that EINP build additional fence gaps to support wildlife movement.

Den site characteristics selected by wild coyotes

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Coyotes (*Canis latrans*) are common mesocarnivores that occupy various environments across North America, including urban areas. Range expansion has occurred due to a lack of predators, increased food availability, and behavioural flexibility.

However, their success in urban environments increases overlap and conflict with humans. Human-wildlife interactions may escalate from nighttime sightings of coyotes to midday attacks on humans and pets, especially if food conditioning occurs. For multiple reasons, understanding the characteristics that coyotes select at their dens is important. First, knowledge of their life history related to denning and pup-rearing is limited, as most of the few studies available have used small sample sizes and have been geographically restricted to the United States. Second, having a secure place to raise pups is crucial for coyotes to reach adulthood, so the availability of appropriate den sites is crucial to coyote recruitment. Third, physical conflict between coyotes and people or pets increases during the coyote breeding and pup-rearing period. Because dens are linked with human-wildlife conflict, predicting their locations is important for management. This study investigates coyote den selection in Elk Island National Park, Alberta, Canada. We are building resource selection functions to compare the characteristics of known den sites with nearby available locations. If coyotes rely mainly on the security of den sites, we predict that selection will be strongest at finer spatial scales, despite differences between urban and parkland habitats. The data has been collected, but analysis is still ongoing. The results of this study will provide further insight into coyote management in urban and non-urban settings.

Exploring the impact of visitor foot traffic on invasive species in national parks

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The natural beauty of National parks attracts a large number of visitors each year yet still faces on-going issues with biodiversity loss. This raises the concern to which extent human-mediated seed dispersal contributes to the introduction of non-native species. Often, these visitors can unintentionally introduce non-native species to these areas, usually through the soles of hiking boots when engaging in recreational activity. We consider what factors may potentially predict or filter the types of plants being brought into these parks. To investigate this, we grew seeds collected from hiking boots in Elk Island National Park and combined the data with park usage and trail location datasets. We found that paved trails had higher foot traffic compared to dirt trails, however, dirt trails exhibited more germinates per volume and a greater diversity of introduced species. These findings suggest that, while paved trails face the highest risk of species invasion due to greater usage, fewer people on these trails use boot scrapers compared to those on dirt trails, potentially exacerbating the problem.

Evaluating drone-based aversive conditioning to mitigate human-wildlife conflict with grizzly bears (*Ursus arctos*) in Kananaskis Country, Alberta

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Managing human-wildlife conflict is an ongoing challenge in protected areas. Wildlife managers mitigate this potential conflict with grizzly bears (*Ursus arctos horribilis*) in Kananaskis Country, Alberta, by using aversive conditioning near hiking trails, facilities, campgrounds, and roadsides. Aversive conditioning exposes bears to negative or stressful experiences to increase their wariness around humans, but the proximity to wildlife that conditioning methods require poses inherent risks to both technicians and bears. My study will explore the use of drones as a safer alternative to conventional conditioning methods. Drones will approach bears and subject them to auditory and visual stimuli without requiring close proximity of bears and people. I will apply drone-based and conventional conditioning in alternating treatment periods and compare bear responses before, during, and after each treatment. I will use GPS and VHF collars to measure the frequency and latency with which bears return to sites after conditioning. My research aims to enhance the safety and efficacy of bear management, promote human-wildlife coexistence, and support healthy grizzly bear populations in critical habitats. This study will build on decades of successful bear management in Kananaskis and could inform coexistence strategies in other regions where humans and bears share space.

Spatial and temporal effects of aversive conditioning on grizzly bears in human-dominated landscapes

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Human-wildlife conflict is increasing in protected areas, particularly where nearby urbanization leads to high levels of human visitation. Conflicts with carnivores and risks to human safety are especially challenging to manage. The management of grizzly bears (*Ursus arctos horribilis*) in Kananaskis Country is an internationally known example of this situation, where human and bear safety are maintained with an innovative aversive conditioning (AC) program. AC is a form of associative learning in which technicians use negative stimuli (e.g., sounds, projectiles, pursuits) to teach bears to be wary of people in high human-use areas. AC is widely used in the mountain parks of Canada, where it clearly causes bears to retreat from technicians in the short term, but there has been no study of its longer-term effects on behaviour and space use by bears. Our research will measure spatial and temporal responses of grizzly bears to AC over the past 20 years in Kananaskis Country. We will use data from GPS collars and AC event records to perform a novel integrated step selection analysis to compare movement and habitat use of grizzly bears from before and after AC events. We will also determine nocturnal activity, before and after AC occurred, while controlling for other variables which are known to affect bear nocturnality. This work will determine the spatial and temporal responses of grizzly bears to AC, allowing wildlife managers to refine AC protocols, which will limit human-bear conflict, promoting human-bear coexistence, thus increasing human and bear safety in Alberta.

Who goes there? A research study of carnivore and herbivore fence crossing events at Elk Island National Park via trail camera analysis

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Habitat fragmentation, driven by roads and fences, disrupts wildlife connectivity and poses significant ecological challenges. Elk Island National Park (EINP), located east of Edmonton, Alberta, offers a unique setting to study fence permeability and its impact on wildlife movement, particularly among carnivores and ungulates. We used trail cameras installed at fence breaches to analyze crossing frequencies and determine how different species navigate these barriers. Our results revealed that carnivores crossed significantly more often than herbivores. This study aims to inform management practices within EINP, supporting population control strategies and predator management programs to sustain ecosystem health throughout the Park. These findings will contribute valuable insights into the role of fence permeability in managing wildlife populations and maintaining ecological connectivity within the Beaver Hills Biosphere.

Impacts of mountain pine beetle disturbance on spider communities: A research study

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Spiders (Areneae) play an important role in numerous types of ecosystems, both as predators and as prey, and are often used as ecological indicators. Spiders occupy specific climatic and structural niches and therefore react strongly to different forms of disturbance. This reaction has been documented in multiple environments, including forest ecosystems. In many parts of western North America, one of the most prevalent contributors to forest disturbances is the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Coleoptera: Curculionidae). Despite the importance of spiders in forest ecosystems and the extent of mountain pine beetle disturbance, there is a lack of literature discussing the interaction between the two. The objective of this study is to determine if disturbance caused by mountain pine beetle impacts the abundance and diversity of spiders. Ground dwelling arthropods were sampled using pitfall traps in lodgepole pine dominant stands with and without recorded mountain pine beetle attacks in areas surrounding Grande Prairie, Alberta. The spider specimens were identified to the family level to assess diversity and abundance. The damage caused by mountain pine beetle results in significant changes to forest architecture and increased light penetration which impacts the climate at the forest floor. Open-area dwelling spiders, such as the families Lycosidae and Gnaphosidae, prefer warm temperatures and low humidity, so it is expected that these families will be more abundant in disturbed areas while forest-dwelling spiders that prefer moderate temperatures and high humidity will be more abundant in undisturbed areas. It is also expected that abundance will increase in disturbed stands

as open-area dwelling spiders establish in these areas while forest-dwelling spiders are still present. Understanding the response of spiders to mountain pine beetle disturbance will fill a current gap in mountain pine beetle research and will provide insight into the interactions between these two important arthropod groups.

Poster Presentations in Paleontology

Dental features of the late Cretaceous small theropod, *Atrociraptor marshalli* (Theropoda, Dromaeosauridae, Saurornitholestinae), from the horseshoe canyon formation (upper Campanian - lower Maastrichtian)

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This study examines the dental features of *Atrociraptor marshalli* to clarify its geological specificity and unique adaptations within dromaeosaurids. *Atrociraptor marshalli* is a recently recognized dromaeosaurid theropod dinosaur from the Cretaceous geological unit, Horseshoe Canyon Formation, of Drumheller, Canada. Due to the rarity of its fossils, analysis of specialized enamel structures called denticles on its teeth are crucial for figuring out the mysteries of its diet and relationships. This study provides additional microscopic observations and statistical analysis on both in-situ and isolated teeth of the holotype specimen of *A. marshalli*. Despite the situation that dromaeosaurid teeth from the Horseshoe Canyon Formation have tended to be identified as *Saurornitholestes langstoni*, a dinosaur that is well known from the older Dinosaur Park Formation, the significance of *A. marshalli* as the Horseshoe Canyon lineage is supported by contrasting its unique ratio of denticle height, width, density, and tooth curvature. In addition, the discovery of flute-like structures common in *Zapsalis* on the premaxillary teeth of *A. marshalli* proposes both noteworthy differences and a close relationship with its sister taxon. This discovery contributes to behavioural studies of dromaeosaurids in North America by indicating that the function of their front teeth is probably for preening. Overall, this examination supports the need for further research to precisely recognize species level differences of the dromaeosaurid teeth in each formation, rather than the teeth being indistinguishable across multiple geological units.

Description of a neonatal hadrosaur specimen from the Oldman Formation (Alberta, Canada)

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New perinatal hadrosaur remains associated with eggshell are reported from the upper Oldman formation of the Milk River Preserve in southeastern Alberta. The nature of the eggshell and the elements preserved from the top of the skull indicate the specimen belongs to a lambeosaurine species, although its jugal shape suggests that it may be a hadrosaurine. The association of large pieces of eggshell with the elements of only one individual suggests that this specimen probably represents the remains of a single egg that was preserved in isolation. Comparisons show that this perinate is 10% larger (in linear measurements) than the largest embryonic specimen of *Hypacrosaurus stebingeri* from Devil's Coulee. Further study is required to identify the species to which this specimen belongs.

Newly described *Chelosphargis advena* specimen from Niobrara, Kansas allows for better understanding of Coniacian Protostegid phylogenetics (Chelospharginae, Protostegidae)

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Although common in the fossil record, marine turtles of the Protostegidae family have been poorly examined in the literature. Little work has been done regarding in-depth descriptions of specimens, comparative analysis and phylogenetic reconstructions. Through the use of C-T scanning data and 3-D modeling software, a detailed description of a new juvenile specimen of *Chelosphargis advena* from the late Coniacian of Kansas furthers the available knowledge of this genus. The cranial material of this Niobrara-Chalk specimen is extremely well preserved, and allows for identification of nearly all dermatocranial elements. Although the post-crania is mostly disarticulated, the majority is still present and permits for a

quality description. This study also serves to provide quantifiable morphological traits for future phylogenetic analysis of the protostegid lineage. This increases the data within the literature, and will facilitate a better understanding of how this now-extinct lineage evolved, thrived, and eventually went extinct.

Investigating the lack of cranial material at the Danek Bonebed: A research study

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The Danek Bonebed is a monodominant *Edmontosaurus* bonebed located in Edmonton, Alberta. Discovered in 1989, the locality was excavated by the Royal Tyrrell Museum until 1991, and was later reopened by the University of Alberta in 2006, which has excavated it ever since. The locality also has material from *Albertosaurus*, ceratopsian, dromaeosaur, and *Troodon* material but the overwhelming majority of the material recovered from this locality is *Edmontosaurus*. Over 1000 specimens assigned to *Edmontosaurus* have been collected from the locality, but there is an interesting lack of cranial material, being represented by under 10% of the material. While the material is expected to be predominantly post cranial, the expected percentage of cranial material (based on the full skeletal inventory of *Edmontosaurus*) is almost double the actual amount. The purpose of this study is to determine whether this lack of cranial material is standard among North American hadrosaur bonebeds through comparison between other similar localities. The sites used for comparison are the Bleriot Ferry Bonebed (an *Edmontosaurus* locality near Drumheller), Choteau Bonebed (a *Maiasaura* locality in Montana), Fox Coulee Bonebed (an *Edmontosaurus* locality near Drumheller), Liscomb Bonebed (an *Edmontosaurus* locality in Northern Alaska), Prehistoric Park Bonebed (an *Edmontosaurus* locality near Drumheller), Ruth Mason Bonebed (an *Edmontosaurus* site in South Dakota), Spring Creek Bonebed (an *Edmontosaurus* locality near Grande Prairie), Standing Rock Hadrosaur Bonebed (an *Edmontosaurus* site in South Dakota), and Wendy's Bonebed (A *Gryposaurus* locality in Southern Alberta). The Danek Bonebed's lack of cranial material does seem to be unusual among North American hadrosaur bonebeds, with the majority of other sites containing almost double the percentage of cranial material. This could mean that the Danek Bonebed could have some unusual preservational quality to it that is as of yet unknown.

Tooth development and resorption in living Teiidae and its implications on tooth replacement in fossil Mosasauridae

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Teeth are a defining characteristic that can be used to construct evolutionary relationships. Despite its diagnostic power, this feature is trivial within squamate (reptile) phylogeny as squamates have a wide variety of tooth attachment types. Considering this morphological variation, internal investigation through hard and soft tissue histology (studying cells under a microscope) is necessary to search for relatedness between living and extinct squamates. This reasoning has been used to discover that mosasaurs share a socketed mode of tooth attachment and three-layered attachment tissue system with extant (living) teiid lizards, like *Tupinambis* (Teiidae); This is something that monitor lizards (Varanidae)— the common modern analog to mosasaurs— do not have. Because of these similarities, further comparative investigation via thin sectioning and histological staining of teiids is necessary to fully understand the tooth attachment geometry, cellular activity, dental lamina movement, and periodontal ligament mineralization during tooth development within Mosasauridae. My current research progress has revealed that teiids have an attachment tissue distribution similar to that of mosasaurs, and through the examination of teiid soft tissues, I have explored how mosasaur soft tissue would behave during tooth resorption. Further, I have revealed that teiids with dental pathologies use the same reparative mechanisms as humans and other amniotes, thus showing evidence of dental homologies between all amniotes. This research is important as it highlights the importance of modern datasets in paleontology, challenges Mosasauridae's widely accepted phylogenetic position besides Varanidae, and adds tooth attachment tissues to the list of squamate diagnostic characteristics.

Poster Presentations in Marine Biology

Habitat suitability and connectivity modeling of reef fishes in the Florida Keys reef tract to inform coral reef restoration planning

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Coral reefs worldwide have suffered devastating loss of coral-cover, biodiversity, and ecosystem function due to escalating stressors. To abate this decline, restoration strategies are increasingly crucial, requiring practitioners to prioritize reefs effectively. To date, the functional roles of reef fishes in determining coral restoration success have received limited attention, yet interactions such as herbivory, invertivory, and nutrient deposition are integral to reef health. Predicting the spatial distribution of these interactions and locating hotspots of beneficial fish communities could inform site selection to increase restoration efficacy. Aiding in these endeavors are seascape connectivity models, which can predict species-specific movement corridors between suitable habitat patches in diverse marine landscapes. The construction of accurate connectivity models requires the quantification of habitat suitability, which remains a complex task. Here, we develop species- and lifestage-specific habitat suitability models (HSMs) for five reef-associated fishes in the Florida Keys, USA, using maximum entropy methods and multiple spatio-environmental predictors including habitat type, metrics of seafloor morphology, and indices of water quality. These HSMs are then input into connectivity models, generating ecologically realistic predictions of species' movement patterns. The selected species - *Scarus coeruleus* (blue parrotfish), *Scarus coelestinus* (midnight parrotfish), *Scarus guacamaia* (rainbow parrotfish), *Lutjanus griseus* (gray snapper), and *Haemulon sciurus* (bluestriped grunt) - exhibit ontogenetic habitat shifts as sub-adults, making them key indicators of seascape connectivity. From our models we identify the strongest spatio-environmental predictors of habitat suitability, elucidating species-seascape interactions that shape patterns of functional connectivity. Areas of convergence between connectivity models indicate local hotspots, where restoration efforts have the greatest potential to benefit from interactions with these key species.

Effects of leaching compounds from artificial aquarium plants on *Daphnia magna* and *Daphnia pulex*

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Enrichment in aquatic animal studies is important for promoting welfare and maintaining animal health and can be categorized by physical, sensory, social, occupational and dietary. However, the risk of potential chemical leaching associated with physical enrichment items has been largely overlooked (i.e., artificial plants or shelter) in animal welfare. Most enrichment items lack information on their chemical composition and have the potential to leach many contaminants into the surrounding environment. In fish and invertebrate research, these leachates have the potential to modify the health of aquatic animals or their reproductive processes. A commonly used enrichment item is plastic plants, which are often made from low-quality materials, which can possibly leach phthalate and metals. The objective of this study is to observe the potential leaching effects from the plastic plants and their effect on invertebrates *Daphnia magna* and *Daphnia pulex* - freshwater crustaceans commonly used in aquatic toxicology. *Daphnia magna* and *Daphnia pulex* to 8 different leachate concentrations (0, 15, 30, 45, 60, 75, 90, 100%). To understand the long-term effects on these organisms, we will conduct a 21-day chronic exposure to a two-week aged leachate. Both mortality and reproduction rates will be measured daily. This study will provide insight into the potential risks of using plastic enrichment items in research and allow for future regulations to be implemented on their use and production.

Poster Presentations in Plant Biology

Impact of humic-based soil amendment on root nodulation and plant growth of soybean (*Glycine max* L.)

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Soybean is one of the most economically important grain legumes grown in Canada. Soybean naturally fixes the atmospheric nitrogen inside the root nodules through their symbiotic relationship with *Rhizobium* bacteria. However, sub-optimum nodulation can reduce nitrogen fixation and plant growth in soybean. Humalite, a humic acid-rich product derived from natural deposits in Southern Alberta, has the potential to enhance nodulation and nitrogen fixation in grain legumes. However, the effects of humalite on soybeans remain unexplored, presenting an opportunity for further research into its role in improving nitrogen fixation and crop performance. This study aims to examine the effects of liquid humalite on root nodulation, plant growth, and symbiotic nitrogen fixation (SNF) in soybean. The experiment will be conducted under greenhouse conditions with four concentrations of liquid humalite (0.2%, 0.4%, 0.8%, and 1.6% v/v) mixed in 0.25X N-free Hoagland's solution. Soybean seeds will be inoculated with *Bradyrhizobium japonicum* USDA 110. After six weeks of growth, various plant growth parameters will be measured, including root length, root surface area, root volume, nodule number, nodule dry weight, and shoot and root biomass. The symbiotic nitrogen fixation capacity will be measured using a ^{15}N-based isotope dilution technique.

Advancements in click chemistry techniques for nascent proteome analysis

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Click chemistry is a set of novel technologies that can be used for labelling of cellular components *in vivo* via efficient and relatively simple chemistry, facilitating enrichment of molecules of interest. One application of this chemistry is to incorporate tags into newly synthesized proteins, where the "click" reaction facilitates the enrichment of exclusively the nascent proteome, washing away the older, high-abundance proteins to intensify signals of interest during mass spectrometry. Pioneered in mammalian cell culture models, these techniques are not equally advanced in plants, in part because of difficulty navigating the unique plant responses to the supplied compounds. We have optimized a protocol for nascent proteome enrichment via click chemistry in *Arabidopsis* that allows for simple and fast tagging of newly synthesized proteins with drug concentrations up to 20-fold lower than previously observed in the literature, using fewer steps, and detecting thousands of unique proteins enriched from small amounts of crude plant matter extract, while simultaneously avoiding previously observed stress phenotypes associated with increased labeling compound concentrations. These improvements grant a more efficient, cheaper, and more accessible workflow for this form of proteomics in plants, allowing for broader and more effective use of these technologies.

A comparative analysis of morphological variations in Galactic Glue and Charlotte's Web cannabis strains

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Cannabis sativa is a chemically varied plant with significant use in research and medicine. The primary cannabinoids, Tetrahydrocannabinol (THC) and cannabidiol (CBD), interact with the endocannabinoid system to affect physiological processes. Strains bred for high THC or high CBD content exhibit distinct biochemical and agronomic traits. This study compares the growth characteristics of two strains cultivated under controlled conditions: Galactic Glue (THC-abundant) and Charlotte's Web (CBD-abundant). Galactic Glue was hypothesized to accumulate more fresh and dry weight than Charlotte's Web, because THC-dominant strains allocate more metabolic resources to biomass and flower development than CBD-dominant strains. Weekly measurements of fresh weight, height, and dry weight were made throughout the flowering period, specifically weeks five through eight of plant growth. Dry biomass was assessed following harvest. In support of the idea, Galactic Glue showed noticeably greater fresh and dry weight accumulation. On average, the fresh weight of Galactic Glue increased by 81%, while 28% of the dry weight was retained. Despite maintaining a constant height development, Charlotte's

Web yielded less biomass than the relative height. Galactic Glue favored denser floral growth, while Charlotte's Web displayed a homogeneous vegetative structure, indicating different metabolic methods. These results imply that biomass distribution is influenced by cannabis concentration, with strains that are higher in THC showing more robust growth. These findings are consistent with earlier research that connected elevated THC biosynthesis to higher biomass and secondary metabolite production. The reduced output of Charlotte's Web would suggest that more resources are being devoted to the creation of CBD rather than the overall buildup of biomass. Understanding the differences in morphology of two distinct strains can help commercial cannabis production choose strains and develop cultivation plans. The molecular foundation of these growth patterns can be further clarified by future studies utilizing metabolomic profiling.

Differences in *Agaricus bisporus* and *Hypsizygus marmoreus* in terms of phenolic content, antioxidant activity, and proximate compositional analysis: A research study

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The brown button mushroom, *Agaricus bisporus*, has served as one of the most common choices of culinary fungi in consumer diets. The *Hypsizygus marmoreus* is a mushroom quickly rising in popularity in North America, with little bioactive analysis done thus far. The focus of this study was to gain a better understanding of the *Hypsizygus marmoreus* while comparing it to a popular and well-studied mushroom, *Agaricus bisporus*. Both mushrooms were separated into their fruits and stems. Bioactive compounds, such as antioxidant and phenolic compounds, were extracted through FRAP, DPPH, and TPC assays. An extraction procedure was developed using ultrasound processing (solvent, power, time), an emerging green technology. Additionally, a proximate compositional analysis was performed to obtain values of ash, protein, moisture, and lipid content from both mushrooms' fruits and stalks. Through these analyses, it was found that the stalks of both varieties of mushroom generally contained a higher percentage of bioactives, opposed to the fruits. Total phenolic content was best extracted with water and 30% ethanol at a lower power and time, whereas total antioxidants were best extracted with 30 and 50% ethanol at a lower power and time. Additionally, the stalk of the *Agaricus bisporus* showed higher protein content than the other samples. This cultivates interest in the stalk of both mushrooms, which is often discarded in culinary settings. Additionally, both *Agaricus bisporus* and *Hypsizygus marmoreus* should be considered as alternative sources for antioxidant and phenolic compound extraction, due to their high values of bioactives obtained using ultrasound processing technology and green solvents (water and ethanol), which work towards low-cost and environmentally-conscious initiatives.

Integrating remote sensing and soil analysis to assess land management impacts on *Quercus acutissima* (Kunugi) forests

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This study, conducted in Azumino, Nagano, a site historically used for traditional wild silk farming, examines the effects of land management practices on soil health and *Quercus acutissima* (Kunugi) by integrating remote sensing and soil analysis. It quantifies evapotranspiration (ET) using SEBAL/METRIC models, derives Normalized Difference Vegetation Index (NDVI) from Sentinel-2 imagery, and assesses soil electrical conductivity (EC), moisture content, soil texture, and nutrient composition across five plots: P1 (slashed-fallowed land), P2 (13 years of continuous cropping), P3 (77 years of continuous cropping), P4 (3 years of continuous cropping), and P5 (bare land). ANOVA and Tukey post-hoc tests revealed significant differences among plots, with P2 exhibiting the highest pH and base cation content, while P1 had the most acidic soils and lower total carbon (TC) and total nitrogen (TN). P2 also had significantly higher EC than other plots, possibly due to increased nutrient solubility or soil salinity. P5 had significantly lower NDVI than P1, indicating poorer vegetation health and soil conditions. Some plots, including P3, P4, and P5, showed no significant differences in key soil properties, suggesting potential resilience mechanisms or unaccounted environmental influences. Multiple regression analysis explained 86.5% of the variance in NDVI ($R^2 = 0.865$), with total nitrogen positively correlated and sodium negatively correlated with NDVI. EC and moisture content were not significant predictors, suggesting indirect or complex interactions. The next phase will apply kriging to interpolate soil properties, assess NDVI trends across seasons, and integrate ET

estimates from remote sensing. Machine learning approaches will be explored to enhance predictive accuracy and identify nonlinear relationships. Findings will inform sustainable land management strategies to optimize soil conditions, enhance Kunugi forest health, and contribute to preserving the historical practice of wild silk farming, supporting ecological and cultural sustainability.

Poster Presentations in Molecular Biology and Genetics

Deciphering the memory code: Elucidating the role of *sec22* as a novel memory suppressor

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Decades of research have led to the discovery of numerous genes involved in normal learning and memory processes. However, recent studies show that genes acting to suppress memory, also exist. Through a targeted small-scale memory screen conducted using *Drosophila melanogaster*, *sec22* was identified as a novel memory suppressor. The ubiquitously expressed sec22 plays a fundamental role in the budding and fusion of vesicles during both anterograde and retrograde transport between the endoplasmic reticulum and Golgi apparatus. Although *sec22*'s role in vesicle transport is well-known, its role in learning and memory is still unclear. Using the aversive olfactory conditioning assay, we found that *sec22* knockdown (KD) in all neurons, dopamine neurons and mushroom body neurons (MBNs), significantly improved memory due to a specific enhancement of learning. *sec22* is part of the Synaptobrevin family of genes consisting of the vesicle fusion and secretion associated genes, *ykt6*, *vamp7*, *syb* and *nsyb*. These genes were interestingly found to all affect memory acquisition as *ykt6* KD in MBNs improved learning while KD of *syb*, *vamp7* and *nsyb*, impaired it. To elucidate *sec22*'s memory suppression mechanism, we first assessed its effects on MBN neurophysiology by conducting $GRAB_{Ach}$ and GCaMP6 *in vivo* imaging and found that *sec22* KD in MBNs increased acetylcholine release but decreased intracellular calcium (Ca^{2+}) in response to odors. The increase in neurotransmitter release despite the decrease in intracellular Ca^{2+} levels demonstrates that the two properties of neurophysiology can be decoupled. Consistent with this, immunostaining and confocal imaging showed that *sec22* KD increased synaptic protein levels in MBNs without altering its presynaptic sites or gross neuroanatomy, suggesting an increase in synaptic vesicle numbers. We postulate that the characterization of *sec22* as a novel memory suppressor could reveal key insights into the cellular mechanisms of memory and may help discover therapeutic targets for memory-associated disorders.

Development of organ-on-a-chip models for studying diseases and drug testing

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Organoid-on-a-chip models are a combination of microfluidic chips with 3D culture of organ-cell lines. Organoid models derived from human induced pluripotent stem cells (hiPSCs) offer transformative potential in drug discovery, diagnostics and toxicity testing. Our research focuses on development of liver and kidney models to investigate metabolic pathways of existing and novel drugs. hiPSCs were used to generate hepatocyte-like cells (HLCs) for liver-specific applications. The HLCs were validated using qPCR, ensuring the complete differentiation. The co-culture of cell lines HMEC-1, THP-1 and HLCs is used to create liver-on-a-chip models simulating the liver sinusoid. Additionally, our research extends to kidney models, targeting the study of uremic toxins to better understand renal pathophysiology. Our kidney-on-a-chip models simulate the proximal convoluted tubule by co-culturing RPTEC and HUVEC cell lines. By developing organoid models that closely mimic human physiology, we can accelerate drug development and improve the relevance of preclinical studies. Traditional animal models are not only costly to maintain but often yield results that are not directly translatable to humans. Our research aims to bridge this gap by creating cost-effective, robust, and physiologically accurate systems that better reflect human biology. Moreover, organoid models can also be tailored to specific needs. The adaptability of organoid models highlights their capability as a cutting-edge tool for driving therapeutic innovation and advancing healthcare solutions.

Investigating the role of extracellular vesicles in cisplatin-induced toxicity

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Cisplatin is a chemotherapeutic drug that is used to treat solid tumors; however, its use is limited by severe side effects. One of these major side effects is cisplatin-induced ototoxicity (CIO). CIO manifests as permanent hearing loss occurring in over 50% of childhood cancer patients treated with cisplatin. Despite the burden of this adverse reaction, there are very few drugs that have been approved for treating CIO. Previous studies have suggested a role of extracellular vesicles (EVs) in protecting against hair cell death, which may involve certain toll-like receptors, such as toll-like receptor 4 (TLR4). Prior knowledge shows that cisplatin can interact with this pattern recognition receptor (PRR), producing reactive oxygen species (ROS) and pro-inflammatory cytokines. Further work is required to elucidate the potential interactions between cisplatin, TLR4, and EVs and how these interactions may be implicated in protecting against cisplatin- induced toxicity (CIT). In this study we attempted to address this gap in knowledge. To do this we used a human embryonic kidney (HEK) cell line (HEKhTLR4 and HEKnull2 cells) and a murine inner ear cell line (HEI-OC1 cells) to monitor the potential influence of TLR4 on these effects, as well as the implications of these effects in protecting against CIO. Our results showed that conditioned media, as well as EVs isolated from this conditioned media, were protective against CIT. These results highlight potential for the use of EVs to mediate against CIT and suggest a way to combat the prevalence of hearing loss in patients treated with cisplatin.

A mechanistic study of DG9-PMO for Duchenne muscular dystrophy: Improved efficacy and localization in skeletal and cardiac models

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Duchenne muscular dystrophy (DMD) is an X linked condition characterized by progressive muscle wasting, due to mutations in the dystrophin gene that cause the absence of the functional dystrophin protein. Available antisense oligonucleotide-based therapies use the premise of exon skipping in order to restore the reading frame of the dystrophin gene. Phosphorodiamidate morpholino oligomers (PMOs) are one class of molecules capable of exon skipping. These treatments however have limited efficacy in cardiac muscle, and level of delivery to the cytoplasm and nuclei of target cells is variable. Cell-penetrating peptide conjugation can improve systemic delivery of the PMOs. The DG9 peptide recently discovered in our lab required that we deduce the previously unknown mechanisms by which DG9 modifies the efficacy of PMOs. Through the use of immortalized DMD cell lines and murine models with a mutated human DMD gene, both with a deletion of exon 45, we focused on mechanisms of uptake and localization, while also looking at the specific impacts on cardiac function, and overall efficacy in different cell types. Using endocytosis inhibition assays and fluorescent cell fractionation spectrophotometry, we discovered that the DG9-PMO uses several endocytotic uptake pathways to enhance intracellular delivery, and has improved nuclear localization compared to the unconjugated PMO. Murine echocardiograms showed strengthened cardioprotection, while cellular transfection assays featured increased efficacy in myoblasts, myotubes and cardiomyocytes, as demonstrated by increased exon 44-skipped dystrophin transcript levels. In murine models, dystrophin restoration was higher than the restoration levels of another cell penetrating peptide R6G, which was previously in clinical

trials. These improvements were observed without detectable toxicity effects. Evaluations present the DG9 peptide as a promising future therapeutic agent for the treatment of Duchenne Muscular Dystrophy, and may address the concern of progressive reduction of cardiac function that is observed in patients.

Investigating the paradoxical role of mGluR5 in traumatic brain injury

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Characterization of novel, recombinantly produced plant protein kinases

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With plant genomes encoding substantially more protein kinases than humans and other non- photosynthetic eukaryotes, many of these protein kinases continue to remain uncharacterized. Here, we use a combination of Gateway cloning and *Escherichia coli* (*E. coli*) to recombinantly produce a family of novel protein kinases with a His 6 -tag for purification and characterization. We will use immobilized metal affinity chromatography (IMAC) to purify these kinases of interest from *E. coli*, isolating the proteins by exploiting the ability of their His6-tag to specifically bind to certain metal ions. Next, we plan to utilize the purified proteins in vitro to gain insight into their enzymatic activities and potential involvement in plant processes, environmental stress adaptations, homeostasis, and growth and development.

Computer modeling of protein kinase inhibitors for alzheimer's disease

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Alzheimer's Disease (AD) is the leading cause of dementia, with the number of cases projected to rise to 131.5 million by 2050. Hyperphosphorylation of Microtubule-Associated Protein Tau (MAP Tau) and the accumulation of Amyloid-β (Aβ) are significant hallmarks of AD, both processes relying on protein kinases. This study aims to identify multi-kinase inhibitors to disrupt these pathological pathways using computational methods. A curated list of kinases implicated in MAP Tau phosphorylation and AD progression was evaluated, docking simulations were performed with 101 known in vivo/vitro kinase inhibitors against seven selected kinases. Virtual screening, molecular docking, and molecular dynamics simulations were employed to predict optimal multi-kinase inhibitors for potential therapeutic use. Thirteen multi-kinase inhibitors were identified, including nine dual inhibitors, three triple inhibitors, and one octuple inhibitor targeting eight AD-related kinases. The octuple inhibitor demonstrated strong literature support, with potency varying from highly effective for GSK3, CDK5, and CK1 to moderate efficacy for other targets. This study provides promising leads for the development of AD therapeutics, offering a foundation for optimizing multi-kinase inhibitors. Additionally, the results highlight candidates for proteolysis targeting chimeras (PROTACs), a cutting-edge drug design approach.

Adapting higher resolution proximity labeling tools for plant systems

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Mapping protein interactions and subcellular localization is important for understanding the dynamic regulatory networks that govern plant growth, development, and stress responses. Proximity labeling (or Proximity-Dependent Biotinylation) is a

useful technique that enables the identification of protein-protein interactions occurring in close proximity within living cells. This method involves using an engineered enzyme to covalently attach biotin to proteins near the bait protein of interest. The biotin-tagged proteins can then be purified and identified via mass spectrometry. In this project, we are adapting a novel proximity-labeling enzyme into plant-compatible vectors to study plant cells. This new enzyme has been suggested to be smaller and more effective in tagging than predecessor enzymes, with enhanced capabilities for precise spatial labeling and shorter reaction times. Preliminary analyses of plasmid construction include colony PCR and agarose gel electrophoresis, which confirmed the presence of similarly sized bands for both the N-terminal and C-terminal fusions. The next steps involve further cloning to express the construct transiently in *Nicotiana benthamiana*. Final validation will include assessing protein expression, labeling efficiency testing, and performing mass spectrometry analysis to identify labeled proteins. This project would help demonstrate an advanced and powerful tool for plant molecular biology. By incorporating novel proximity labeling technologies into plant systems, we aim to potentially improve the quality and efficiency of studying protein-protein interactions and subcellular proteomics in plants.

Potential treatment for neurotrauma: The antiseizure drug retigabine reduces negative effects

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Inhibition of ROMO1 and ovarian cancer proliferation: A research study

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Of the estimated 3000 Canadian women diagnosed with ovarian cancer in 2024, it is predicted that 2000 women will die from this cancer. Related to this, recent evidence suggests that a mitochondrial protein, reactive oxygen species modulator 1 (ROMO1), is upregulated in cancers of the female reproductive system and promotes aggressive cancer proliferation. ROMO1 appears to promote cancer growth by inducing mild/physiological levels of reactive oxygen species (ROS) in the mitochondria that ultimately drives downstream oncogenic transcription factor activation, notably targeting the cell cycle control protein p27. We have identified three Health Canada approved anti-schizophrenic drugs that may inhibit ROMO1 and therefore prevent downstream oncogene activation. Our project has characterized that three anti-schizophrenic agents from the same drug class have potential anti-tumor effects on cultured ovarian cancer cells. Using two epithelial ovarian cancer cell lines, OVCAR8 and SKOV3, treated with varying concentrations of drug and vehicle control (DMSO) for 24 hours, were used for live-dead cell imaging, immunoblot analysis, and cell counting experiments. Live-dead imaging following the drug treatment period revealed that the three drugs produced significant cell death compared to the vehicle control. Furthermore, immunoblot analysis revealed that p27 protein levels were increased across three biological replicates of increasing drug concentrations, suggesting that the drugs may inhibit the cell cycle through the inhibition of ROMO1. Future experiments will investigate other potential cell death pathways that these drugs may modulate via ROMO1 inhibition. Given that this proof-of-concept study seeks to expand the clinical uses of a Health Canada approved compound, the results of this study could be rapidly translated into a clinical trial and, if positive, accelerate the translation of this drug to improve patient care. Finally, by demonstrating that targeting ROMO1 in cancer cells is safe and effective, we anticipate that future therapies be developed to target ROMO1.

Mechanisms of retinal pigment epithelium degradation in PROMININ-1 deficient frog

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PROMININ-1 (PROM1) variants are associated with human inherited blindness. Prom1-null frogs have dysmorphic photoreceptor outer segments, and they accumulate large deposits of cellular debris in the outer segment layer and disordered

retinal pigment epithelium (RPE) as they age. The origins of these deposits and the mechanism of RPE degradation is unknown. A daily task of the RPE is to phagocytose spent photoreceptor outer segment discs, subjecting them to significant oxidative and metabolic stress over our lifetime. We hypothesized that dysmorphic prom1-null outer segments increase oxidative stress in the RPE, leading to the accumulation of indigestible waste and RPE death. We were also interested in whether microglia were associated with the outer segment layer deposits. We used immunohistochemistry and epifluorescence microscopy to examine three potential contributors to prom1-null associated retinal degeneration: microglia (IB4 isolectin, LAMP1), oxidative stress (E06 anti-oxidized phospholipid antibody, LAMP1), and/or dying RPE (TUNEL). We observed microglial infiltration into the outer segment layer of prom1-null tadpoles. Microglia in prom1-null retinas had a reactive ameboid morphology and upregulated LAMP1 (lysosomal) expression, distinct from microglia observed in wildtype retinas. E06 labeling was apparent in the deposits and in the RPE of prom1-null retinas, compared to wildtype animals, indicating oxidative stress. TUNEL labeling did not indicate any cell nuclei undergoing apoptosis or necrosis. In conclusion, we have evidence of secondary toxic effects of oxidative stress on the RPE and/or microglial infiltration in the subretinal space as contributors to prom1-null associated retinal degeneration. So far, however, we have not seen evidence that there are dead cells in the deposits. These results are significant because these deposits are also very similar to those seen in humans with age-related blindness. Understanding what they are and where they come from will help with the development of future treatments for inherited and age-related blindness.

Unveiling the cage-like structure of Müller glia and retinal pigment epithelium around cone photoreceptors in zebrafish

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Photoreceptors are the primary light-sensing cells of the retina, with morphologies precisely tailored to their function. In particular, a large sensory ending known as the outer segment (OS) is adapted to efficiently capture photons. While prioritizing the phototransduction process, photoreceptors outsource many maintenance tasks to supporting cells - Müller glia (MG) and retinal pigment epithelium (RPE). MG and RPE are considered to be physically separate, with each cell type approaching photoreceptors from an opposite direction. Nonetheless, we recently discovered through confocal microscopy an overlap between MG/RPE apical processes alongside cone photoreceptors in the zebrafish retina. We next used Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) to obtain a more detailed and three-dimensional view of interactions in the outer retina. The imaging revealed a cage-like arrangement of MG and RPE apical processes around the OS of short cones. Further, MG processes feature a defined structure as they extend towards the tip of the OS: initially narrow, the distal region expands to house distinct and organized bundles of actin. RPE processes are less ordered but contain abundant endoplasmic reticulum. Using confocal microscopy, we found that MG/RPE contact is established shortly after the OS develops. In conclusion, our data reveal extensive and unexpected interactions in the outer retina, reshaping our understanding of how photoreceptor homeostasis is supported. Current experiments are investigating conservation across species and how disrupting contacts alters retinal health and function.

Optimization of the in-vitro transcription of engineered self-amplifying RNA for human gene therapy

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Self-amplifying RNA (saRNA) is a new approach for human gene therapy. Deriving from the single-stranded, positive-sense RNA backbone of alphaviruses, it serves as another model for RNA-based therapies. This model gained traction following the success of Pfizer-BioNTech and Moderna's COVID-19 mRNA vaccines. While this success established the foundation for RNA-based therapies, there are still several limitations to this. Such limitations include its short lifespan leading to the

need for more frequent dosing that elicits a strong immune response. saRNA directly addresses these limitations of mRNA by requiring less frequent dosing because it encodes its own replication machinery that prolongs its effect. However, given the emergence of saRNA as a technology, there is a lot more research to be done regarding its immunogenicity. We are seeking to establish saRNA as the new standard – translating advancements made in mRNA over to saRNA. To start, we are applying known optimizations in mRNA's in-vitro transcription, precisely the effects of different RNA polymerases, reaction temperatures, base modifications, 5'-methyl G capping, and poly (A) tailing. The resultant product's immunogenicity will be measured using toll-like receptor-3, tumor necrosis factor alpha, and the interferon responses in the human monocytic cell line, THP-1. The production of high-yield, lowly immunogenic saRNA is vital for its transition to preclinical applications of this technology.

Characterizing dynamic changes in pre-synaptic proteome of memory circuits during learning and forgetting: A research study

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Learning and forgetting are essential for cognition, behavior, and adaptation across the animal kingdom. Much of our understanding comes from *Drosophila melanogaster* whose simple nervous system provides a powerful model for studying these processes. Memory formation is orchestrated by intricate neural circuits and molecular mechanisms, with synaptic plasticity at its core. Recently, the role of synaptic proteins has gained considerable attention with the presynaptic proteome emerging as a critical player in modulating cognitive processes. Among them Bruchpilot (BRP) is crucial for organizing active zones and maintaining synaptic integrity which is fundamental for learning and memory. Previous research has shown that homeostatic presynaptic structural remodeling in Mushroom body neurons (*Drosophila's memory circuits*) is vital for memory formation and consolidation. However, we lack a comprehensive understanding of how dynamic changes of synaptic proteome that underlie memory processes and behavior. To address this, I have developed a neuron-specific, optogenetic, BRPs-fused LOV-TurboID labelling system, which enables temporally controlled labelling of pre-synaptic proteomes in defined memory circuits. This tool will allow us to track protein dynamics at presynaptic active zones as flies form and forget memories. So far, we have optimized the conditions for this system and aim to capture and compare proteomes before and after learning and forgetting to identify key proteins serving as either memory substrates or regulators. Using RNAi-based gene disruption memory screen I will narrow down potential key players in memory and forgetting pathways in the adult *Drosophila* brain. This research will significantly broaden our understanding of memory functions, as well as provide tools for studying synaptic proteome dynamics in other neural processes and circuits. Given the conservation of genetic pathways, insights from Drosophila may shed light on active forgetting in humans, a process essential for cognitive flexibility and preventing information overload and could have broader implications for developing treatments for memory-related disorders.

Characterizing lodgepole pine candidate mountain pine beetle host quality genes

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Lodgepole pine (*Pinus contorta* var. *latifolia*) is the co-evolutionary host of mountain pine beetle (MPB; *Dendroctonus ponderosae*), a bark beetle that has devastated millions of hectares of pine forests in western Canada. Even following severe MPB outbreaks, individual trees may survive or evade mass attack by MPB. Using population genomics, the Cooke Lab has identified a genetic fingerprint for this multigenic trait that distinguishes the progeny of MPB survivor trees from the progeny of MPB-killed trees. We are now investigating underlying biological mechanisms for this resilient phenotype. As part of a larger study, we are conducting functional analyses of a subset of genes from the genetic fingerprint to determine if any of these genes impact host quality of lodgepole pine to MPB. Host quality encompasses lodgepole pine traits that affect MPB host selection, colonization, and reproduction. We have cloned full-length cDNAs of these genes and identified putative functions based on sequence similarity to functionally characterized genes in angiosperm species. These putative functions include growth regulation, abiotic stress management, and pathogen defense signalling. Quantitative RT-PCR of a subset of these genes revealed that some of these genes are differentially expressed in mature lodgepole pine in response to MPB attack and *Grosmannia clavigera*, a fungal symbiont of MPB and a lodgepole pine pathogen. Currently, we are conducting biochemical complementation assays in *Arabidopsis thaliana* to provide further clues on gene function as we are unable to

generate transgenic pine trees to complete these assays. Knockout Arabidopsis plants for the putative ortholog have been grown and transformed with the pine ortholog cDNA. These will be subjected to pathogen challenge with *Botrytis sp.* to assess if the pine cDNA may restore defense. Through characterizing these genes, we may potentially uncover novel genes and associated biological mechanisms that confer lodgepole pine resilience to MPB.

GM1 brain ganglioside as potential therapeutic for post-traumatic seizures after traumatic brain injury

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Vein patterning by auxin production: A research study

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Auxin, or indole-3-acetic acid (IAA) is a hormone commonly associated with plant development and expression. Biosynthesis of auxin is usually tryptophan dependent and is catalyzed by aminotransferases TAR1, TAR2 and TAA1 to an auxin intermediate indole-3-pyruvic acid. This is a reversible reaction by the enzyme VAS1. Other pathways for auxin biosynthesis are only produced in stress conditions or specific to Brassicaceae. It is unclear how auxin is involved in vein patterning and how it is distributed in the leaf. We hypothesized that by identifying the distribution of TAR, TAA and VAS genes in the leaf we could predict how the amount of indole-3-pyruvic acid would affect the amount of auxin. This would give better insight into how auxin is mediated for vein patterning. A better understanding of how these genes interact will further the plant genetics field and the investigation into leaf vein patterning.

Investigating the MlaYZ system that maintains outer membrane lipid asymmetry in *Pseudomonas aeruginosa*: A research study

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The outer membrane (OM) of Gram-negative bacteria displays an asymmetric bilayer of glycerophospholipids (PLs) distributed in the inner leaflet and lipopolysaccharide (LPS) in the outer leaflet. Maintaining this lipid asymmetry is crucial to the cell's integrity and functionality as it provides a barrier against toxic environmental stressors such as antibiotics. How this lipid asymmetry is maintained has primarily been investigated within *Escherichia coli*, resulting in a poor understanding of the mechanisms involved within other bacterial species. Consequently, this work will investigate the bacteria *Pseudomonas aeruginosa*. Previous research recently identified new Mla proteins, MlaY and MlaZ, that are thought to be involved in maintaining lipid asymmetry. More specifically, it is believed that MlaZ removes PLs from the outer leaflet and transfers them to MlaY to be degraded. However, how PLs are transferred from MlaZ to MlaY remains unknown. We hypothesize PLs are removed from the outer leaflet through direct protein-protein interaction between MlaZ and MlaY. To visualize whether an interaction is occurring, we must tag the mlaYZ operon. Research by Randi Guest, a professor at MacEwan University, has created a functionally tagged mlaZ gene; however, tagging the mlaY gene has presented difficulty (unpublished). This is presumably due to the ribosome binding site of mlaZ being located in the C-terminus of mlaY. We will design an operon such that the ribosome binding site of mlaZ will succeed mlaY, to allow the addition of a successful β-lactamase tag on the C-terminus of mlaY. This will grant sufficient machinery to investigate an interaction between MlaZ and MlaY using pBPA photocrosslinking and western blot analysis. This research will further the current understanding of how lipid asymmetry is

maintained within P.aeruginosa to provide insight into our understanding of how the OM protects Gram-negative bacteria from Antibiotics.

Poster Presentations in Microbiology

Salmonella Typhi regulates the relative expression of typhoid toxin subunits via a network of interacting two-component-systems sensing diverse environmental cues

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Salmonella enterica serovar Typhi is a human-specific facultative intracellular pathogen that causes typhoid fever, a life-threatening disease endemic in developing countries. *S.* Typhi produces typhoid toxin, a unique AB-type toxin that appears to contribute to the acute symptoms of typhoid fever. Typhoid toxin is found in two forms, distinguished by their binding subunits, PltC or PltB. Typhoid toxins are produced by intracellular *S.* Typhi, and expression of the two binding subunits appears to be controlled by different, interacting two-component systems (TCS) responding to various environmental cues. Here, we examine how *pltC* expression is regulated via the two-component system SsrAB using beta-galactosidase assays and *lacZ* reporter strains, as well as Western blots. We demonstrate that SsrAB is necessary for *pltC* expression, but that the global two-component systems PhoPQ and EnvZ/OmpR are additionally required under some environmental conditions. We aim to unravel the complexities of *pltC* gene regulation and elucidate the roles of two-component systems in typhoid toxin expression. This study has important implications for developing novel therapeutics for Typhoid fever, especially as the incidence of multi-drug-resistant *S.* Typhi rises.

The microbiome of gills from coastal crab species provides clues for invasive species success

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Carcinus maenas, the European green shore crab, is a successful invasive species. Consequently, since the 1990s, the establishment of *C. maenas* in Pacific coastal waters has resulted in critical ecological impacts on both ecosystems and native crab species populations. *C. maenas* has a broad physiological tolerance and thrives under fluctuating conditions of salinity, oxygen and nutrients. The gill and its associated microbiome are at the interface of the crab environment and may hold clues to their ecological success. Gill microbiomes hold a diverse set of microbes that can augment animal immunity, respiration and removal of nitrogenous waste. This study compared the posterior gill microbiomes for coastal crab species from the Pacific west coast: *Metacarcinus magister*, *Metacarcinus gracilis*, and *Cancer productus*, which are native to this ecosystem, and the invasive *C. maenas*. Additionally, we included the gill microbiome of *C. maenas* from its native European habitat. All of the gill microbiomes contained *Robiginitomaculum sp.*, *Pseudahrensia sp.*, *Rhodopirellula sp.*, *Sulfitobacter sp.*, *Altereythrobacter sp.*, and *Bdellovibrio sp.* However, the complete communities of gill microbiomes from the three native crab species were significantly different than those from the two *C. maenas* microbiomes ($p<0.01$). The gill microbiomes of the two *C. maenas* populations were not statistically different. *Nitrosomonas sp.* which are ammonia-oxidizing bacteria were detected in the *C. maenas*, *C. productus*, and *M. gracilis* microbiomes, but only *Nitrospirota sp.* which are complete ammonia oxidizers were present in the *C. maenas* microbiomes. The planctomycete *Feurista sp.* was only present in the *C. maenas* microbiomes at high abundance (42.9±12.4%) in the Pacific coastal specimens and at lower abundance (5.84±2.55%) in the European specimens. These differences in gill microbiome composition provide clues for the success of *C. maenas* over native crab species.

Characterization of Bcc phage DC1's receptor

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Bacteriophage (or phage) therapy provides an alternative therapeutic to antibiotics, through the use of bacteria-specific viruses, for the targeted killing of infection-causing bacteria. The *Burkholderia cepacia* complex (Bcc) are an agriculturally

and clinically relevant group of highly antibiotic-resistant bacteria that can cause damaging crop losses, notably to onions, and serious, potentially lethal, infection in those with cystic fibrosis. Bcc's characteristic antibiotic resistance, as well as clinical and agricultural relevance, makes phage therapy a promising route for treatment development. DC1 is a myoviridae phage that has been found to infect members of the Bcc, thus showing potential as a candidate for a phage treatment provided its receptor is identified. Currently, work is being done to identify DC1's true primary receptor with previous studies indicating that it is potentially related to the type II secretion system and glycosyltransferases. Here, further work is being conducted to identify the receptor through the generation and genetic analysis of spontaneous DC1 resistant mutants in 4 different members of the Bcc. Mutations in glycosyltransferase proteins have once again been associated with conferring this phenotype and thus efforts to understand these proteins' role in phage adherence are being undertaken. A large frameshift mutation was also identified in the tolA gene that is thought to be a potential candidate for phage binding; however, further work must be done to confirm if this is true.

Investigating novel "hybrid" AB5 toxins and their implications in toxin evolution and therapeutics

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AB5 toxins are secreted protein complexes that are important virulence factors for many bacterial pathogens. They comprise of an active "A" subunit that manipulates host cell biology and a pentamer ring of delivery "B" subunits that bind host cell receptors to mediate toxin uptake and trafficking. This common structure suggests that all AB5 toxins are evolutionarily related, and yet there are substantial differences between AB5 toxin families in their enzymatic activities and target receptors. The Fowler lab recently discovered two novel "hybrid" toxins encoded in rare *Salmonella enterica* strains consisting of the A subunit from one family of AB5 toxins with B subunits from a different family. These toxins are genuine AB5 toxins; they have been shown to assemble canonically and elicit toxicity in cultured human cells. This phenomenon nods to a mechanism of AB5 toxin evolution; the shared assembly architecture allows subunits of distinct toxin families to combine to form novel toxins. It also opens the door to engineering new combinations of toxin activities with different cell-targeting properties for therapeutic purposes. This project involves cloning and purifying toxins with different A/B subunit combinations and investigating their ability to form stable complexes as well as their effects on cultured human cell lines. The knowledge gained from this project will contribute to our understanding of bacterial toxin evolution and will play a role in the emerging field of AB-toxin-based therapeutics.

Optimization of glycosylation pathway to improve human therapeutic proteins

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O-glycosylation occurs when sugars are post-translationally attached to specific serine or threonine residues on proteins. These sugars, called glycans, have a variety of cellular functions including cell-to-cell communication, protein folding and controlling protein degradation. One of our labs' main focus is making O-glycosylation operons that contain all of the components required to glycosylate proteins in bacteria. Using these operons we are increasing glycosylation of various therapeutic human proteins, such as cytokines. By increasing glycosylation this will help increase their serum half-life, so they can survive in the body longer. This project focuses on the second step of the glycosylation pathway, adding a galactose molecule onto a N-acetylgalactosamine molecule to create the core 1 glycan structure. This important step is currently done in the lab using a Campylobacter jejuni enzyme, which works well, but struggles with heavily glycosylated areas, such as the mucin-like domain we often work with. To characterize other β-1,3-galactosyltransferases the reaction conditions, temperature, substrate concentrations and co-factors were optimized. While characterizing bacterial galactosyltransferases, from *Burkholderia cenocepacia* and *Bacillus cereus*, it was discovered that they did not show any enzymatic activity. Thus the focus moved on to various eukaryotic enzymes, from *Drosophila melanogaster* and *Biomphalaria glabrata*. Characterization continues to find the best possible galactosyltransferase to replace the current enzyme, which will help optimize the glycosylation pathway.

Sending mixed signals: Investigating the crosstalk and evolution of two-component systems in *Salmonella*

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Salmonella enterica is an enteric bacterial pathogen with a unique ability: it can persist within the cells of an animal host, effectively hiding from the host's immune system. It produces sensor proteins to detect changes in its intracellular environment during infection, and cognate regulator proteins that control gene expression in response to the environment. Both proteins increase the efficiency of *S. enterica*'s attack. The RstAB system is of particular interest, where RstB is the sensor protein and RstA is the regulator protein. *S. enterica* demonstrates perplexing, uncommon interactions between its sensor and regulator proteins: while RstA/RstB interact in predictable ways as partners in related intestinal pathogens like *Escherichia coli*, recent research has found that RstA does not require RstB to function in *S. enterica*. Furthermore, we have found that the rstA and rstB genes are separated by coding sequences in *S. enterica*, but not in *E. coli* and other related bacteria. This study aims to utilize DNA mutations and sequencing, as well as protein analysis, to investigate the nature of the functionally detached relationship between RstA and RstB in *S. enterica*. Potential other factors and sensor proteins that could be influencing these interactions will also be explored. The results of this research can play a vital role in understanding the evolution of *S. enterica* and the nature of its intracellular pathogen-host interactions.

Characterization of the exopolysaccharide depolymerase of *Erwinia amylovora* phage AW3

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Erwinia amylovora is a Gram-negative bacterium, and is the causative agent of the disease fire blight in Rosaceous plants. As is true of most bacteria, the antimicrobial resistance (AMR) profile of *E. amylovora* continues to increase each year. Additionally, a critical part of the infection cycle of *E. amylovora* is the formation of a strong biofilm in the vascular tissue of plants, further increasing its innate AMR. For these reasons, *E. amylovora* is a strong candidate for bacteriophage therapy. Bacteriophages (or phages) are viruses which infect and lyse bacterial cells. They have many advantages over conventional antimicrobials, and notably do not drive widespread AMR profiles. Some phages may also possess proteins which confer specific advantages to their use. Notably, exopolysaccharide (EPS) depolymerases (DPs) degrade those extracellular sugars which comprise biofilms, and phages which possess EPS DPs are especially effective against *E. amylovora*. A phage possessing an EPS DP specific to *E. amylovora*, named AW3, was previously isolated. My objective has focused on the cloning and expression of the EPS DP of phage AW3 in an *E. coli* system, and to test its efficacy as a stand alone treatment for the biofilms of *E. amylovora*. The DP has been shown to be effective in crude *E. coli* lysate, and does not require protein purification. Spotting of this EPS DP lysate has been shown to clear the EPS of *E. amylovora* on solid media, and is anticipated to reduce the level of EPS in a liquid culture. The AW3 DP efficacy in liquid will be assessed using an EPS precipitation assay to quantify the level of intact EPS following DP treatment. It is hoped that the results of this research will have significance for treating agricultural *E. amylovora* infections, as without a proper biofilm formation *E. amylovora* demonstrates greatly decreased virulence.

Optimizing industrial viability of *Methylomicrobium album* BG8 through adaptive evolution to low pH conditions for increased acid tolerance

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Methane is a major driver of climate change, with 28 times the warming potential of carbon dioxide over a 100-year timescale. However, the natural biological processes of methanotrophs offer a viable means of mitigation. This diverse cohort of microorganisms utilize methane as their sole carbon source, converting this harmful pollutant into high-value products such as biopolymers and biofuels. Among these methanotrophs, *Methylomicrobium album* BG8 is of particular interest for industrial applications due to its rapid growth rate, high yields, and adaptability to varying nutrient conditions. However, while methanotrophs thrive under controlled laboratory conditions, industrial-scale cultivation presents challenges. Temperature fluctuations, pH variations, and inhibitory byproducts often reduce growth, yields, and robustness. Low pH in

particular, is problematic for the neutrophilic *M. album* BG8. During fermentation, organic acids are often produced, causing metabolic strain that slows growth and diminishes product yield. This research employs adaptive evolution and sequential passaging to develop a strain of *M. album* BG8 capable of thriving under low-pH conditions, optimizing it for industrial bioproduction. Adaptive evolution is an effective strategy for inducing advantageous mutations in fast-growing microbes. By gradually lowering the pH over successive generations, this approach produced a strain of *M. album* BG8 capable of growing in a pH 4.0 buffer. Its stability was confirmed by returning it to neutral conditions, then re-exposing it to low-pH conditions, where it maintained comparable growth. To elucidate the adaptation mechanisms, whole-genome sequencing, RNA sequence analysis and metabolomics will be conducted comparing the parental strain of *M. album* BG8 with the low-pH adapted strain. By overcoming the pH limitation that hinders industrial scalability, this research aims to develop a low-pH adapted strain of *M. album* BG8 more optimized for industrial bioproduction. The findings will also provide insights into the mechanisms of acid stress adaptation.

Soil microbial necromass contribution in northern temperate grasslands

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Grasslands cover 25% of the terrestrial surface globally and hold 30% of global soil carbon. Livestock grazing management practices, including stocking rates and grazing systems, can alter the amount of carbon held in grassland soils, potentially altering the effects of climate change. Microbial necromass can contribute up to 50% of the soil organic carbon pool and is therefore an important component of soil carbon sequestration. However, there is limited information on necromass attributes in northern temperate grasslands. The aim of this study is to assess the effects of grazing management and environmental controls on grassland soil necromass in temperate grasslands of western Canada. Soil samples were extracted from two soil depths (0-15 cm and 15-30 cm) at 48 grasslands across Saskatchewan with varying stocking rates and grazing systems. Amino sugar biomarkers (glucosamine and muramic acid) will be quantified using High-performance liquid chromatography (HPLC) to measure necromass. Results are expected to indicate how different grazing systems (e.g., continuous, vs simple rotational, vs adaptive multi-paddock) and associated management metrics (e.g., stocking rate, stocking density and rest period) affect soil microbial necromass. Additionally, relationships between climatic factors (e.g. mean annual rainfall) and soil properties (e.g. soil pH, particulate organic carbon, mineral-associated organic carbon and soil necromass) will be explored. The results of this study will provide valuable information characterizing necromass in grassland soil carbon pools and evaluate necromass sensitivity to specific grazing management practices.

Phages of *Burkholderia cepacia* complex clinical isolates

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The *Burkholderia cepacia* complex (Bcc) is a group of Gram-negative opportunistic pathogens that can cause potentially fatal infections, particularly in patients with Cystic Fibrosis and Chronic Granulomatous Disease. Due to the extreme antibiotic resistance exhibited by the Bcc, these infections are often difficult to clear with conventional antimicrobials. This necessitates the development of alternative therapies, such as "phage therapy" (PT). PT is the use of bacterial viruses, or phages, for the treatment of bacterial infections. PT has shown tremendous promise, but host specificity presents a challenge for treating Bcc, which has great genetic diversity. The objective of this study is to increase the number of phages available for Bcc PT. This was done through the use of Mitomycin C, which induces prophages within clinical isolates to enter into the lytic cycle. Two novel phages, LH1 and LH2, have been isolated, purified, and sequenced from two Bcc isolates from a single patient over several years. LH1 has a genome size of 29,624 base pairs, with lytic activity against 24 Bcc strains. LH2 has a genome size of 42,403 base pairs, with lytic activity against 11 Bcc strains. In conclusion, two novel Bcc-specific phages have been isolated and partially characterized. Further studies to examine their therapeutic potential are underway.

Phage and *Burkholderia gladioli* isolation from soil and *Gladiolus* bulbs

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Bacteriophage (phage) therapy is an emerging area of research that can be used as an alternative method of treatment for antibiotic resistant bacteria. Phage therapy is a promising substitute for antibiotics due to its specific nature, and for its ability to replicate at the site of infection. *Burkholderia gladioli* is an opportunistic bacterial infection that is often found in immunocompromised individuals, particularly cystic fibrosis patients, and is commonly isolated from rice, corn, orchids, onions, mushrooms, soil and the flower genus *Gladiolus*. *B. gladioli* is resistant to most traditional antibiotics, making it a possible candidate for phage therapy. Isolation of novel strains of bacteriophage from soil and *Gladiolus* bulbs can be performed to find a phage that is compatible with *B. gladioli* infections that previously could not be treated. We have performed spot testing of soil and plant filtered lysates from 58 different samples on overlays of 12 different *B. gladioli* bacterial strains, in order to obtain phage plaques, which is indicative of potentially new phages that can then be purified and characterized. This approach has led to 11 possible novel phages identified from both soil and *Gladiolus* bulbs, but further research is required to determine their identity and to purify them. The isolation of previously undiscovered *B. gladioli* strains can occur through the use of selective media, such as *Burkholderia cepacia* Selective Agar (BCSA), and confirmed using PCR and Sanger sequencing. 58 *Gladiolus* bulbs were tested to identify 2 new putative *B. gladioli* strains. The isolation of these new *B. gladioli* phages and strains will increase the size of our libraries, and permit improved opportunity or isolating and characterizing additional *B. gladioli* phages for use in phage therapy applications.

Poster Presentations in Physiology and Development

Sex, drugs, and social cues: Examining zebrafish anxiety in a modified novel tank dive test

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Zebrafish (*Danio rerio*) are an established model for studying anxiety-like behaviours due to their sensitivity to environmental stressors and pharmacological manipulations. However, there is significant variability in zebrafish behaviour in response to anxiety-inducing drugs, influenced by factors such as tank dimensions, genetic differences, and social environments. This study investigated the behavioural effects of acute exposure to anxiety-modulating compounds using a modified novel tank dive test with taller and narrower dimensions designed to isolate 2D behavioural parameters and optimize vertical exploration. Zebrafish were administered anxiogenic agents chondroitin sulfate (0.1 g/L) and beta-carboline (B-CBL, 10 μM), as well as anxiolytic agents delta-9-tetrahydrocannabinol (THC, 1.0 mg/mL), ethanol (0.8%), and beta-caryophyllene (BCP, 1.0%). Behavioral parameters, including geotaxis, swimming velocity, and immobility, were assessed to determine anxiety-like and locomotor responses. Both anxiety-increasing and anxiety-decreasing agents increased bottom-dwelling behaviour, indicating that the modified tank dimensions influenced zebrafish responses. Chondroitin sulfate did not affect time in zones but uniquely caused a sex-specific increase in male swimming velocity. Both ethanol and THC reduced swimming velocity and increased immobility, consistent with their sedative properties, however, BCP and B-CBL had no effect on locomotion. To explore social buffering effects, the study also tested whether the presence of drug-treated fish altered the behaviour of observer fish. No significant behavioural changes were observed in the reverse social buffering paradigm, suggesting that social influence in this context does not extend to observer effects. These findings emphasize the importance of tank dimensions in assessing anxiety-like behaviours and highlight the dynamic interaction of pharmacological, environmental, and biological factors in zebrafish research.

Investigating the role of ketones in regulating inflammation

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Liver-derived ketones are recognized for their role as metabolic substrates that provide vital energy (ATP) for multiple organs during fasting and reduced carbohydrate intake. However, beta-hydroxybutyrate (βOHB), the primary ketone body, has also shown anti-inflammatory properties. Recently, βOHB supplementation has been demonstrated as an effective treatment in several rodent models of inflammatory diseases, such as sepsis and heart failure. These findings raise the question of whether ketones are a necessary part of the inflammatory response potentially acting as a safeguard against excessive activation. To address this, we generated a line of mice with reduced circulating ketone levels. This was achieved by knocking out the rate- limiting ketogenic enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2). This was accomplished via the flox-Cre system, with Cre recombinase delivered through an adeno- associated virus under the control of the albumin promoter, achieving hepatocyte-specific deletion of HMGCS2. We then exposed these mice, along with wild-type control mice, to the inflammatory stimulus lipopolysaccharides (LPS). After 24 hours, we assessed the overall health of the mice using a modified frailty index and collected plasma and vital organs for later comparison of tissue mRNA expression of the pro-inflammatory cytokines Il-1b, Il-6, and Tnf-a. Surprisingly, we found that a reduction in circulating ketone levels did not significantly impact the inflammatory response to LPS, evidenced by no change in behaviour scores nor transcript levels of the three pro-inflammatory cytokines. Furthermore, we did not find any significant differences in plasma levels of alanine aminotransferase, aspartate aminotransferase, or blood urea nitrogen, markers of liver and kidney function. Together, these findings suggest that ketones may not be as crucial to the inflammatory response, or that other mechanisms compensate for the loss of ketones. Given that ketone supplementation has been shown to treat inflammatory diseases, further investigation into the role that ketones play in regulating inflammation is warranted.

The role of acidic sphingomyelinase in Parkinson's disease

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Acidic sphingomyelinase (ASMase) converts sphingomyelin into ceramide and phosphorylcholine on membranes. Although increased ASMase levels have been observed in various neurological disorders, their role in Parkinson's Disease remains unclear. Parkinson's disease mutations in a gene called GBA1 leads to a loss of glucocerebrosidase activity, which can be mimicked by the inhibitor conduritol-β-epoxide (CBE). Our lab discovered an increase in ectosomes, extracellular vesicles shedding from the plasma membrane, containing misfolded alpha-synuclein. We also found a decrease in sphingomyelin and an increase in ceramide with CBE treatment. Given ceramides' role in the generation of extracellular vesicles, we hypothesize that increased ASMase activity is responsible for stimulating ectosomes in Parkinson's disease. To model Parkinson's disease, we treated primary cortical neurons with CBE. Using an Amplex™ Red Sphingomyelinase Assay Kit, we observed an increase in ASMase activity in primary cortical neurons. Next we performed live-cell microscopy on the ectosomes labeled with the plasma membrane marker mVenus-CAAX. We discovered SMase inhibitors reduced ectosome shedding induced by CBE. These findings support a role for increased ASMase activity in stimulating ectosome formation in Parkinson's disease. Future directions will focus on elucidating whether ASMase is recruited to the plasma membrane in primary cortical neurons treated with CBE and dopaminergic neurons from Parkinson's disease patients.

Conditioned to crave: Investigating ethanol seeking in zebrafish using colour preference

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Conditioned colour preference is an experimental task that can be used to study addiction via investigation of drug-seeking behaviours in zebrafish (*Danio rerio*). Using coloured visual cues is advantageous due to ease of replication, relevance to

natural preferences, and test simplicity. This study used unbiased stimuli to test ethanol-exposed zebrafish at different drug withdrawal durations. Zebrafish were dosed in 0.8 % vol/vol ethanol or with habitat water (controls) while being exposed to one of two colours (yellow or red) for 1 hour per day over a period of 21 days. Fish were tested following their set withdrawal period (2-, 4-, or 8-days later) in a two-way yellow and red maze for 10 minutes. Motion-tracking software was used to track their movements and quantify duration of time spent in zones. Red conditioned fish showed a main effect of ethanol and a significant increase in time spent in red compared to time in yellow at 8-days of withdrawal but not at 2-days or 4-days of withdrawal. Yellow conditioned fish showed a main effect of withdrawal but did not show any zone preference during the 2-, 4-, or 8-days of withdrawal. The significant preference for red at 8-days of withdrawal, but not at yellow, suggests that red may be a more salient pairing with ethanol's effects or there could be possible differences in colour perception and preference due to withdrawal. Conditioned colour testing can be used to examine seeking behaviour and is beneficial in a behavioural battery of tests to examine drug addiction.

Sepsis alters renal and hepatic mitochondrial respiration

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Sepsis is estimated to underlie 20% of all global deaths. Males appear to be more susceptible to sepsis-induced organ injury than females, although the mechanisms underlying these sex-differences are unclear. Here, we studied the impact of fecal slurry-induced peritonitis (FIP) on mitochondrial function in male and female mice to gain insight into the sex-specific metabolic consequences of sepsis. C57BL/6N mice were injected with fecal slurry (FS, 0.55 mg/g) or vehicle. Buprenorphine (0.5 mg/kg, at 4h), Ringer's Lactate (15 mL/kg, at 12h) and Imipenem (25 mg/kg, at 12h) were administered post FIP-induction. At 4h, 12h, and 24h post-FIP, mice were euthanized, and tissues were collected; liver and kidney homogenates were assessed for mitochondrial function by high resolution respirometry. Biochemical assays were used to assess mitochondrial content and oxidative stress (8-oxo-dG). FIP caused: (1) no changes in renal mitochondrial content, but reduced content in the liver of males (P=0.012), but not females (P=0.095) by 24h; (2) reduced respiration through complex(C)II in the renal medulla of males (P=0.002), but not females (P=0.75), as early as 4h post-FIP; (3) increased respiration through CI in kidneys of both males (P=0.03) and females (P=0.006) by 12h post-FIP, but reduced respiration through CI in the liver of males (P=0.031) and females (P=0.0005) by 12h post-FIP; (4) increased liver 8-oxo-dG in females (P=0.01), but not males (P=0.35) by 12h post-FIP. This work may provide insights into the sex differences in susceptibility to sepsis-induced organ dysfunction.

The effects of copper toxicity in *Daphnia pulex* under Arctic relevant water chemistries

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Living under fire: Comparing physiological responses of two freshwater crustaceans to Australian bushfire ash

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Wildfire prevalence has increased drastically around the world, exacerbated by climate change, resulting in devastation to ecosystems and the organisms therewithin. Australian bushfires are of particular concern given Australia's extensive biodiversity and numerous endemic species. While terrestrial impacts are often the primary concern for fires, the aquatic environment is also at risk. Ash from fires can directly descend into aquatic environments or be introduced as runoff, and which can release heavy metals and contaminants such as polyaromatic hydrocarbons. The physiological effects of bushfire ash on aquatic animals are not well understood, nor is their subsequent coping and recovery ability. Two native Australian freshwater species, the long- armed shrimp (*Macrobrachium australiense*) and the blue yabby (*Cherax destructor*), were

selected to examine their physiological responses following exposure to Australian bushfire ash. We found that the shrimp were significantly more sensitive to ash than the crayfish. Metabolic oxygen consumption was measured, and the enzymatic activity of lactate dehydrogenase was assessed in three tissue types (gills, hepatopancreas and muscle). Thus, insight is gained into the species method of cellular respiration. The sensitivity and effects of ash exposure are important to adequately evaluate the effects of bushfires. The ability for animals to contend with ash exposure will allow for identification of critical areas where monitoring and conservation efforts should be made following bushfires.

The toxic effects of nickel in the aquatic Arctic environment

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The Arctic environment is rich with resources needed for green technologies - in particular critical minerals like Nickel (Ni). The risk, however, is that resource extraction is particularly environmentally traumatic, and could cause metal contamination in this unique environment. The characteristics of an Arctic environment are niche including low temperatures, decreased water hardness, fluctuating water pH, and varying day-night cycles. Current risk assessment models for resource extraction are based on temperate species/environments, unrelated to Arctic environments. Therefore, this study aims to provide a baseline for the development of applicable risk assessment models and highlight the importance of physiochemical characteristics in the Arctic with respect to organism physiology and toxicology. This study will use the freshwater species *Daphnia pulex* to evaluate the effects of acute (48 h) and chronic (21 d) Ni exposure, under Arctic relevant hardness and pH. Less than 24-hour old neonates acclimated to three environmental pHs (7.0, 7.6, and 8.2) will be exposed to a range of Ni concentrations where the median lethal concentration (LC50) and median effect concentration (EC50) will be determined and compared to studies performed under conventional water hardness and pH. Overall, this research will address the knowledge gaps regarding the physiological effects of Ni exposure under Arctic conditions, which will provide a baseline for risk assessment models, as well as promote sustainable industrial practices.

Exploring virtual reality for better peer connections in healthcare

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Peer support programs provide an opportunity for individuals with spinal cord injuries (SCI) to communicate and consult with experienced peers in a safe environment. This study explored using spatial meeting technologies to enhance virtual peer networks for SCI patients. A mixed method, a comparative, cross-over cohort design was employed to evaluate the effectiveness of spatial meeting technologies against traditional video conferencing systems, like Zoom. Participants completed two phases of weekly peer support sessions: one using Zoom and the other using spatial meeting environments in Virtual Reality (VR). Each session was facilitated by trained coordinators, with VR providing customizable, immersive settings to foster meaningful interaction between peers. Participants received VR headsets for the study and underwent individualized training to ensure familiarity with the equipment. After completing eight peer support sessions (4 via Zoom, 4 via VR), participants were interviewed via Zoom and were asked to reflect on their experience. Interviews were analyzed using a thematic qualitative method, and four themes were recognized: Building Deeper Connections, where VR facilitated stronger interpersonal bonds; Immersive Engagement, with VR reducing distractions and encouraging focus; Elevated Mood and Empowerment, as participants experienced greater confidence and positivity; and Facilitating Natural Interactions, where VR offered a more fluid and dynamic conversational environment compared to Zoom. These results demonstrate the potential of VR to improve the quality and depth of peer support for individuals with SCI. By fostering emotional well-being and enhancing interpersonal connections, VR presents a promising avenue for expanding access to effective peer support in healthcare.

Poster Presentations in Immunology and Infection

Exploring recurrent and metastatic breast cancer treatment with oncolytic vaccinia virus in vitro

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Cancer is the second leading cause of death globally, with nearly 1 in 6 deaths attributable to cancer. After decades of research, the fight continues to develop more effective therapeutics. One promising new therapy, oncolytic viruses, uses viruses that infect and promote tumor cell destruction, stimulating an immune response against cancer. In this way, it is a therapeutic cancer vaccine. Previously, a vaccine targeting breast cancer produced a robust immune response and effectively eliminated tumors in most mice, but other treatment outcomes were observed such as recurrence and metastasis. The project objective was to examine factors influencing vaccine treatment outcomes. Our hypotheses were tested by sequencing recurrent and metastatic breast cancer cells, testing oncolytic virus infectivity in vitro, and completing cell growth curves. We found that there were no mutations in the metastatic or recurrent tumor cells in the gene encoding the peptide that is targeted by the vaccine. We found that the oncolytic virus trended toward reduced infectivity in the metastatic and recurrent tumor cells, but this difference was not statistically significant. The metastatic and recurrent tumor cells also had significantly increased growth rates compared to the cells that seeded the tumors. Our results show that during tumor treatment with the oncolytic vaccinia virus, the anti-tumoral immune response may not be sufficient to eliminate tumors, allowing for recurrence. These observations show that to increase the efficacy of our oncolytic vaccinia virus in recurrent and metastatic cancer, vaccines may need to be administered in higher doses to overcome cell resistance to infection.

Expression of ST8Sia6

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Glycans, or carbohydrates, linked to cell surface proteins are one of the most significant and complex biological molecules that play a role in human health and disease. Sialic acid (Sia) is found at the outermost position of glycan chains, facilitating a key role in cell-to-cell signaling and ligand-receptor interactions. PolySia is a long homopolymer of α2,8-linked Sias that differs from widely expressed α2,3- and α2,6- linked monoSia. There is considerable knowledge surrounding the critical roles of polySia in cell migration, and more recently, the relationship between sialylation and immune attenuation. However, one type of Sia that is often overlooked is oligoSia. Due to the lack of tools, such as commercial antibodies, there is very limited data on this glycan. This project aims to shed light on the mystery that is oligoSia by investigating the biosynthesis of oligosialylated proteins. α2,8-sialyltransferases (ST8Sia) catalyze the transfer of Sias from sugar donor molecules to substrate proteins. ST8Sia3 and ST8Sia6 are likely to be responsible for synthesizing oligoSia. While ST8Sia3 is mostly limited to the nervous system, ST8Sia6 mRNA is more broadly expressed and increases in many cancers, including breast cancer and melanoma, suggesting a potential role in cancer progression. However, the endogenous protein targets of ST8Sia6 remain unknown. We investigated ST8Sia6 in a bacterial system and human embryonic kidney cells. While the expression in bacteria was strong, we observed little to no activity on test proteins. Elucidating the products of ST8Sia6 may enhance our understanding of the role of oligoSia in health and disease and allow us to identify new mechanisms supporting oligoSia biology.

The pivotal role of TSLP in mediating the immune response to asthma

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Asthma is a complex disease encompassing two main endotypes (type 2-high and type 2-low asthma), which manifest similarly as coughing, wheezing, and shortness of breath. Recent pharmaceutical developments have aimed to inhibit alarmin cytokines released by airway epithelial cells, a key effector cell involved in allergic inflammation. An important alarmin cytokine is thymic stromal lymphopoietin (TSLP), which is a prominent trigger of immune responses, particularly those involved in type 2-high and type 2-low asthma. However, the mechanism of TSLP secretion and the allergens that trigger its release are poorly characterized. Cytokine secretion has been shown in other secretory cells to utilize a unique vesicular trafficking mechanism involving recycling endosomes, which mediate endocytic, exocytic, and recycling pathways in epithelial cells. We hypothesized that allergen exposure in epithelial cells triggers de novo TSLP release and that this release is dependent on recycling endosome trafficking. In this study, we utilized an immortalized cell line (BEAS-2B) and two primary cell cultures, normal human bronchial epithelial (NHBE) cells, and asthmatic human bronchial epithelial (AHBE) cells to study how allergen extracts such as German cockroach (CE), American house dust mites (HDM), Timothy grass, and birch pollen influence TSLP secretion. We utilized immunolabeling, imaging and quantification to assess how these allergens affect TSLP secretion. We applied the protein synthesis inhibitor verrucarin A to inhibit de novo protein synthesis and actinomycin D to inhibit DNA transcription. Our findings show that administering inhibitors during allergen stimulation blocked TSLP synthesis in BEAS-2B, NHBE, and AHBE cells, suggesting that newly synthesized TSLP is generated following allergen exposure. We also found novel co-localization of TSLP with the recycling endosome regulator Rab11a using high-resolution immunofluorescence, suggesting the potential involvement of recycling endosomes in TSLP secretion. Our results provide new insights into TSLP trafficking in airway epithelial cells during exposure to allergens.

Oil sands processed affected waters (OSPW) bioremediation methods with murine macrophages as the biological screen

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Oil sands processed affected waters in Northern Alberta are a growing environmental concern due to the lack of prior risk assessment of the tailing ponds. Naphthenic acids (NAs) are a natural component of bitumen and are generally thought to be the main source of OSPW toxicity. Active and passive water treatments are the two methods that are used for bioremediation to effectively eliminate toxicity in OSPW. Active water treatment uses the addition of chemicals and energy for remediation, whereas passive water treatment incorporates natural processes such as constructed wetland treatment systems to degrade contaminants. The advanced oxidation process (AOP) is a form of active treatment that generates hydroxyl radicals that can disrupt the molecular structure of NAs, which leads to possible reduced toxicity of OSPW. Even if energy- efficient AOP treatment innovation is available, combining active and passive treatment remains ideal. Semi-passive treatment incorporates both active and passive treatment, outweighing the disadvantages of both methods. In-vitro analysis using RAW 264.7 murine macrophage cell lines can detect OSPW toxicity which makes it a valuable biological tool for assessing OSPW toxicity. Nitric oxide (NO) and pro-inflammatory cytokine quantification were used to determine the toxicity of OSPW in treated waters. Comparing both two treatment processes using our cell bioassay will help inform future bioremediation development methods.

Using immunohistochemistry to identify the optimal alcohol-based fixation time for juvenile *Biomphalaria glabrata* snails

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Fixation is the chemical treatment of tissues to prevent autolysis and degradation, preserving tissue morphology for downstream assays. The efficacy of fixation depends on the prudent selection of parameters including the type of fixative and

fixation time that vary across different tissue types. This challenge is apparent in fixing mollusks like the aquatic snail *Biomphalaria glabrata*. Due to their robust mucosal interfaces, cross-linking fixatives like formaldehyde lack sufficient protein substrate to stabilize the tissue. However, preliminary work has demonstrated the potential of alcohol-based fixatives like Carnoy's and Methacarn that fix tissues by introducing hydrogen bonds. Additionally, these fixatives might penetrate tissues quicker, although an optimal fixation time remains uncharacterized. By determining an optimal alcohol-based fixative and fixation time for juvenile *B. glabrata*, this study aims to refine the fixation protocols for freshwater snails to facilitate a deeper study into the molecular and physiological constituents of the epithelial-mucosal interface. This study determines the efficacy of Carnoy's and Methacarn fixation of juvenile *B. glabrata* at different time points. *B. glabrata* are fixed with Carnoy's or Methacarn, then immunostained for alpha-tubulin and a *B. glabrata* toll-like receptor (TLR) called BgTLR that is expressed in the BS-90 strain. Finally, nuclei are stained with Hoescht before epifluorescent and confocal imaging to quantify relative fluorescence. We hypothesize that different fixation times change the viability of tissue samples for downstream immunohistochemical assays because the fixative will have varying capacities to penetrate the tissue and denature the target proteins. Because of the role of BgTLR in mucosal immunity, we expect to find most BgTLR signals localized to the haemocytes and mucosal epithelial cells. These findings not only substantiate previous studies on BgTLR, but open up opportunities for future molecular approaches requiring *B. glabrata* fixation.

Proteomic study of host factors involved in influenza virus infection

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Influenza A viruses (IAV) are a group of single-stranded RNA viruses from the family Orthomyxoviridae. They are largely responsible for seasonal flu outbreaks in humans and the H1N1 subtype is implicated in both the 1918 and 2009 flu pandemics. Over the years, various transcriptomic and proteomic methods have been used to study proteome changes in influenza infected cells. However, recent advances in the sensitivity and resolution of mass spectrometers has enabled deeper proteome coverage and identification of low abundance proteins, many of which may be involved in viral replication or host response mechanisms. Here, we employed label free quantitative proteomics to determine proteome changes in A549 human lung epithelial cells infected with A/PR/8/34 (H1N1) at 8, 24, and 48 hours post infection, compared to uninfected controls. Using an Orbitrap Exploris 480 (Thermo Scientific) in data-independent acquisition (DIA) mode, we identified over 4500 protein groups per sample, including 12 viral proteins. Overall, 295 host protein groups were significantly increased and 446 were significantly decreased over the course of infection. Proteins involved in innate and adaptive immunity, cell cycle regulation, and proteasomal degradation were enriched in response to infection, while lysosomal and mitochondrial proteins were downregulated. Many of these proteins have never been identified by previous studies, and may serve as novel biomarkers of infection or targets for antiviral therapies. Next, we intend to implement a single-cell proteomics workflow previously developed in our lab to study how individual cells respond differently to IAV infection.

Examining the role of T-cell receptor signaling strength in intestinal T-cell development

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The immune system is an important bodily system that eliminates foreign disease-causing microbes. T-cells play an important role in this system, with multiple different T-cell lineages serving different functions. Intraepithelial lymphocytes (IELs) are a unique subset of T-cells that reside in the small intestine. Researchers have previously discovered that during T-cell development in the thymus, IEL progenitors (IELp) require strong T-cell receptor (TCR) signals that typically cause cell death. Why these progenitors require these strong signals is unclear. We have found that IELp expresses a protein called CD137, and without CD137, IEL generation is impaired. To understand why IEL generation was impaired in the absence of CD137, this study examined the strength of TCR signals using Helios and Nur77 expression as surrogates. T-cells were harvested from wildtype, BimKO, and CD137KO mice, stained using antibodies against various immunological markers, and analyzed by flow cytometry. BimKO and CD137KO mice were used because the former has an expanded IELp and IEL population and the latter has an absent IEL population. In the wild-type mouse, the CD137+ IELp population had increased Helios expression relative to the CD137- IELp population. Additionally, the IELp population from the wildtype mouse had increased Helios expression relative to the BimKO and CD137KO mice. Taken together, this data suggests that CD137 may

play an important role in the generation of IEL populations through regulation of TCR signaling strength. Future studies will further investigate the generation of IELs by looking at downstream signals.

Investigating the immunostimulatory effects of oil sands process-affected water and commercial naphthenic acids using mammalian macrophages

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Alberta has one of the largest oil deposits in the world. A substantial amount of these oil sands undergo surface mining and bitumen extraction, a process requiring large quantities of water. This water is referred to as oil sands process-affected water (OSPW) and has been shown to contain inorganic and organic components that can induce toxicity responses in a variety of organisms. Some of the major toxic constituents of the organic fraction are naphthenic acids (NAs), a family of complex carboxylic acids typically containing at least one 5-or 6-membered aliphatic ring. NAs are found in complex mixtures in OSPW and are extremely difficult to extract; however, some NAs are commercially available for industrial purposes. In this study, we investigated the sublethal effects of OSPW and individual commercial naphthenic acids (cNAs) on mammalian macrophage cells. The cells were exposed to OSPW and cNA samples for 24 hours, then analyzed for nitric oxide and cytokine production using a nitric oxide assay and a multiplex cytokine assay as indicators of immunotoxicity. Results showed significant immunostimulatory effects of OSPW and cNAs on the cells, as well as distinct sensitivities of cells to individual cNA structures. This study highlights the potential use of mammalian macrophages as a bioindicator for assessing the immunotoxic effects of OSPW components and suggests that macrophages are an effective sensor of NAs. Additionally, our lab is currently exploring the interaction mechanisms between cNAs and immune cells to determine whether macrophages possess specific receptors for recognizing NAs.

Investigating the mechanism behind the enhanced oncolysis of σ1 mutant reoviruses

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Many cancer treatments currently available have off-target effects, creating a need for highly targeted therapies. Reovirus can be used as a cancer therapy because it selectively infects and kills cancer cells while causing no disease to healthy cells. This makes reovirus an ideal candidate for targeted treatment of specific cancers. However, clinical trials using the wild-type reovirus serotype 3/T3DPL strain as a cancer therapy have shown underwhelming results, highlighting the need for improvement. That being said, we predict that reovirus requires modifications to increase its potency in a tumor environment. This project explores how mutations Q217H, R219Q and N312R in the reovirus cell attachment protein, σ1, enhance cancer cell killing. The mechanisms by which reovirus has improved due to these mutations remain unknown. The first objective of this study is to investigate whether these mutants spread and infect cancer cells further from the initial site of infection compared to wild-type reovirus. Plaque assays and immunofluorescence revealed that only mutants Q217H and R219Q have increased viral spread relative to wild-type reovirus. This may allow for better dissemination of reovirus infection. The second objective is to investigate whether these mutants bind less or more to cancer cells at 37°C compared to wild-type reovirus. Virus binding on the surface of cancer cells was evaluated using binding assays and flow cytometry. It was determined that all mutants exhibit reduced binding at 37°C in comparison to wild-type reovirus. Reduced binding may allow the viruses to travel greater distances to establish infection. This data suggests that increased viral spread and reduced binding to cancer cells may be involved in enhancing the oncolytic capacity of reovirus. These findings provide deeper insights into what mechanisms drive improved cancer cell killing.

Polysialic acid on chemokine receptor CCR10 in a heterologous expression system

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Polysialic acid (polySia) is characterized by a chain of α2,8-linked sialic acid residues and has been linked to various diseases, including autoimmune and cancers, making it a critical area of research. PolySia is crucial in modulating immune cell migration through chemokine and chemokine receptor interactions to sites of inflammation or infection. The role of polySia has been primarily studied in the innate immune system, with limited research looking into the adaptive immune system. This has prompted us to investigate the role of polySia in B cell migration. The lab has previously identified C-C chemokine receptor type 10 (CCR10), a G protein-coupled receptor as a potentially polysialylated receptor on T and B cells through proteomics analysis. We hypothesize that CCR10, expressed in a heterologous system, will be polysialylated and play a role in mediating B cell migration. To test this hypothesis, we have cloned the CCR10 gene into a mammalian expression vector and will transfect it into the MCF7 human breast cancer cell line, a heterologous system that lacks endogenous cell surface polySia. By expressing the transfected receptor, we suspect that polySia will be transported to the cell surface and interact with the transfected CCR10 receptor. We will look for the presence of polySia and CCR10 on the cell surface through flow cytometry. Additionally, to identify the site(s) of polysialylation on CCR10, we will mutate predicted polysialylation sites on CCR10 and perform similar transfections into the MCF7 cell line. This study aims to confirm the presence and role of polySia in B cell migration, to aid in the development of therapeutics for immune-mediated diseases.

Conflicts of Interest

There are no conflicts of interest.

Authors' Contributions

LM: served as ‘Primary Organizer’ for the conference, helped draft the conference abstract booklet, reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
LI: served as ‘Student Presentations’ volunteer for the conference, helped draft the conference abstract booklet, reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
SP: served as ‘Student Presentations’ volunteer for the conference, helped draft the conference abstract booklet, reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
ML: served as ‘Secondary Organizer’ for the conference, helped draft the conference abstract booklet, reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.
CL: served as ‘Student Presentations’ volunteer for the conference, helped draft the conference abstract booklet, reviewed the abstract submissions and ensured that they adhered to correct formatting standards, and gave final approval of the version to be published.

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