

Dr. R.E. Peter Biology Conference, March 9-11, 2022 – Conference Schedule & Abstract Book



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**Note: Correction added after original version published on April 04, 2022.
We regret any inconvenience caused.**

Abstract

The R. E. Peter Biology Conference will be held March 9-11, 2022. This conference was originally organized by the Biology Graduate Students' Association to honour Dr. Peter (1943-2007) and his contributions to our department, as well as showcase the diverse research conducted by students in the Department of Biological Sciences at the University of Alberta. The conference has grown to include students from other research institutes in the Edmonton area and now invites students in any department whose work aligns with biological sciences, to encourage cross-disciplinary research. The 2022 conference consists of both oral and poster presentations by graduate and senior undergraduate students from the University of Alberta and MacEwan University.

Keywords: Alberta; biology; ecology; evolution; molecules; physiology; development; immunology; genetics

Table of Contents

Oral Presentations in Ecology and Evolution	pg. A01-A04
Oral Presentations in Entomology	pg. A04-A05
Oral Presentations in Immunology and Infection	pg. A05-A06
Oral Presentations in Microbiology	pg. A06-A07
Oral Presentations in Molecules and Cellular Genetics	pg. A07-A07
Oral Presentations in Physiology and Development	pg. A07-A08
Oral Presentations in Plant Biology	pg. A08-A09
Poster Presentations in Ecology and Evolution	pg. A09-A12
Poster Presentations in Immunology and Infection	pg. A12-A14
Poster Presentations in Microbiology	pg. A14-A15
Poster Presentations in Molecules and Cellular Genetics	pg. A15-A17
Poster Presentations in Physiology and Development	pg. A17-A18

Conference Abstracts

Oral Presentations in Ecology and Evolution

Carbon dynamics and microbial communities associated with winter flow in a lake dominated discontinuous permafrost environment

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In the western Canadian subarctic, climate change is mobilizing vast stores of carbon previously held in permafrost and altering the flow of water from land to aquatic systems; these changes should fundamentally affect stream chemistry and

ecology. Warming or changing flow regimes can alter the configuration of supra-permafrost taliks, which are pockets of perennially unfrozen ground in a permafrost environment; these have received limited attention for their potential role in facilitating movement of materials in permafrost-affected landscapes. Taliks provide connections between unfrozen areas of the soil during winter, which has the potential to alter the source and chemistry of flow to streams. Across many northern regions, the development and expansion of supra-permafrost taliks may contribute to increased winter/spring flow. The specific influence of taliks during different seasons on subarctic rivers is poorly understood. Winter flow through taliks contributes to the development of icings, which are sheet-like masses of layered ice above the ground surface that can be used as an archival tool to study the chemical nature of winter flow. In this study we are investigating the biogeochemical effects of taliks within the River Lake and Baker Creek catchments near Yellowknife, NT. We performed detailed water sampling from April to June 2021. In March, we collected ice cores from six icings to investigate temporal changes in talik water chemistry over winter. Chemical analyses of water and ice samples is underway; biological processing was assessed using incubation experiments to determine DOC loss over time. To understand how seasonal changes in water chemistry affect ecological function, microbial community structure will be assessed using 16-S-rRNA techniques. Early results show striking differences in water quality between thawed icing water and open water flow. These results will help inform predictions of how climate warming may change seasonal water chemistry at watershed and larger geographic scales.

Response of boreal songbird communities to energy sector linear features of varying width: Do all linear features have the same disturbance effect? How much does “width effect” matter?

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Linear features such as seismic lines, pipelines and powerlines are very common in Alberta, and they vary in width substantially. However, in provincial bird models used for regulatory decision making, all linear features are treated as being the same width. This raises a question: are these decisions grounded in fact? This study is designed to understand community-level responses of boreal songbirds with the goal of determining the effects of linear feature width on bird communities in an effort to assess how important linear feature width is. In addition, the interactive effects of linear feature density and vegetation recovery that may influence the effects of linear feature width will also be assessed. Here we suggest the term “width effect” and hypothesize that boreal songbird communities change as a (a) function of linear feature width and will test if (b) the width of linear features depends on how the importance of width depends on landscape context (density of linear features) and/ or (c) linear feature width impacts depend on the vegetation regrowth on the linear feature. The study area spans the entirety of Alberta. Sampling sites were selected representing different vegetation types including upland and lowland spruce, pine, mixedwood and deciduous forests, graminoid fens and shrubby wetlands. Bird communities in forest-edge habitats associated with linear features (seismic lines, pipelines, and powerlines) were surveyed primarily by Autonomous Recording Units (ARUs) (n ~1000) in addition to avian point counts and mobile surveys. Vegetation attributes on linear features were also measured at each survey point. For the analysis, community composition, density, richness, and functional diversity will be quantified. This study will provide important information that is vital to improve impact assessments and restoration of linear features considering the fact that the effects of linear features vary as the width gets changed.

Preliminary results from a community-based aversive conditioning program of urban coyotes (*Canis latrans*) in Edmonton

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Coyotes (*Canis latrans*) are increasingly common in urban areas across North America where they may occupy residential neighborhoods, den underneath houses, and approach or attack people or pets. Many governmental entities recommend aversive conditioning, or hazing, as a humane way to deter urban coyotes from approaching people, but there are few guidelines for implementing this technique. This project aims to develop and refine the use of aversive conditioning by members of a community as a cost-effective and humane tool to reduce human-coyote conflict. In 2021, the program used a before-after-control-impact design in treatment and control neighbourhoods from February-May wherein 76 volunteers in 28 neighborhoods patrolled designated areas for both coyotes and attractants. When coyotes were observed, volunteers measured the overt reaction distance and flight initiation distance as they walked towards the coyote. When volunteers were within 40 m of coyotes, they either receded (control) or hazed (treatment). Volunteers spent 569 hours patrolling, submitted 656 patrol forms, described 64 coyote sightings, and conducted aversive conditioning five times. Coyotes receded from volunteers 80%

of the time when they were observed and 100% of the time when they were conditioned. Coyotes were observed again in neighborhoods an average of 9.72 days later when no conditioning occurred and 37.4 days later when it did. These results suggest that aversive conditioning can increase wariness and reduce daytime sightings of coyotes in residential areas, but more data will be gathered in 2022.

Host preference, infectivity, and pathogenicity of *Phasmarhabditis californica* in terrestrial slugs in Alberta, Canada

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The nematode, *Phasmarhabditis hermaphrodita* is currently marketed as a biocontrol agent against pest gastropods in the UK and Europe where the species is naturally found. However, Canada had no records of any *Phasmarhabditis* species, until a recent discovery of a parasitic Canadian strain of *Phasmarhabditis californica* in Edmonton, Alberta. The presence of a novel parasitic nematode strain does not suggest the immediate action of its use as a bio-control agent against slugs in Canada; major knowledge gaps should be first addressed before any decision is informed and implemented. The aim of this project therefore is to study the host preference, infectivity, and pathogenicity of *P. californica*. A field study was conducted from July to October 2021 to survey slugs from selected agricultural sites, nurseries, and greenhouses in Alberta. Slug-associated nematodes were recovered from 43 of 1331 slugs collected during this survey. Molecular analyses are underway to identify the nematodes. The host preference of *P. californica* will be determined using chemotaxis assays with slug mucus from different slug species. Preliminary data suggests that *P. californica* prefers the cues (mucus) of *Deroceras reticulatum*, one of the major pest slugs in agriculture and horticulture, over *Arion fasciatus*. Other major slug species, *Deroceras laeve*, *Ambigolimax valentianus* and *Arion rufus* will also be tested to establish a hierarchical attraction index of the nematode for each host species. The infectivity and pathogenicity of *P. californica* will be determined by exposing different slug species to the nematode. I will measure changes in the rate of feeding and mortality of control and infected slugs for 20 days or until they die, whichever comes first. The results of these findings will establish baseline knowledge on *P. californica* which will be useful for agronomists in developing a potential biocontrol product that could be used in the absence of *P. hermaphrodita*.

The influence of longevity on parental investment: A meta-analysis

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Life-history theory strives to develop our understanding of how natural selection shapes organisms reproduce successfully, given trade-offs faced. This includes the trade-off between investment in current versus future reproduction investigated through parental investment theory. Parental investment theory states that parents won't invest all of their available energy in caring for their current brood since it will come at the expense of their future reproductive opportunities. In altricial birds, as nestlings hatch and age parents must invest additional energy to meet the increasing demand of their current brood, for example by increasing regularity of feeding visits. By how much should they increase investment? The trade-off between current and future reproduction is mediated by species longevity which influences residual reproductive value (RRV), that is, the remaining expected reproductive output of that species. Existing research has been both observational and experimental, with experimental work manipulating nestling numbers in current broods. Differences in RRV should equate to differences in how species respond to brood size manipulations i.e., how much they invest in their current brood as the demand of that brood increases. My plan is to assess this via a meta-analysis of published experimental papers wherein brood size was enlarged or reduced and resulting changes in parental investment monitored. My aim is to evaluate whether the trade-off between current and future reproduction is mediated by species longevity or, more simply, 'do longer-lived species respond differently to brood size manipulation experiments when compared to shorter lived species?' The answer to this question cannot be obtained within the confines of a single study as it required comparison across multiple species with different lifespans. Finding the answer, via meta-analysis, will contribute to our understanding of how natural selection shapes organisms with different life spans to maximise reproductive output.

Fool me twice: Personality-related recapture bias in black-capped chickadees (*Poecile atricapillus*)

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There is growing awareness that within populations, individuals often exhibit repeatable differences in behaviour, a phenomenon termed animal personality. Animal personality can have important ecological and evolutionary consequences and may also impact population-level inferences if recaptured individuals are not representative samples. Certain behavioural types, such as bolder or more aggressive animals may have a greater probability of being captured and recaptured for study. Thus, ecological inferences based on these samples may be subject to personality-related (re)capture bias. Using a sample of approximately 100 previously captured black-capped chickadees (*Poecile atricapillus*), we explored whether individual recapture probability is affected by repeatable behavioural measures (exploration, handling aggression, feeding rate, boldness, information sampling) and sex (a proxy for social dominance). We found no evidence for recapture bias which suggests that we can make valid population level inferences based on our recaptured samples. This is encouraging; however, we cannot generalize these results. Limited literature controls for survival and/or emigration confounds, which hinders our ability to conclude whether our null results can apply across taxa and ecological contexts. We encourage ecologists to investigate possible sampling biases within their study populations to enable future conclusions about the conditions under which recapture bias is versus is not expected.

The sub-lethal impacts of chlorinated water on rainbow trout: Methemoglobin (MetHb) formation and reduced swimming performance

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Chloramines are acutely toxic disinfectants that are added to drinking water to rapidly kill microorganisms. Rainbow trout are naturally found in Alberta and are common species used for regulatory decision making. When trout are exposed to chloramine, it rapidly oxidizes the oxygen-carrying pigment hemoglobin to form methemoglobin (MetHb), which cannot bind oxygen. This study is designed to examine the impact of short term, non-lethal chloramine exposure on MetHb formation and clearance from the blood stream. We also examined the role that MetHb formation plays in swimming performance of exposed rainbow trout. We hypothesized that a short-term non-lethal chlorinated water exposure would result in MetHb formation, and this would be associated with a significant impairment in swim performance. To examine kinetics of MetHb formation, groups of fish (n=6) were exposed to 0.5mg/L chloramine for 5, 15, 30 mins and 1hr and immediately tested for MetHb presence in blood. [MetHb] was maximal by 30 min and reached ~8% of red cells after 30 min exposure. We also examined MetHb clearance in fish exposed to 0.5mg/L for 30 minutes and allowed 3, 6, 24 and 48 hrs recovery. Although MetHb was cleared rapidly from the blood, it still persisted with ~0.2% 48 hrs post-exposure. The impacts of chloramine on swim performance were then investigated in swim tunnels. Maximum sustained speed (UCrit) and oxygen consumption were both reduced following 30 min chloramine exposure. Additional histology, RT-PCR and biochemical assays will be performed to assess other sub-lethal indicators of chloramine exposure. Understanding the effects of chloramine, a commonly used disinfectant, on rainbow trout will allow for better assessment of their health, quality of life and our impact on the environment.

Oral Presentations in Entomology

Effect of natal and colonized host species on female host acceptance and male joining behaviour of the mountain pine beetle (Coleoptera: Curculionidae) using lodgepole pine and jack pine

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The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae) uses aggregation pheromones to concentrate conspecifics for host colonization of chemically defended host trees. Host selection is primarily done by female beetles can sequester α -pinene as a monoterpene ester as larvae, as a precursor to the production of the aggregation pheromone trans-verbenol. In the historic range of the mountain pine beetle, the preferred host is lodgepole pine (*Pinus contorta*), but eastward spread has resulted in the colonization of jack pine (*Pinus banksian*). Pheromone release in females is essential for males to join the beetle aggregation to overwhelm host tree defenses. I will use a 2×2 factorial design for laboratory bioassays to test for the effect of phloem consumption in the natal and colonized host aggregation pheromone production and response in mountain pine beetles. Female beetles reared from lodgepole pine and jack pine will be introduced into freshly cut bolts of either lodgepole or jack pine. Male beetles will be subsequently inoculated into the entrance hole of each bolt. Pheromone production will be measured and male time to entry into the female gallery will be measured. I expect to find differences in pheromone production based on host feeding in both the natal and reproductive host.

Mountain pine beetles in the eastern leading edge encounter jack pines, which have more α -pinene in the phloem than lodgepole pines, which is also why female beetles reared from jack pines should have higher rates of host acceptance of both pine host materials than females reared from lodgepole pines. This study based will fill the existing knowledge gaps in the understanding the role of α -pinene-trans-verbenol-based female pheromone production and male host acceptance of mountain pine beetle in western North America.

Context matters: Abundance of *Pterostichus melanarius* (Coleoptera: Carabidae) in pulse agroecosystems and implications for conservation biological control

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The changing climate is facilitating an expansion of insect pests into regions previously unoccupied. One important insect pest is the pea leaf weevil (PLW) *Sitona lineatus* L., (Coleoptera: Curculionidae). PLW primarily targets field pea, *Pisum sativum* L. (Fabaceae), and faba bean, *Vicia faba* L. (Fabaceae), which are important crops worldwide. The shift towards conservation biological control (CBC) has created a new focus on preserving landscape heterogeneity and natural enemies. Carabid beetles, such as *Pterostichus melanarius*, are being evaluated for their potential to provide CBC services and contribute to an integrated pest management (IPM) strategy for PLW. We conducted research to determine the effects of crop type and transect location on *P. melanarius* abundance over a three-week period within one growing season in Alberta, Canada. Pitfall traps in field pea and faba bean in the Alberta capital region were used to capture carabids. We determined that *P. melanarius* abundance did not vary between field pea and faba bean sample sites. On the contrary, we saw *P. melanarius* abundance in interior transects to be greater than transects in field margins. We also noticed a significant interaction between sampling date and transect type, with abundance in the field interior being greater than the field margins throughout the sampling weeks. The findings suggest that *P. melanarius* occupies a similar spatio-temporal distribution to PLW, however, more research is needed into diet composition to gain a better understanding of CBC potential.

QTL analysis of virus resistance in honeybees

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As in many other organisms, viruses have been linked to serious illness in honey bees (*Apis mellifera*), and thus significantly contribute to ongoing worldwide colony losses – a major threat to agricultural pollination. Israeli acute paralysis virus (IAPV) is a particularly debilitating disease of honey bees, able to infect all stages of their life cycle. While it has been observed that susceptibility toward IAPV infection (as well as other viruses) varies across different lineages, little is known about the underlying causes of these differences. This study sought to identify genomic regions that closely correlate with virus resistance in honey bees by quantitative trait locus (QTL) analysis. Experimentally, two lines differing in their IAPV susceptibilities were backcrossed, producing resulting workers with varying degrees of IAPV susceptibility. Subsequently, workers were sampled, and individual worker susceptibility was assessed by inoculating each bee with IAPV. After genotyping-by-sequencing across the entire genome, the workers' survival was then related across genotypes ~70,000 variable genetic markers. In addition, the titres of IAPV alongside other, naturally occurring viruses were quantified and used in additional QTL analyses. I will report my preliminary findings in the context of potential candidate genes for IAPV resistance in honey bees, and discuss the prospect of breeding virus-resistant honey bees for sustainable beekeeping.

Oral Presentations in Immunology and Infection

Short-chain fatty acid differentially regulate apoptosis and T-cell interactions of the two main subtypes of colorectal cancer

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The two main subtypes of colorectal cancer (CRC) are characterized by microsatellite instability (MSI), due to deficient DNA mismatch repair, and chromosomal instability (CIN), due to chromosomal rearrangements. MSI CRC patients have a better prognosis than CIN patients because they induce higher anti-tumour immunity. Immune signaling and gene expression of the intestinal epithelial cells can be influenced by microbially-derived short-chain fatty acids (SCFAs). In healthy epithelial cells, SCFAs are used for energy; however, cancer-driven changes in metabolism can cause SCFAs to increase

CRC cell death. Due to this, as well as their role in regulating immune signaling, SCFAs are linked to a better prognosis in CRC. We hypothesize that MSI CRCs will be more susceptible to SCFA mediated apoptosis and cytotoxic (CD8+) T cell-mediated killing than CIN CRCs. To model MSI and CIN CRC, we used CRISPR-Cas9 knockouts of Mlh1^{-/-} and Rad51^{-/-} respectively in the MC38 mouse CRC cell line. Western blot and qPCR were used to understand how SCFAs, butyrate and propionate, influence cell death regulation in the absence of T cells and investigate how SCFAs modulate immune signaling. To investigate if SCFAs influence CRC cells' interactions with antigen-specific CD8+T cells, we used flow cytometry to measure OT-1 CD8+ T-cell activation and killing of OVA-expressing CRC cells. The SCFAs were able to induce apoptosis in both CIN and MSI CRC cells, but apoptosis is activated sooner in the Mlh1^{-/-} cells. Even though Rad51^{-/-} cells had higher cytokine expression upon SCFA stimulation, when we co-cultured CRC cells with OT-1 CD8+ T cells, the Mlh1^{-/-} cells expressed more MHC1 and were better at activating T cell-mediated killing. SCFAs sensitized MSI CRC cells to CD8+ T cell-mediated recognition and killing more effectively than CIN CRC cells. Therefore, the differing prognoses of MSI CRC and CIN CRC may be influenced by microbial-derived SCFAs.

Oral Presentations in Microbiology

Determining gene regulatory networks of *Methylomonas denitrificans* FJG1 using a combined machine learning approach

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Machine learning (ML)-based algorithms have greatly advanced data-driven analysis in cell biology in recent years. These algorithms are powerful tools for classification and prediction problems, excelling within the data-rich environment of increasingly cost-efficient, high-throughput biological datasets like transcriptome data. RNA-Seq transcriptome data captures gene expression information necessary to understand cell regulatory mechanisms, captured as gene regulatory networks (GRNs). GRNs underlie almost all cellular phenomena, thus, accurate GRN maps are essential for understanding gene function, especially in aiding the identification and prioritization of candidate genes for functional analysis. ML algorithms offer a robust, computational approach to modeling GRNs, inferring complex regulator-target relationships automatically from large scale transcriptome data that would otherwise take considerable time and effort to construct by hand. In this study, we determined GRNs for *Methylomonas denitrificans* FJG1 under hypoxia with two types of growth media, analyzing time-series RNA-Seq transcriptome data using ARACNE to produce a simplified GRN to visualize network edges, and Random Forest (RF) to produce a more exhaustive network. We used known differences between nitrate and ammonia growth conditions to validate the network produced by ARACNE, confirming it was a subset of Random Forest. This combination of human-curated and AI-corroborated validation capitalizes on the strengths of both types of validation. In cases like FJG1, where many genes and functional details are unknown, manual validation is difficult, therefore using an additional ML algorithm increases confidence. By the same token, adding human validation of key genes strengthens confidence for networks determined by ML methods. With many regulator-target relationships confirmed, there is confidence that network connections inferred by this method can lead to the discovery of previously unknown regulatory relationships, thereby expanding knowledge of FJG1 cell physiology. ML-based GRN modeling can produce faster, more automated workflows for studying cell physiology, greatly enhancing research and industrial applications of transcriptome data.

Detecting antibiofilm activity in phytochemical extracts from local invasive weed species

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The development of antibiotic resistant pathogens is a serious global healthcare concern. Treatment for pathogens that develop biofilms is particularly difficult because biofilm formation provides an extra physical barrier that enhances a pathogen's resistance to antibiotic treatment. New antimicrobial treatment options must be discovered to address this challenge. Invasive weeds are of increasing interest because of their potential to produce antimicrobial chemicals. Studies on the phytochemicals produced by local invasive weed species have been limited thus far, despite showing strong potential as a novel antibiofilm treatment due to their noxious and allelopathic nature. This project examined phytochemical extracts of invasive weeds from the local Edmonton, Alberta environment and aimed to find evidence of extracts demonstrating antibiofilm activity. Invasive weeds were collected from local environments, dried, and extracted using different liquid solvents (hexane, ethyl acetate, or methanol). After solvent removal, the phytochemicals were redissolved in DMSO to prepare phytochemical extracts for testing. Previously completed disk diffusion assays identified general antibacterial activity in these extracts, indicating

potential for the extracts to also demonstrate activity specifically targeting biofilms. An antibiofilm assay was developed, in which biofilms of gram-positive and gram-negative bacterial species were grown in 96-well microtiter plates. Work was done to optimize the method for use with various bacterial test strains and was validated using the known antibiofilm agent trans-cinnamaldehyde as a positive control. This antibiofilm assay was then used to identify inhibiting or killing effects of phytochemical extract treatments on biofilm cultures, with initial experiments showing evidence of antibiofilm activity. These extracts will be further studied to identify the specific phytochemicals causing the observed antibiofilm effects, and to assist in the development of new potential antimicrobial agents to treat antibiotic resistant pathogens.

LPS binding phages, antibiotics, and the complement system for potential irresistible antibacterial treatment of *B. cenocepacia*

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Burkholderia cenocepacia is an opportunistic multi-drug resistant (MDR) pathogenic bacterium of the *Burkholderia cepacia* complex capable of causing sepsis. Discovery of novel therapeutics is necessary to effectively treat the devastating infections that *B. cenocepacia* causes. Phage therapy is emerging as an alternative treatment option for MDR bacterial infections but has not been used on a large scale to date. This is partially due to the extreme specificity phages exhibit towards bacterial host strains as conferred by their bacterial receptors. We have characterized 12 phages which utilize *B. cenocepacia* as a host. Most of these phages were found to adhere to the lipopolysaccharide (LPS) layer of the outer membrane as the primary receptor to infect the cells. Like antibiotic resistance, resistance to phage infection is a concern, and alteration of the LPS that the phage uses to adsorb to the host cell can prevent phage access to the cell. LPS is a well-known bacterial virulence factor that is used to evade the innate immune system, including the complement system. Complete cellular LPS prevents complement proteins from binding to the bacterial cell surface and marking it for cell lysis by the complement cascade. In contrast, partial or no LPS was confirmed to render *B. cenocepacia* sensitive to complement in both in vitro and in vivo assays. Additionally, the loss of LPS led *B. cenocepacia* to be more sensitive to some antibiotics. Cells treated with LPS-binding phages that mutate to lose LPS to prevent phages from adhering, concurrently become sensitive to the effects of antibiotics and complement. Therefore, the combination of phages with antibiotics and complement can serve to enhance treatment therapy and prevent the evolution of resistance. Future work will focus on combination treatments using multiple phages, antibiotics, and the complement system to clear *B. cenocepacia* infections without the development of resistance.

Oral Presentations in Molecules and Cellular Genetics

Cloning and expression analysis of lignin-associated genes in white spruce during bud burst

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White spruce (*Picea glauca*) is a keystone species in North American boreal forests. One of the major insect pests of white spruce is the spruce budworm, SBW (*Choristoneura fumiferana*), a needle defoliator that can result in impeded host growth. Spruce budworm preferentially feeds on expanding foliage because it is more nutritious and less fortified, making younger foliage more palatable. Lignin, an important plant chemical that provides structural support to growing tissues, can also play a pivotal role in the plant's defense mechanism against pests and pathogens. Our lab has established that histochemical changes related to lignin deposition coincided with budburst stages (i.e., bud burst phenology) in white spruce and correlates with needle toughness. Thus, we hypothesize that regulation in lignin gene expression is correlated with lignin deposition, needle toughness, and ultimately with feeding preferences of ESB larvae. The main objective of this study is to test this hypothesis by developing transcript profiles for white spruce lignin biosynthesis genes with quantitative RT-PCR during the course of bud burst. These results will also be used to validate white spruce bud burst RNA-Seq data. I have identified and cloned a subset of candidate lignin biosynthesis genes in white spruce. I am currently working on optimizing qRT-PCR primers for these genes, and quantitative RT-PCR will be carried out this semester. Two-way ANOVA will be used to analyze the effect of the bud burst stage on transcript abundance. These data will contribute to a fine-scale profile of lignification in expanding needles to better understand how lignification contributes to white spruce foliar palatability the phenological window of opportunity for spruce budworm.

Oral Presentations in Physiology and Development

Zebrafish imprint to an amino acid mixture

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Animals constantly receive sensory information from their environments which dictate their behavior. Olfaction (smell) is a particularly important sense influencing how animals find food, avoid predation, and mate. How an animal perceives environmental stimuli is influenced by their early-life environment, some animals imprint to odorants found in their environment forming long-lasting memories during a brief sensitive window during early development. This imprinting effect influences how animals behave in the presence of an imprinted odor when encountered in future life stages. My project aims to determine whether larval zebrafish imprint to an amino-acid mixture during their early development given that they utilize olfaction of amino-acids to dictate their behavior. This will be assessed by observing how adult zebrafish behave in response to this same amino-acid mixture as fully grown adults and comparing the resulting response to zebrafish that were not imprinted to the amino-acid mixture. If evidence of imprinting behavior is found, I will then examine zebrafish behavior in response to each individual amino-acid within the imprint mixture to determine if they imprinted to the amino-acid mixture as a whole (configurative imprinting), or to the individual components within the mixture (elemental imprinting). To date, I have established that zebrafish do imprint to an amino-acid mixture consisting of L-Leucine, L-Valine, and L-Lysine. I predict that zebrafish will imprint to the entire mixture rather than to each/one amino-acid within the mixture.

Multigenerational adaptation of daphnia magna to sunscreen UV filters

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Organic ultraviolet filters (UVFs) such as avobenzene, octocrylene and oxybenzone are widely used in sunscreens and other personal care products to protect against harmful ultraviolet radiation. Their use in sunscreens leads to widespread environmental contamination due to the leaching of these chemicals during recreational activities, posing a threat to both freshwater and marine aquatic systems. The majority of research regarding the toxicity of UVFs has utilized short-term exposures or high concentrations that are unlikely to be encountered in an environmentally realistic exposure scenario. This study sought to model the long-term effects of chronic UVF exposure to the freshwater invertebrate, *Daphnia magna*, as well as their adaptation potential over four subsequent, continuously exposed generations. The effects of sunscreen contamination were quantified across a variety of endpoints, including subtle changes in biotransformation and metabolic gene expression, behavioural alterations in response to environmental stimuli, developmental delays, and changes to overall reproductive capabilities.

Interaction between metal exposure and social behaviour in three-spined stickleback (*Gasterosteus aculeatus*)

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Social context influences all aspects of life, including how organisms perceive and interpret stimuli. The condition of aquatic environments worldwide is worsening due to anthropogenic runoff and climate change, creating challenges for aquatic species to navigate. Animal social behaviours can affect how they interact with their environment, how they experience stress, and can influence physiological responses that may affect how organisms cope with toxicants in their environment. We have sought to understand how the social group size of Three-Spined Stickleback (*Gasterosteus aculeatus*) influences how these individuals respond to metal (copper) contamination in their environment. Behavioural (activity level, foraging) and biochemical (enzyme assays, copper accumulation) assessments were conducted. Observing response to copper after isolation and group exposures may highlight the importance of accounting for the social environment when assessing risk. The interplay between toxicology and social behaviour is not well understood, and as such is not considered in risk assessment standard practice. This potentially leaves a significant gap in our understanding of organismal response to toxicants and may leave species unprotected. Therefore, this research could have a broad influence on policy and water quality guidelines. With more species in jeopardy than ever before we must consider all factors that affect the survivability of key species, that humans and other organisms rely on.

Oral Presentations in Plant Biology

Development of microsatellite markers for *Cypripedium passerinum*

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In the age of the Anthropocene, there has been a steady and exponential decline in global plant biodiversity leading to an increase in conservation efforts. Key to an effective conservation strategy is an assessment of the genetic diversity of the vulnerable population. Microsatellites are a type of tandem repeat found in the DNA of all eukaryotes that have proven useful in assessing genetic diversity because of their genomic abundance, high mutation rate, and resulting high levels of polymorphism. This project aimed to develop microsatellite markers for the endangered orchid, *Cypripedium passerinum*, to elucidate genetic variation in populations within the Wagner Natural Area in central Alberta. Fast Isolation of AFLP Sequences Containing Repeats (FIASCO) was used to generate three different microsatellite-enriched libraries using AC, AT, and AAG probes. Of the 687 clones in these libraries, 58 have been sequenced and 10 microsatellite primer pairs have been developed. Currently, these designed primer pairs are being evaluated for their ability to detect polymorphisms within the *C. passerinum* population at Wagner Natural Area. This project's findings will help contribute to the existing knowledge and conservation of *C. passerinum* individuals within and outside of the Wagner Natural Area.

Poster Presentations in Ecology and Evolution

Monitoring root uptake of microplastics into plants with an application for environmental transmission of infectious prion material

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The horizontal transmission of Chronic Wasting Disease (CWD), the prion disease that affects cervids, involves environmental factors. Soil and plant matter may act as reservoirs of infected material after an diseased animal has excreted bodily fluids into the environment. Soils contaminated with infectious material may facilitate root uptake of such material into the stems and leaves of the plant. Herbivores such as cervids are directly exposed to the aerial parts of plants and established findings have identified the uptake of amino acids, whole bacteria, and nano/microplastics by plant roots (Wright 1962, Gorbatsevich et al., 2013, Liu et al., 2022). To understand the uptake of protein molecules into the aerial parts of plants, we look at the uptake of non-infectious alternative mimics of similar size and structure to establish whether uptake is truly occurring and what method is best for monitoring such processes. This method can then be applied to understanding and further exploring the uptake of infectious prions into plants as it relates to environmental transmission. It is assumed that if the microbeads are uptaken through the roots, larger molecules such as prions could also potentially be uptaken to the leaves and stems, consumed by herbivores, and then horizontally transmitted. Fluorescently labeled carboxylated microspheres in the plant sections were observed using a confocal laser scanning microscope (Zeiss LSM 700) at the specific fluorescence excitation/emission wavelengths. The preliminary data indicates that microparticles may be uptaken and that such methods, with modification, may be applied to prion uptake within plants.

Woodpecker bioacoustic data optimization

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Acoustic data collected through the use of autonomous recording units (ARUs) is increasingly becoming a standard for wildlife monitoring. However, due to their infrequent vocalization rates, birds belonging to the group Picoides (woodpeckers) are understudied. This research hopes to gain insight of how to better sample for woodpecker species. To achieve this goal, ARUs were placed at locations throughout Alberta and scheduled to record at set intervals throughout the spring and summer months. Here I present my preliminary findings of woodpecker detection rates throughout the year. In this research, I explore how detection probability varies across the following woodpecker species: *Colaptes auratus* (Northern flicker), *Dryocopus pileatus* (pileated woodpecker), *Picoides pubescens* (downy woodpecker), *Leuconotopicus villosus* (hairy woodpecker), and *Sphyrapicus varius* (yellow-bellied sapsucker). By determining the time of year woodpeckers have the highest likelihood of detection, woodpecker species can be studied more accurately. This is especially important because woodpecker species are increasingly becoming the the focal point to many conservation efforts due to their role as keystone species.

How does individual variation affect movement? Two case studies using western Hudson bay polar bears (*Ursus maritimus*)

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Animal movement is a fundamental ecological process tightly linked to population energetics, access to resources and response to environmental change. While environmental factors that influence population movement have been well studied, the effect of individual factors (e.g. sex, age, reproductive status, body condition, personality) on movement dynamics is not well understood for the majority of animal species. I argue that understanding the effect of individual factors on movement would increase the accuracy of habitat selection or energetics models, and is especially important for migratory species or species experiencing environmental change. Here, I explore how individual factors affect movement in two studies on western Hudson Bay polar bears (*Ursus maritimus*), who migrate annually between the ice and the land and are severely affected by the shorter ice seasons resulting from warming temperatures. Using Argos GPS collar and eartag data, my first study tests whether sex and age affects speed, arrival/departure date and home range, and my second investigates the overlap between home ranges measured in the same individual for different years. I outline the experimental design of my studies, as well as their preliminary results and possible future directions.

A comparison of three invasive plants (*Agropyron cristatum*, *Bromus inermis*, *Poa pratensis* subsp. *angustifolia*) performance in their native range in Eurasia and introduced range in Canada to test for enemy release hypothesis

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The enemy release hypothesis (ERH) is one of the most prominent hypotheses to understand non-native plant invasions. It predicts that when a species is introduced into a new range, it is released from co-evolved enemies in its native range, such as soil pathogens, favouring a higher plant fitness in the introduced range compared to the native range. Despite its intuitivity, the ERH has received mixed support in empirical research. We verified the ERH in a growth experiment using three grasses that are native to Eurasia's steppe grasslands and non-native and invasive in North American prairie grasslands: *Agropyron cristatum* (crested wheatgrass), *Bromus inermis* (smooth brome), and *Poa pratensis* subsp. *angustifolia* (Kentucky bluegrass). We planted seeds from populations in the native and non-native range (14 seed populations for *A. cristatum*, 16 populations for *B. inermis*, and 17 populations for *P. pratensis* subsp. *angustifolia*) into sterilized soil inoculated by one of four treatments (local control soil, native soil, introduced soil, and control soil), yielding a fully crossed design of seed and soil origin (total number of pots: 752). We quantified germination success and plant growth via the maximum length of leaves, the number of leaves (assessed every two weeks), and above and belowground biomass (to be assessed at the end of the experiment). If the ERH is true, we expect to see the highest germination success and growth in the following order of soil treatments: control > introduced > native > and local control. Overall, this study would contribute to a better understanding of the invasion pathway and explain the invasiveness of these three invasive species.

Distribution and diversity of terrestrial isopods (Oniscidea) and their symbionts in Alberta

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Sowbugs (Isopoda: Oniscidea) have been introduced to North America from Europe throughout the past several centuries. Sowbugs have symbiotic associations with a variety of organisms, including parasites that have vertebrates as final hosts (Acanthocephala), and many species of sowbugs inhabit urban environments. Although sowbugs are undeniably present in Alberta, there are no published records of sowbugs from Alberta or other Canadian prairie province as of this date. It is also not known whether any sowbug-associated symbiotic species have been introduced to the province. Our preliminary collections have revealed four terrestrial isopod species in the Edmonton area (*Cylisticus convexus* (De Geer), *Trachelipus rathkii* (Brandt), *Porcellio spinicornis* Say, *Porcellio scaber* Latreille). The two potentially symbiotic taxa we have seen so far (Acari and Nematoda) likely represent phoretic and trophic relationships respectively. Future work includes broader surveys of the province to determine if other sowbug species are present in Alberta, and to evaluate whether sowbugs in Alberta are restricted to urban environments. Collected sowbugs will be examined for symbionts to determine whether any non-native symbiotic species are present, and whether sowbugs may be acting as hosts for any native symbiont species.

Post-management dietary dynamics in spottail shiner (*Notropis hudsonius*) and trout-perch (*Percopsis omiscomaycus*) from 2009-2013 in Lac la Biche, Alberta

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Piscivorous fish stocking in freshwater lakes across Canada is a popular restoration strategy used to sustain fisheries as they provide quick recreational fishing benefits. However, fluctuations in predatory abundance could impose top-down effects on lower trophic level fish species like spottail shiner (*Notropis hudsonius*) and trout-perch (*Percopsis omiscomaycus*) resulting in dietary shifts. In 2005, the Lac la Biche Alberta Fisheries Restoration Program was created to address depressed walleye (*Sander vitreus*) populations due to overfishing. Predatory double-crested cormorants (*Phalacrocorax auritus*) were culled and their eggs oiled, and walleye stocks were added to the lake in 2006 to restore walleye populations. The objectives of this research are to understand the diets of spottail shiner and trout-perch and to observe if the spottail shiner diet shifted after management. Stomach contents of thirty-five spottail shiners and ten trout-perch across Lac la Biche, Alberta from 2009-2013 were analyzed for four prey groups: macroinvertebrates, zooplankton, phytoplankton, and microplastics. Phytoplankton was the most consumed prey group in both spottail shiners and trout-perch, representing over 61% and 78% respectively of their diets. Phytoplankton and zooplankton consumption decreased over time whereas macroinvertebrate consumption increased. Sphaeriidae, Chironomidae, Chyrididae, and Stephanodiscaceae were consumed throughout all sampled years, although there were fluctuations in Cyprididae, Chironomidae, and Sphaeriidae between years, possibly demonstrating top down-effects on macroinvertebrate species prey abundance. An average of four microplastics were found in the stomachs of both trout-perch and spottail shiner and consumption remained stable throughout 2009-2013. Evidence of top-down effects on spottail shiner and trout-perch diets due to walleye stocking is still unclear, as other possible indicators like pollution, could pose potential alternative stressors on feeding behaviors of lower trophic level species. However, this study increases the knowledge of spottail shiner and trout-perch diets, fosters research on understanding this little-studied biota, and informs future policy and management practice.

Tooth replacement in snakes: Synapomorphy or chaos?

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Serpentes (snake-lizards) are the most diverse clade of lizards with nearly 4000 living species. All living species have highly modified skulls for increased cranial kinesis. Most of these modifications relate to unique feeding styles, generally involving the ingestion of large prey (i.e. macrostomy). One such adaptation to snake's feeding style is highly recurved teeth that help hold prey as they 'walk' their jaws over their prey to completely engulf them. The degree and consistency of tooth recurvature observed in snakes is not observed in other lizards and is considered a uniting feature. Early investigations of the tooth replacement in snakes demonstrated they developed in soft tissue epithelia and suggested that the crowns began development in an orientation parallel with their respective jawbones, changing in orientation as they moved to a functional position. Surveys of alcohol preserved, and dissected specimens corroborated this pattern, and it became engrained as another synapomorphy, characterized as horizontal tooth replacement. This, however, was never quantified for statistical relationships, nor observed in any living specimens. This leaves uncertainty around the validity of "horizontal tooth replacement" as a synapomorphy for all snakes as it may be a consequence of both the tooth recurvature and the postmortem effects on soft tissues housing replacement crowns. Using micro-CT scanners, thawed snake heads from frozen collections were scanned to examine their jaws and teeth. Representatives were selected from most major snake clades for taxonomic diversity. Functional and replacement crowns from each tooth family were segmented and their orientation to the jaw was measured. These angles were examined for their variance between jawbones and taxa for any patterns and significant changes in tooth crown angles throughout development into an attached tooth. Early results suggest a much more complex pattern of tooth replacement with a high degree of variability across taxa.

Stressful decisions: Effects of simulated herbivory on root foraging behaviour and plant growth in sunflower (*Helianthus annuus* L.)

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Optimal foraging theory broadly describes how organisms can optimize energy intake compared to output while foraging. While it is usually discussed regarding animals, the same principles can describe foraging behaviours in plants. In below-ground systems, plants can optimize foraging by preferentially aggregating roots into high quality nutrient patches compared to neutral or low quality patches, termed foraging precision. Under heterogenous soil conditions, sunflower (*Helianthus annuus* L.) is known to show high precision foraging in which individual plants aggregate roots in high quality nutrient patches. However, plants are subject to natural stressors such as herbivory that may influence the ability of an individual to forage precisely and will influence foraging decisions. Additionally, plants can tolerate herbivory with compensatory growth of tissues that allow a damaged plant to achieve around equal fitness as if it were undamaged. The goal of this experiment is to understand the impact of a discreet stressor on foraging precision and growth, and how this behaviour changes over time. To test this, 30 sunflowers were grown in window boxes and presented with a high quality nutrient patch and a control patch. After two weeks of growth, the stress treatment was applied, which simulated herbivory through clipping true leaves of the plants, and two levels of clipping intensity (high and low) were applied. The sunflowers continued to grow for 2 additional weeks following treatment to quantify changes in foraging behaviour and digital scans of the roots were taken throughout the growth period to compare root proliferation in patches. I predict that sunflower will show an immediate reduction in root foraging precision after application of the clipping treatment as a response to stress. Following treatment, root foraging precision should gradually increase over time until returning to the baseline precision, as the stressor is discreet which allows for recovery in behaviour.

Effects of host plant quality and microsporidian infection on forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) performance and disease susceptibility

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The cyclic population dynamics of forest tent caterpillar (FTC) (*Malacosoma disstria* Hbn.) (Lepidoptera: Lasiocampidae) are driven by a variety of factors including delayed-density dependent mechanisms such as disease. We measured the performance and microsporidia infection load of FTC when reared on four different diets, including trembling aspen foliage (*Populus tremuloides* Michx.), sugar maple foliage (*Acer saccharum* Marshall), a standard artificial diet, and an artificial diet fortified with lyophilized trembling aspen foliage to determine if diet interacts with microsporidia infection to alter FTC performance and their susceptibility to infection. There were no interactive effects between diet and microsporidia infection on adult performance of FTC, but diet affected FTC susceptibility to infection. Adult FTC had lower rates of infection when reared on fresh aspen foliage or an aspen-fortified artificial diet, compared to the other diet types. While diet and microsporidia infection do not interact to effect adult FTC performance, they may interact to effect larval performance as susceptibility to microsporidia infection varies by diet. The findings of this study help to increase our understanding of how disease and plant quality effect FTC and ultimately their population dynamics. Additionally, this study provides more information on tri-tropic interactions involving disease.

Poster Presentations in Immunology and Infection

The stimulation potential of mitochondrial DNA on cGAS in colorectal cancer subtypes

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Colorectal cancer (CRC) is the third leading cause of cancer death in Canada. The chromosome instable subtype (CIN) is characterized by chromosomal abnormalities leading to reduced patient prognosis because of lowered anti-tumor immune responses. Microsatellite instable subtypes (MIN), characterized by mutations in microsatellite regions of the genome, have an increased immunogenicity and therefore better patient outcomes. cGAS/STING, a cytosolic DNA (cyDNA) sensing pathway capable of inducing anti-tumor responses, may play a role in this enhanced immunity. Genetic instability in MIN may increase DNA release into the cytoplasm from the nucleus and mitochondria, leading to stronger immune response. Mitochondrial DNA (mtDNA) is of particular interest due to the bacterial origin of the mitochondria. Thus, we hypothesize

that mtDNA is more stimulatory of cGAS than nuclear DNA with an enhanced effect in MIN cells. Using mouse MC38 CRC cell lines with mutations in Mlh1 to model MIN and Kras and Rad51 for CIN, cyDNA stimulations were performed by delivering cyDNA to the cytoplasm of Vector MC38 cells with Lipofectamine 3000. MIN and CIN cells were first treated with ethidium bromide to deplete mtDNA to obtain mtDNA-depleted cyDNA. Different vector cells were stimulated with equal amounts of mtDNA-depleted cyDNA and nondepleted cyDNA to determine the stimulatory potential of mtDNA on cGAS. Western blot results of IRF7 and TBK1 phosphorylation shows decreased activation upon stimulation with mtDNA-depleted cyDNA indicating the potential for mtDNA to be more stimulatory than nuclear DNA in cGAS activation potential. To further test the stimulatory nature of mtDNA, isolated mtDNA will be used to increase the mtDNA proportions in our stimulations of vector cells. This will allow us to observe whether increased mtDNA in the cytoplasm enhances cGAS/STING activation. We hope to understand the mechanism behind the immunogenicity of MIN cells to aid in developing immunotherapy treatments for CRC patients.

Characterizing reovirus' adaptation to laboratory cell culture environments

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Mammalian orthoreoviruses ("reovirus") infect humans without causing disease and are repurposed into cancer therapies. Numerous laboratories have propagated reoviruses for decades leading to diversity between lab strains. The Shmulevitz lab previously found differences in viral replication kinetics and cytokine induction between lab strains, provoking the question of which features of laboratory reoviruses truly reflect "wild" reoviruses found in the natural environment. Moreover, will adapting wild reoviruses possess any unique features from laboratory strains that could benefit anti-tumour activities? Reovirus parental strains collected from primary effluent samples were passaged 3 times in tumorigenic L929 mouse fibroblast cells to permit adaptation. From the adapted lysates, large and small plaque-forming viruses were selected from one parental virus, and assessed for their ability to replicate in L929 cells. Reoviruses possess an inner and outer capsid, and uncoating of the outer capsid is critical for viral transcription and onset of infection. Binding and uncoating of the wild reoviruses were evaluated in L929 cells. Cell-binding activity was similar between the viruses although the small plaque-inducing viruses demonstrate more variable $\sigma 1$ protein levels, previously been associated with infectivity. The parental wild strain uncoats slower than the established laboratory strains and big plaque-forming viruses uncoat faster than small plaque-inducing viruses in L929 cells. Sequencing data showed mutations on the outward-facing surface of the $\sigma 13$ protein, possibly associating with the faster uncoating phenotype observed in big plaque-forming viruses. Together, these results suggest that wild reoviruses are maladapted to L929 cells and exhibit poor binding and uncoating. In three passages, large plaque-forming isolates reflect the ability to uncoat in L929 cells. In the future, we plan to 1) dissect the mutations in reovirus associated with adaptation to various cancer cell lines, 2) explore the cytokine induction profiles wild reoviruses to understand the relationship between reovirus genotype and virus-host interactions.

Generating antigenically-distinct and cleavage-resistant versions of therapeutic reovirus

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Mammalian orthoreovirus is a non-pathogenic virus with oncolytic abilities and is being explored for use as a cancer therapeutic. Some obstacles inspire exploration into how reovirus's oncolytic potential may be enhanced. One obstacle is that its cell attachment protein ($\sigma 1$) is inactivated by metalloproteases in breast tumors, causing reduced binding and infectivity. Another challenge lies with $\sigma 1$ being the dominant target of virus clearance by immune system antibodies. Neutralization of the virions upon subsequent doses may impede oncolysis. To overcome these challenges, we aim to generate an array of recombinant reoviruses with new $\sigma 1$ proteins from multiple diverse reovirus isolates using reverse genetics and co-infection. New reoviruses will be assessed for metalloprotease cleavage avoidance using Western blot analysis and replicative capabilities by standard plaque assays. The recombinant reoviruses generated here with diverse $\sigma 1$ may allow for multiple treatments without neutralization. Preliminary results have demonstrated the successful cloning of eight different wild $\sigma 1$ sequences into the reverse genetics system, but protein expression and virus rescue have yet to be optimized. As an alternative to reverse genetics, I am simultaneously exploring the use of co-infecting cells with both the lab strain and a wild strain of reovirus to produce reassortant viruses expressing wild $\sigma 1$. These viruses will also be assessed for metalloprotease avoidance and replicative capacity to observe their potential for overcoming obstacles in oncolytic therapy. By identifying diverse $\sigma 1$ proteins in reovirus possessing these qualifications, we will be poised to test

if they improve tumor clearance in breast cancer models. Thank you to the NSERC USRA for providing stipend support for this project.

Pathophysiology of hypereosinophilia in the brain

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Hypereosinophilia is a rare but significant condition, wherein eosinophils infiltrate cells, release inflammatory cytokines, and may lead to systemic organ and damage. It could precipitated by allergic diseases, infection, or hypersensitivity reactions and lead to rapid changes in mental status. However, the mechanism of central nervous system-related changes in patients with hypereosinophilic syndrome is still poorly understood. Here, we conducted a scoping review to synthesize current evidence in the literature. We conducted a review of the literature followed by a qualitative narrative synthesis following ENTREQ guidelines. Databases including PubMed/MEDLINE, EMBASE and Google Scholar were screened, and no time, setting, or language restrictions were imposed on the search strategy. Keywords in our search included: "eosinophilia", "mental", "consciousness", "psychological", "neurologic", "nervous system" and "HES". Primary research articles such as case studies, systematic reviews and meta-analyses, were included. Experimental and animal studies were excluded. We identified 14 articles that met our inclusion criteria. Of the 14, ten discussed the presence of mental changes including stroke, temporal arteritis, leptomenigeal dissemination, memory deficits and dysarthria associated with hypereosinophilia. Three were systematic reviews identifying 143 cases and evaluating outcomes and discussing risk factors. Two major mechanisms including encephalopathy, which can result in states of confusion, and multiple embolic strokes and/or an increased presence of ischemic lesions in the brain accumulated the greatest mentions in literature. Cerebrovascular disease was an important co-morbidity as well as a significant risk factor characterized or mentioned by 12 studies. The evidence on changes in mental status in patients with hypereosinophilic syndrome is currently limited and of low quality involving retrospective studies, case studies, and reviews; thus, more rigorous studies are warranted.

Exploring immune cell EVs: Identifying polysialic acid on human T cell extracellular vesicles

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Polysialic acid (polySia) is a long homopolymer constructed from α 2,8-linked sialic acid monomers. It has been identified as a post translational modification on cell surface proteins. It is well characterized in the context of the nervous system and the tumor microenvironment, where it shows involvement in cell migration, cell adhesion, and contact dependent differentiation. Its restricted expression has been shown to reflect host physiology and pathology. Recently, polySia has been identified to be present on various immune cells and has been demonstrated to be secreted by T cells. Despite these findings, polySia is still severely understudied and little is known about its expression on immune cell surface proteins and the role that it plays in the immune response. Herein, we show the secretion of polySia from T cells on extracellular vesicles (EVs) and explore possible roles for polySia in facilitating distant cellular interactions and in migration of EVs. Specifically, we show expression of polySia on the surface of Jurkat T cell EVs and primary T cell EVs using ELISA assays and Western blotting. This is the first demonstration of polySia expression on human T cell EVs, and our results lay down the foundation for further research examining EV's use of polySia.

Poster Presentations in Microbiology

Bioautography as a method to screen for antimicrobials compounds in noxious weeds from Alberta

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The growth of antimicrobial resistance poses a serious problem to global public health. Infection by microbes resistant to multiple antimicrobials is often associated with a higher mortality rate and treatment cost. As such, finding novel antimicrobial compounds is an important part of combating antimicrobial resistance. Many current antimicrobial drugs are derived from natural sources, mainly fungus and bacteria. Plants are often less studied and therefore offer great potential for the discovery of novel antimicrobials. Noxious weeds in Alberta are of particular interest because their phytochemical composition has not been fully studied, and they have been shown to disrupt soil microbe composition. One of the main

hurdles of studying natural products is the slow and inefficient extraction and isolation of bioactive compounds. TLC-bioautography aims to shorten the identification of bioactive compounds by coupling the separation and testing of compounds together. To this end, this project's aim is to develop a reliable method for bioautography in order to quickly screen for antimicrobial compounds present in plant extracts, in particular noxious weeds from Alberta. Our method for bioautography was developed using plant extracts with known antimicrobial compounds, such as oregano oil. We optimized the separation of the compounds on TLC before directly overlying it with Mueller-Hinton Agar. The agar was subsequently inoculated with bacteria and incubated before being treated with stains to visualize bacterial growth. Lack of bacterial growth was observed around spots on the TLC plate indicating antimicrobial activity. Overall, our preliminary results show bioautography has potential in quickly screening for antimicrobial compounds from natural sources. Having a method that can quickly screen for antimicrobials will help shorten the research time on our weeds of interest, and more broadly shorten the time it takes to isolate new and effective antimicrobials.

Identification of genes involved in complement resistance in *Burkholderia cenocepacia* K56-2

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Antibiotic resistance is continuously increasing amongst bacterial pathogens, making treatment difficult and leading to poorer outcomes for patients. *Burkholderia cenocepacia* is a Gram negative bacteria that is highly problematic because it is resistant to many classes of antibiotics. It is particularly dangerous to cystic fibrosis patients, and oftentimes the infection spreads into the blood. To be present in the blood suggests that this species has mechanisms to resist the complement system. The complement system is an important player in both innate and adaptive immunity, and involves many proteins which opsonize pathogens or cause their lysis. *B. cenocepacia* must avoid destruction by the complement system in order to be present in the blood, so it must have genes for complement resistance. The goal of this research project is to characterize the genes in *B. cenocepacia* involved in resistance to complement, by using a *B. cenocepacia* K56-2 plasposon insertion mutant library where the mutants of interest grow normally in LB but are static in serum. Through cloning the insertion sites from the chromosomes of these mutants, sequencing the insertion, and comparing the sequence to genome databases, the genes involved in complement resistance will be identified. So far, genes from the TraK family of proteins and polysaccharide biosynthesis proteins have been identified, and work is ongoing to identify different genes in other mutants. Future work will involve testing the role of these identified genes in complement resistance. For example, first the presence of a capsule around these mutants must be verified, and then experiments will be performed to determine its involvement in complement resistance. There is currently not much work being done on *B. cenocepacia* and its complement resistance, and this research can contribute to finding new targets for treatment of *B. cenocepacia* complement resistant infections.

The gut microbiota and its role in atopic asthma modulation

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Gut dysbiosis likely precedes the development of allergy. Murine models support the importance of gut microbiota in shaping immune maturation and tolerance. Gut microbiota may affect allergic asthma susceptibility by modulating type 2 immunity, influencing immune development and tolerance, regulating basophil populations, and promoting intestinal barrier function. Using a systematic review approach adhering to PRISMA guidelines, we elucidate and summarize some of the main mechanisms by which the gut microbiota may mediate allergic asthma development. These findings are important within the context of understanding clinical and therapeutic interventions to mitigate inflammation and poor prognostic markers in both paediatric and adult populations.

Poster Presentations in Molecules and Cellular Genetics

Assessing differential expression of enzymes in ticks following cold exposure

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The survival of overwintering ticks is influenced by several biochemical changes induced by the onset of cold temperatures. *Dermacentor andersoni* and *Dermacentor variabilis* ticks are able to increase levels of various biochemical components following cold exposure, however the mechanisms underlying these changes are unknown. This research focused on

identifying well-known metabolic enzymes in these ticks in order to establish a mechanism for the increase in metabolites observed following cold exposure. The enzymes explored included glycogen phosphorylase, trehalose-6-phosphate synthase, and phosphofructokinase. As specific nucleotide sequences for each enzyme are not known in *D. andersoni* or *D. variabilis*, degenerate primers were developed by searching GenBank for homologous sequences in related organisms. RNA to be processed to cDNA was extracted from individual *D. andersoni* and *D. variabilis* ticks following mechanical homogenization. The resulting cDNA was then subject to PCR using combinations of degenerate primers for each enzyme, and gene products were observed using agarose gel electrophoresis. From the pool of degenerate primers designed for PCR, three glycogen phosphorylase primer sets and one trehalose-6-phosphate synthase primer set produced promising results. These resulting DNA fragments were excised from the gels to allow for ethanol precipitation of DNA. Following the successful sequencing of the resulting DNA to establish the true sequence amplified, future research will focus on deploying cold treatments to assess if an increase in enzyme expression exists following tick exposure to cold temperatures. This research will reveal potential mechanisms underlying the observed biochemical changes in ticks, as well as provide a comprehensive research design to be used for future studies identifying other genes expressed in cold exposure.

Role of planar cell polarity on the closure of the superior fissure during eye development

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During development, a transient fissure forms within the inferior eye to guide vasculature into the eye. If this fissure remains open, a condition called coloboma develops, which causes about 10% of childhood blindness. We have reported the existence of a superior fissure in the eye in addition to the inferior fissure and shown that if it does not close properly, superior coloboma, a similar but distinct condition to coloboma, develops. We have identified six candidate disease-causing genes in both the core and the alternate planar cell polarity (PCP) pathways in a group of patients with superior coloboma. We have shown that loss of core PCP signaling components delays the closure of the superior fissure in zebrafish. As such, we hypothesize that the successful closure of the superior fissure requires both the alternate and the core PCP pathway and that loss of both core and alternate pathway components will result in failure of superior fissure closure. We are currently identifying downstream genes whose expressions are regulated by *vangl2*, a core PCP component, and the levels of expressed proteins in and around the superior fissure. We will perform multi-omics analyses using RNA-seq and mass spectrometry comparing the *vangl2*-mutant with wildtype zebrafish. I will also generate zebrafish mutants in our candidate alternate PCP pathway genes *fat2* and *fat4*, using CRISPR-Cas9. The formation and closure of the superior fissure will then be visualized to select mutant zebrafish with an abnormal superior fissure and perform multi-omics analyses. Finally, I will cross zebrafish mutants of the two pathways and perform multi-omics analyses to investigate the interaction and the combinatorial effects of both pathways on fissure closure. Taken together, this project will address the gaps in our knowledge of the causality of the superior fissure closure and the prevention, diagnosis, and treatment of superior coloboma.

Role of periocular neural crest stem cells in choroidal fissure closure

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The choroid fissure is a transient structure formed in the ventral eye during embryonic development. Failure of the choroid fissure to close results in ocular coloboma, a congenital ocular disorder characterized by gaps in several ocular tissues such as the iris or retina. In recent years, several different cell types have been implicated in choroid fissure closure, including the periocular mesenchyme (POM). Neural crest cells (NCC), a major component of the POM, can be found to migrate into the choroid fissure once it has formed, and there is increasing evidence to suggest that the NCC play an important role in the closure of the choroid fissure. Therefore, the purpose of this research is to observe the effects of removing NCC on gene expression in the choroid fissure. To identify genes expressed by NCC during fissure closure, zebrafish embryos were treated with caffeic acid phenethyl ester (CAPE) to prevent the formation of NCC or an equivalent volume of DMSO as a control. The heads of CAPE and DMSO-treated embryos were subjected to RNA-sequencing. Transcripts that were changed in mutants represent potential genes expressed in NCC during fissure closure. We are currently designing RNA probes for these targets to complete RNA in situ-hybridization to validate the expression of these transcripts in the POM. However, as CAPE indirectly inhibits NCC formation, the results need to be verified in another model. The results of these experiments will help to determine which candidates will be further explored. To address this, I will directly inhibit NCC formation by injecting zebrafish with *tfap2a* and *fox3* morpholino antisense oligonucleotides. I am currently in the process of optimizing the amount of each morpholino needed to eliminate all NCC in the fissure. Once this is achieved, I will conduct RNA sequencing

experiment to determine which genes are altered in the absence of the NCC. The results of these experiments will contribute to our understanding of the mechanisms of choroid fissure closure.

Effect of virulence factor protein FadA on WNT/B-catenin signaling in CIN and MSI colorectal cancers

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Colorectal cancer (CRC) is a heterogeneous disease that can be divided into two main subtypes, Microsatellite instable (MSI) and Chromosomal instable (CIN). CIN CRC is characterized by inherent genetic instability mainly caused by a mutation in the adenomatous polyposis coli (APC) gene whereas MSI CRC is characterized by deficient DNA mismatch repair (MMR) mechanisms contributing to the accumulation of mutations in microsatellite regions in the genome. CIN CRC is more abundant clinically and is associated with poor patient prognosis when compared to MSI CRC. Interestingly, both CRC subtypes differ in the regulation of a major signaling pathway, the WNT/B-catenin pathway, that is known to modulate cell growth and developmental processes in the cell. The role of WNT signaling in CRC is evident through aberrant regulation of the effector, B-catenin, and its subsequent localization to the nucleus to allow for expression of Wnt-related genes. Ubiquitous WNT activation leads to gene expression signatures associated with increased cellular proliferation and induction of cancerous migratory pathways. One of the ways in which the Wnt pathway can become dysregulated is through exogenous activation of the pathway independent of its normal activating stimulus. Recently, the pathogenic microbe *Fusobacterium nucleatum* has been implicated in accelerating CRC growth through its influence on the WNT/B-catenin pathway. We aim to elucidate the mechanistic basis of *F. nucleatum* pathogenesis through the virulence factor protein FadA and the associated consequences involved with how it contributes to CRC development by dysregulating WNT/B-catenin signaling. We will specifically study this interaction by utilizing murine CRC cell lines engineered to model MSI and CIN instable CRC subtypes. Our research aims to better predict patient prognosis as it relates to the influence of cancer-promoting microbes and genetic instability on the WNT/B-catenin pathway in CRC.

Poster Presentations in Physiology and Development

The effects of avobenzene on olfactory imprinting in zebrafish (*Danio rerio*)

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Fish use odorants such as amino acids to avoid predators, locate and navigate to their home spawning ground, and to find food. When larval fish are exposed to these odorants a lifelong memory can form in a process termed imprinting. When later exposed to these odorants as adults, the odorant will elicit an altered behavioural response comparatively to non-imprinted fish. This imprinting process coincides with larval neurodevelopment. A recent report outlined that avobenzene, an organic ultraviolet filter used in personal care products, disrupts proper neurodevelopment in zebrafish (*Danio rerio*). Imprinting's reliance on neurodevelopment leaves it susceptible to disruption when neurodevelopment is interrupted. This study aims to illuminate the extent avobenzene disrupts olfactory imprinting in zebrafish. Disruption to olfactory imprinting would decrease the fitness of these organisms, leaving fish susceptible to death as they take too shallow of a stream home, or unable to find a mate due to sexual selection. In this examination larval zebrafish were allowed to imprint to an amino acid mixture while being exposed to various concentrations of avobenzene. Then, as adults, their behavioural responses to the imprinted odourants were observed to determine if avobenzene disrupts olfactory imprinting. Lower concentrations of avobenzene induced an avoidance response to the imprinted odourants that elicited an attraction response in the control fish. At higher concentrations a neutral response was observed, indicating that imprinting was removed. Avobenzene, thus, does disrupt olfactory imprinting, and these personal care products need to be re-examined to limit human caused fitness declines.

Effects of cannabidiol exposure during early development on zebrafish escape response

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Cannabis is a commonly used drug and its use is increasing. There are concerns about the effects of cannabis use during pregnancy on the embryo's health. From previous experiments, exposure to cannabis or its metabolic by-products, including cannabidiol (CBD), during early development affects neural and motor development. This experiment specifically

investigates the effects of CBN exposure on auditory and mechanical escape response early in development. Zebrafish were exposed to varying concentrations of CBN immediately after egg fertilization until 24 hours post fertilization. At 5- and 6-days post fertilization (dpf), embryos were placed in an open field under a microscope and presented with an acute auditory stimulus. At 2 dpf, embryos were placed in an open field and presented with a mechanical stimulus, as auditory escape responses are not developed at 2 dpf. High escape response rates indicate functioning sensory hair cell and motor neuron development. Given the previous findings of CBN-induced alterations to sensory hair cell and motor neuron development, it is expected in both experiments that zebrafish will display altered escape responses to the respective stimuli. No significant trends have been observed in the preliminary results from the auditory escape response experiments. The full results for the auditory escape response are expected by the end of January and the mechanical escape response by early February. These results are important in further understanding and characterizing the effects of cannabis on neural development. While much research has focused on other compounds in cannabis, including tetrahydrocannabinol (THC) and cannabidiol (CBD), comparatively little work has focused on CBN. This topic is of growing importance; as cannabis use increases, the potential for use during pregnancy also increases.

Understanding the evolution of novelty: An exploration of the sex determination pathway of *Caenorhabditis tropicalis*

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The hermaphrodite/male *Caenorhabditis* systems, *C. elegans* and *C. briggsae*, are robust for the study of the function and the evolution of eukaryotic signal transduction pathways that regulate developmental decisions. In these species, the number of sex (X) chromosomes determines the sex of the organism, either male (X0) or hermaphrodite (XX), and their sex determination genetic pathways are well-defined. Under the assumption that sequence homologues to sex determination pathway genes found in *C. elegans* and *C. briggsae* will allow for the alignment of functional domains based on sequence conservation, this project will entail the use of a third male/hermaphrodite *Caenorhabditis* species, *C. tropicalis*, which is not closely related to either *C. briggsae* or *C. elegans* but has a male/female common ancestor with both species, and also possesses a closely related male/female sister species (*C. wallacei*). By studying the sex determination pathway of *C. tropicalis* and its underlying molecular mechanisms using a reverse genetics (CRISPR/Cas9) approach, we will determine if it adopts sex-determining strategies like *C. briggsae*, *C. elegans*, or neither. In doing this, we hope to address how genomes evolve novelty, what the purpose of these male/hermaphrodite sex-determining factors were in the male/female common ancestor, produce an overall greater understanding of the evolution of the sex determination pathway in eukaryotic systems, and further develop the robust *Caenorhabditis* system for future research in the evolution of the signal transduction pathways regulating developmental decisions.

Conflicts of Interest

The authors declare that they have no conflict of interests.

Authors' Contributions

MB: Communications for the R.E. Peter 2022 Biology Conference, coordinated with other universities to recruit student presenters and maintained communications with URNCST.

JS: Primary Event Organizer for the R.E. Peter 2022 Biology Conference, drafted the conference abstract booklet, and gave final approval of the version to be published.

MT: Secondary Event Organizer for the R.E. Peter 2022 Biology Conference, managed student information for dissemination on the R.E. Peter website and in the conference abstract booklet.

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