

Ontario Ecology, Ethology, and Evolution Colloquium

2021 Digital Conference Program

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OE3C 2021 Planning Committee University of Guelph

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How does wastewater effluent affect benthic macroinvertebrate communities in summer and winter?

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Effluent discharged from municipal wastewater treatment plants (WWTPs) is known to be a major source of contamination which can impact community structure, population size, and biodiversity of aquatic ecosystems. Despite the importance of understanding these impacts, few studies have assessed how wastewater effluent effects benthic macroinvertebrate communities in both summer and winter months. In this study, we aimed to assess the impacts of wastewater effluent on benthic macroinvertebrate communities near two WWTPs in Hamilton Harbour during summer and winter. Macroinvertebrate samples from Dundas and Woodward WWTPs were collected and enumerated to family level using various macroinvertebrate family identification guides. We found few seasonal biodiversity differences for abundance, family richness, family diversity, family evenness, and average tolerance. There were, however, strong increases and decreases in biodiversity with distance from the wastewater outfall, with the pattern dictated by the WWTP studied. The benthic macroinvertebrate community composition differed between seasons and with distance from the outfall for both WWTPs. These findings were further explored with similarity percentages analysis which identified family groups and functional feeding groups that majorly contributed to these differences between season and site. The results of our study give a better understanding of the impacts of wastewater effluents on benthic macroinvertebrate communities and aquatic ecosystems as a whole. We also raise questions about the cause of the observed biodiversity differences between the two treatment plants. As well, the results indicated few seasonal biodiversity differences in Hamilton Harbour. By conducting our study across seasons, our findings will further contribute to the recent and growing interest in winter ecology, and provides a new focus for ecotoxicology, a field where seasonal differences are seldom studied.

Impact of urban development on the long-term viability of a Blanding's turtle population

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Habitat change and fragmentation due to urbanisation have had negative impacts on turtle populations worldwide, and developers are often required to counter effects of habitat loss with mitigation measures, as a condition of project approval. We studied an urban Blanding's turtle (Emydoidea blandingii) population in Kanata, ON to assess whether urban development was compatible with long-term turtle population viability. We monitored turtle locations and survival in 2017-2020 (during ongoing development) using telemetry and mark-recapture methods, respectively. We compared our data to data collected in 2010-2013 (prior to major development) to assess whether population viability had declined through increased habitat loss, decreased survival, and lower population size. Between 2010 and 2020, wetland area and Blanding's turtle habitat suitability declined by 13.6% and 9.8%, respectively, in the study area. Concomitantly, the number of turtles visiting the development area was reduced by 25%, which was also reflected in a 30% decline in mean home range size of resident animals. Individual turtles studied during both periods (n=4) exhibited a 62% decline in estimated home range size. Blanding's turtle population estimates for the study area declined from 81 to 25 adults during the last decade, with declines being especially pronounced in the female cohort. During the study, several turtles were killed by vehicles on the main road bisecting the study area, and modeled impacts of one or two adult turtle road mortalities per year resulted in a 100% risk of population quasi-extinction during the next 50 years relative to a stable population experiencing no road mortality. Population viability analysis results suggests that two road mortalities per year could explain the decline in the population size over the past decade. Our interpretation of the relevance of these demographic changes relate primarily to the role of habitat loss in forcing turtles onto roads. We infer that our estimated road mortality rate is not sustainable and mitigation measures required as a condition of development approval (culverts, fences) were not successful in providing adequate protection for the turtle population. We conclude that currently, urban development and associated mitigation measures are not compatible with viability of at-risk

freshwater turtle populations, meaning that regulators must revise development approval conditions in order to remain compliant with Species-at-Risk legislation.

Is limiting similarity more evident for positively cooccurring plant species?

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Despite general acceptance of classical niche theory, few analyses of field data have produced evidence of limiting similarity, which postulates that resource restrictions limit niche overlap between coexisting plant species. Traditional assessments of species coexistence typically substitute with cooccurrence data representing snapshots of community composition. However, these data do not account for the temporary presence of negatively co-occurring species, which may be competitively excluded due to trait convergence and consequently higher niche overlap. Their inclusion in community data sets may confound assessments of limiting similarity, which expect lower niche overlap between co-occurring species. Reducing noise in community data sets may increase our understanding of plant community assembly and its drivers, allowing for the prediction of changes to such assemblages in response to species invasions and anthropogenic disturbances, among other factors. To test whether niche overlap differs between negatively and positively co-occurring species pairs, we compared presence-absence and trait data from 13 perennial herbaceous communities. As functional traits are generally expected to reflect species-specific niche requirements, mean trait distance data were used to infer niche overlap. We calculated mean trait distances for eight morphological traits, chosen due to their relevance in existing literature: maximum height, specific leaf area (SLA), leaf area, leaf dry mass, leaf dry matter content, leaf water content, leaf nitrogen content, and seed dry mass. Mean trait distances of all positive pairs were compared to those of all negative pairs within each site, as site-specific data for all eight traits were not always available and the number of replicates differed between sites. We expected that positive pairs would have higher mean trait distances, reflecting trait divergence as expected under limiting similarity. Although positive and negative pairs did not differ significantly for most sites and traits, significant differences in SLA, maximum height, and leaf area were found in some sites. In all these cases, SLA was lower and maximum height was higher for negative pairs, while leaf area differed between sites. The inclusion of more trait data may reveal similar relationships in the remaining sites and possibly support the use

of parametric tests. However, other factors, such as environmental gradients, could be more prominent drivers of community assembly. Future work may explore the implication of a trait-based null model approach, and whether altering significance levels of pairwise co-occurrence tests increases findings consistent with limiting similarity.

Influence of host species on unisexual *Ambystoma* communities

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Unisexual vertebrates are often regarded as evolutionary dead ends with weak adaptive capacities and low genetic diversity. This traditional understanding presumes that unisexuals lack sufficient diversity to adapt to a changing environment, which should limit both their spread across heterogeneous landscapes as well as their persistence following landscape-level perturbations. Several examples now illustrate that some unisexual systems can persist for extended periods. One such system is the unisexual Ambystoma which comprise a monophyletic all-female lineage of nuclear hybrids with a complex reproductive system that can produce communities of mixed-ploidy animals with variable genome dosages. Unisexual Ambystoma require sperm from a suitable host species for successful reproduction, meaning that these communities can only persist when they are syntopic with populations of their host. Importantly, presence of multiple sperm-donor host species can have multiplicative effects on the diversity of unisexual communities, and in this way contribute to the adaptive potential of unisexuals as well as their ability to persist in new and changing environments. On Pelee Island, unisexual *Ambystoma* are syntopic with two compatible host species, *A. laterale* (LL) and A. texanum (TT), together comprising an isolated and unique Ambystoma complex. From 2015-2018, tissue samples were collected and genomotyped from 1181 adults and 843 larvae at sites across the island. Genomotype composition varied significantly across site and was unassociated with spatial proximity. Diploid unisexuals (LTs) made up the largest fraction of salamanders at all sites. Average ploidy was generally higher in larvae than adults at any given site. Overall, A. texanum made up only ~4.3% of all samples, and A. laterale made up ~0.15% (i.e., 3 individuals). The relative abundance of diploid sperm donors at a given site ranged from 0.3%-12.5%. Comparison to samples collected 30 years ago indicates that these communities have shifted significantly in composition. A. laterale has been lost from some historic sites, and the relative abundance of L-biased unisexuals (e.g., LLTs) has declined. Our results highlight that unisexual community structure varies over time and space, likely in accordance with variation in the underlying biotic and abiotic habitat conditions. This work

illustrates that unisexuality is not necessarily an evolutionary dead-end when access to genetic material from syntopic host species enable unisexual communities to diversify and match their current environment.

Effect of mode of larval development on the demographic responses of four northeastern Pacific intertidal gastropods to the extreme 1997/98 and the 2015/16 El Niño events

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Life history traits that simultaneously affect dispersal ability could have large effects on the demographic and evolutionary responses of species to global climate change. Yet long-term population studies of ecologically similar species that differ in life-history traits are rare. Four species of a model genus of herbivorous gastropods (Littorina spp.) occur on Northeastern Pacific shores. Two species release egg capsules that develop into free-swimming larvae and two species develop directly from attached egg masses into crawling juveniles. I compared the changes in the abundance and size-structure of *Littorina* with different modes of development to natural environmental variation between 1993 to 2020. Two extreme El Niño events, 1997/98 and 2015/16, occurred during that period and both caused increased poleward transport of larvae in winter by the El Niño-augmented Davidson current. These extreme events were correlated with a significant increase in recruitment of the two species with planktotrophic larval stage that was synchronous at two sites. The second event was also correlated with a large decrease in the normally abundant direct-developing species which, may have been caused by increased thermal and desiccation stress during emersion exacerbated by reduced mean wave-height. This differential effect of extreme El Niño events on the population dynamics of marine species that vary in mode of development has not been described previously and should be incorporated into predictive models of climate change.

Priority effects alter interaction outcomes in a legumerhizobium mutualism

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Priority effects occur when the order of species arrival affects final community structure. Mutualists often interact with multiple partners in different orders, but if or how priority effects alter interaction outcomes is an open question. In a natural field setting, we paired the legume Medicago lupulina with two nodulating strains of Ensifer bacteria that vary in nitrogen-fixing ability. We inoculated plants with strains in different orders with a time lag, and then measured interaction outcomes. The first strain to arrive primarily determined host plant performance and final relative abundances of rhizobia on roots (i.e. final community composition), indicating priority effects altered the outcome for both partners in the symbiosis. Plants that received effective microbes first and ineffective microbes second grew larger than plants inoculated with the same microbes in opposite order. We discuss how two mechanisms of priority effects, niche preemption and niche modification, may explain the trends we see in this legume-rhizobium symbiosis. Our results show that mutualism outcomes can be influenced not just by partner identity, but by interaction order, demonstrating how community ecology theory has importance in host-microbiome interactions and plant health. Furthermore, the result that microbial priority effects can influence the outcome of a mutualism is relevant to the evolutionary and ecological maintenance of variation in mutualist quality. Hosts receiving high-quality mutualists early can better tolerate low-quality symbionts later, indicating priority effects may help explain the persistence of ineffective symbionts by reducing the cost to the host. On a broader scale, the composition and abundance of soil microbes are heterogeneous across space, and may change seasonally, providing opportunities for priority effects to affect the success and distribution of plants across the landscape. When a plant colonizes a new site, associating with an effective partner first could mitigate costs of associating with less-effective partners later that may not be adapted to the host. By contrast, when a plant colonizes an environment where effective partners are less frequent,

the plant could associate with ineffective partners first and experience priority effects that reduce its performance regardless of future effective partners, constraining plant distribution. Overall, interaction order may play an important role in plant-microbe symbioses, plant health and distribution, and the maintenance of variation in partner quality.

The effect of temperature on *Ceratina calcarata* offspring body size

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Insects reared at high temperatures are oftentimes smaller than those reared at cool temperatures, a trend called the Temperature-Size Rule. Bees offer a unique perspective to studying the Temperature-Size Rule since many species are mass-provisioners. This means that their adult body size is directly constrained by the size of the one-time pollen mass provided to them by their mother. Temperature during development may also affect final offspring body size in bees through a change in cell size or number. Ceratina calcarata is a small, solitary, mass-provisioning carpenter bee native to eastern North America that nests in the exposed pith of raspberry canes. The objective of this study is to investigate the effect of developmental temperature on C. calcarata adult body size and to assess whether mass-provisioning bees conform to the Temperature-Size Rule. We hypothesized that if bees reared at different temperatures are different sizes, and that difference is not due to maternal input, they must be different sizes due to developmental temperature. To test this hypothesis, we used trap-nests to catch C. calcarata females and then moved these nests to sunny or shady locations. We recorded the internal nest temperature, monitored the mothers' foraging activity, and measured the final size of their offspring. We made molds of the compound eyes of the offspring using clear nail polish, and using a microscope, measured the size of 30 of their centroid ommatidia to calculate their average ommatidia size. After accounting for other factors known to influence body size in C. calcarata, we found a significant treatment effect; offspring born in the hotter (ANOVA; $F_{(1,256)}$ =17.73, p<0.001) sunny nests were significantly smaller (LMM; $F_{(1.57.15)}=2.81$, p=0.099) than those born in the shade. Additionally, average ommatidia size did not differ between treatments (ANOVA; $F_{(1,34)}=1.34$, p=0.255), suggesting that the difference in body size is likely due to a decrease in the number of cells rather than a reduction in cell size. This indicates that C. calcarata conforms to the Temperature-Size Rule. Since the mass-provisioning behaviour of mothers did not differ between treatments (LMM; Pollen mass; $F_{(1.5.75)}=1.15$, p=0.327. ANOVA; Foraging time; $F_{(7.5)}=0.529$, p=0.786), body size differences are due to nest microclimate

alone. Previous research has found that larger bees have higher survival during overwintering, are able navigate faster, carry more pollen and fly further. Since larger individuals have higher fitness than smaller individuals, the nesting location chosen by a mother may have fitness consequences for her offspring.

Scientific telephone: How an estimate of lichen ecosystem dominance became "fact"

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Proper citation of peer-reviewed research is one of the pillars of scientific knowledge and progress. When there is a breakdown in this process, the result is at best confusion, and at worst misinformation leading to action or lack thereof, causing harm. Here we present an example of "scientific telephone", where a commonly cited statistic about lichen ecosystem dominance (an estimation that 8% of the world's terrestrial surface is dominated by lichens) has become misconstrued and misused through time. We traced the original source of the statistic back to two papers published in 1987 and 1995, neither of which were peer-reviewed. We then tracked all citations in peer-reviewed literature of those papers for the statistic (63 papers in total), some of which became a citation source instead of the original articles. We identified at least 9 instances of the statistic's use in popular science media, including in a recent widely publicized mycology book. We found that citations for this statistic have slowly increased through time, and there was a notable increase in citations upon its inclusion in a popular lichen biology textbook published in 2008. We also found that the intent and nuance of the stated statistic has changed, with earlier works hedging the number as an estimate and later works stating it with more certainty. Most alarmingly, we have found several instances of the 8% statistic itself being altered, with anywhere from 6-10% reported, despite citation of the original source. This is significant because lichen terrestrial dominance is a commonly used statistic to garner the public's interest in lichen biology, and because it implies that this question of how much of the world is dominated by lichens has already been answered when it has not, deterring future researchers from approaching this interesting and significant research avenue. Importantly, this case serves as a warning and example of the power of misinformation even through the peer review process, and how we as researchers, reviewers, and editors must take care to be thorough, vigilant, and check our sources.

Novel biodegradable tracer particle to study aquatic dispersal processes

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The effects of land use changes on stream hydrology and riverbed habitat are pervasive for aquatic organisms because these affect the transport of biologically relevant particles such as propagules (e.g., gametes, larvae, and fragments) and those containing nutrients or contaminants. Freshwater mussels may be especially sensitive to these changes since their juveniles are transported freely in the water column and must settle in viable habitat on the riverbed to recruit. Unfortunately, it can be difficult to study these systems directly for a number of reasons. Physical models are used to study hydrodynamically-mediated dispersal when it is not feasible to release live organisms or their propagules. Large quantities of non-biodegradable particles are required, which can create environmental problems. Consequently, we developed a novel biodegradable and non-toxic physical model (alginate microbeads) with modifiable size and density. The microbeads were designed specifically to simulate the physical characteristics of juvenile freshwater mussels ($\rho = 1200 \text{ kg m}^{-3}$) (Unionidae) and a neutrally buoyant particle ($\rho = 1000 \text{ kg m}^{-3}$) to model their dispersal in the field. We released the microbeads at a site with measurements of high-resolution riverbed elevation survey data. Microbead captures in drift nets and specially designed sedimentation traps revealed patterns of transport and entry into the riverbed. The neutrally buoyant microbeads drifted farther downstream than the ones modelled after mussels. The decline in microbead capture rates with distance downstream correlated with negative exponential and power model predictions, which is consistent with dispersal studies on larval mussels and other taxa. A mathematical model that accounts for turbulence offered similar predictions to the simpler exponential and power models. The physical model developed in this study provides an environmentally-friendly resource to study the dispersal of aquatic organisms and propagules. This study informs management and conservation efforts on population augmentation planning, and how land use changes to river habitats may affect dispersal. The facile customization of biodegradable polymers makes them ideally suited to study other

dispersal phenomena in the field, as their potential uses reach beyond the microscopic and nearneutrally buoyant particles featured in this study.

Ectoparasite community assemblages found on Peromyscus maniculatus across varying degrees of ixodid tick exposure

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Ectoparasites are fundamental to ecosystems, playing a key role in trophic regulation. Fleas, mites, and ticks are common hematophagous ectoparasites that infest shared mammalian hosts. One common host in Ontario, Canada, is the deer mouse (Peromyscus maniculatus). As the climate warms and the geographic ranges of blacklegged ticks (Ixodes scapularis) and wood ticks (Dermacentor variabilis) expand, their introduction to new ecosystems may alter current ectoparasite communities. At three different sites where exposure to ticks varied (both in terms of tick diversity and abundance), ectoparasite community structures found on deer mouse hosts were compared and analyzed, focusing on species co-occurrences and habitat partitioning on the host. We predicted that when varying tick species were prevalent, ticks would dominate the micro-habitat niches often inhabited by other parasites, thereby significantly altering parasite community structure. Our results suggest that blacklegged ticks and wood ticks could have a positive association with each other, but a negative or random association with other ectoparasite species, even when they do not occupy the same micro-habitat niche. Sampling site played a significant role in community assemblages as well, possibly due to the differences in tick exposure. As the ticks' ranges expand and they become more abundant, it is important to understand how their prevalence can potentially alter the dynamics in an ectoparasite community, affecting the transmission of pathogens that may spread within an ecosystem, from one host to another.

Impacts of hybridization by domestic apple (*Malus domestica*) on native crabapple (*Malus coronaria*) in southern Ontario

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Introduced species, including those domesticated for food production, can have diverse impacts on native biological diversity. For example, through hybridization, domesticated species can have negative genetic and demographic effects on native congeners and may alter the balance between asexual versus sexual modes of offspring formation. However, the relative importance of these effects and their implications for native species' abundance and persistence are poorly understood. In southern Canada, domestic apple (Malus domestica), a diploid species originating in Eurasia, regularly hybridizes with the native, tetraploid, sweet crabapple (Malus coronaria), which is capable of sexual and asexual seed production. While there is little evidence of introgression into the crabapple genome, the impacts on conspecific seed production and asexuality are unknown. My study examines the impacts of hybridization by M. domestica on intraspecific M. coronaria seed production and asexual seed formation. Four pollination treatments (open pollination; open pollination plus supplemented M. coronaria pollen; M. coronaria pollen only; and M domestica pollen only) were applied to each of 26 M. coronaria trees at rare Charitable Research Reserve. Resulting fruit were assessed for seed production, and the source of pollen (intra vs inter specific) and mode of embryo formation (sexual vs asexual) for each seed was inferred from the ploidy of embryo and endosperm tissue estimated using Flow Cytometric Seed Screening (FCSS). With the sexual hybrid seeds, DNA extraction, microsatellite analysis, and parental exclusion methods are being used to determine the source of hybrid (M. domestica) pollen, recognized varieties or feral domestic trees. To evaluate the demographic impacts of hybridization, I examined the relationship between frequency of hybrid seeds and conspecific seeds among fruits and trees. To examine the impacts of hybridization on asexual seed formation, I examined the relative frequency of asexually (vs sexually) formed embryos in hybrid vs non-hybrid crabapple seeds. Preliminary analyses suggest that there is no demographic cost of hybridization since hybrid seeds are not produced at the cost of conspecific seed. However, there

appears to be evidence that hybridization does affect the proportion of seed that are asexually derived, which may represent a unique consequence of hybridization by domesticated species on a native congener. This study will provide novel insights into impacts of hybridization in plants, which may have significant impacts on population dynamics and genetic structure of native species.

David vs. Goliath: Does competition influence plant size?

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Plant competition is considered an important force for regulating species coexistence in highly productive communities. According to traditional theory, a large body size is indicative of a competitive advantage. However, this notion of a large mature size being advantageous (the 'Size Advantage' hypothesis) contrasts with basic observations that small species are ubiquitous and abundant within plant communities. If competition tends to be intense in natural, crowded environments, and large species are competitively superior, why are there so many small species? In order to test the Size Advantage hypothesis, we sought to determine if more intense competition among neighbours contributes to a loss of small species. We performed a neighbour removal experiment using transplants in an old-field community to quantify competitive intensity and importance of treatment plots. Competition metrics were assessed for each plot using the dry aboveground biomass of each transplant collected at the end of the growing season. Plots in our study community are characterized by having high competition intensity and moderate-high competition importance. Using multiple linear regression analyses, we found no evidence that competition intensity or importance in a given plot had any effect on mean height. Instead, other factors like the proportion of native species in a plot, plot-level available nitrogen, and plot-level phosphorous had a significant negative relationship with mean height. Therefore, having a large body size may contribute to less efficient resource use, which limits large species membership in plots with lower phosphorous and nitrogen. This research clarifies the role that plant size plays in competition and coexistence. Examining the relationship of size advantages and species loss can also add to our understanding of ecosystem diversity and community assembly rules.

Spatiotemporal variability of core and rare microbiota in Tamiasciurus hudsonicus microbiomes

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Microbial communities are becoming an increasingly accessible and interesting avenue of research in long-term ecological study systems. With an existing microbiome dataset from the Kluane Red Squirrel Project, we tested four hypotheses regarding the drivers of fecal microbial community composition. Our four hypotheses include: (1) core microbiota are essential functional members of the host-associated microbiomes of a given species, (2) rare microbial taxa are functionally additional or accessory to the core microbiota of a host-associated microbial community, (3) the movement of microbiota between hosts increases as time progresses throughout the sampling season, and (4) seasonal variations will alter the distribution of core and rare microbiota in hostassociated microbial communities. To test the predictions associated with each of our four hypotheses, we initially processed the amplicon sequencing data from Ren et al (2017) using the QIIME2 bioinformatics platform. After sequence processing with QIIME2, performed multivariate statistical analyses in R. Our statistical analyses included local regression to assess the temporal distribution of microbiota and principle coordinates of neighbour matrices to identify potential spatial patterns in the physical distribution of microbiota. We replicated all analyses for both the core microbiota, which are the most common microbiota across all microbiome samples, and the rare microbiota in all the red squirrel microbiome samples. We have confirmed several contributors to microbial community assembly for both the core and rare groups of microbial taxa. The location of the sampled squirrels in their local environment has been identified as a key contributor to ecological dynamics in both core and rare microbiota. Core microbiota, identified using an occupancy-abundance model, were observed to vary less along spatial gradients than the rare microbiota. The distribution of rare microbiota within the red squirrel microbiome metacommunity was highly localized in each host population. In addition to the localize spatial variation, the presence of rare microbiota in microbiome samples varied more seasonality than that of the core microbiota. Specific red squirrel behaviours that occur seasonally, such as mating and pilfering, are

the likely contributors to the spatial and temporal distribution of all microbiota. The spatio-temporal replication of the data in our study was an essential component to understanding assembly of microbial communities. Incorporating microbiome data into long-term ecological research such as the Kluane Red Squirrel Project is essential to getting a comprehensive view of the health of the entire system.

Ecosystem-level CO2 responses to increased atmospheric nitrogen deposition in an Ontario meadow hayfield of varying soil texture

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The ongoing increase in atmospheric deposition of nitrogen (N) derived from fossil fuel burning into ecosystems has important implications for soil health, species diversity, and nutrient cycling, impacting the long-term stability of many ecosystems. Grassland ecosystems are one of the most important terrestrial carbon sinks that could be utilized to mitigate climate change due to their high productivity and rapid turnover of fine roots, resulting in high potential for enhancing carbon inputs into sequestered soil carbon pools. Eastern Ontario is currently experiencing atmospheric N deposition levels of approximately 10 kg N ha⁻¹ yr⁻¹. The long-term consequences of enhanced atmospheric N deposition on grasslands are difficult to predict because their responses to increased N availability may be strongly influenced by factors such as soil texture and moisture availability and ecosystem-level responses to these factors are not yet fully understood. The objective of this research is to determine the relative impacts that low-level N additions (simulated 2050 atmospheric N input rates) and soil texture will have on net ecosystem exchange (NEE) and its component fluxes gross ecosystem production (GEP) and ecosystem respiration (ER). The simulated enhanced atmospheric N deposition experiment was set up in 2005 and consists of six replicate treatment and control plots on both clay-based soil and sand-based soils within a meadow hayfield in eastern Ontario. The component fluxes of NEE (GEP and ER) were measured once per week over the growing season (6) times) using a LI-COR 6400 infrared gas analytical system fitted with a customized ecosystem flux chamber (30cm height; 706.9 cm² area). The impacts of the N addition treatment on ecosystem fluxes are compared between the two soil types. GEP and NEE fluxes are correlated with shoot biomass in the collars and ER with soil moisture, soil temperature and root biomass. Results indicate that NEE and its component fluxes are determined by the interaction between N addition and soil texture, with the latter factor having the strongest influence. Soil texture strongly influences moisture and nutrient availability, limiting plant growth in sand-based soils and causing a significantly lower capacity to take

up carbon compared to clay-based soils. The results of this study will improve our understanding of the interactive effect of soil texture in determining the responses of meadow grassland plant communities to future atmospheric N deposition, and therefore will improve management capacity to optimise species diversity for conservation value and carbon storage.

Enhancing selective fish passage by manipulating discharge through a fishway: An experimental field test

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Barriers are pervasive in aquatic habitats, and their presence fragments the connectivity of aquatic habitats. Barriers such as culverts, road crossings, and dams limit the movement of fishes between habitats required for feeding, breeding, and rearing offspring. Additionally, fragmented subpopulations can be subjected to environmental and demographic stochasticity. These impediments to movement reduce gene flow, inhibit breeding, and increase juvenile mortality, resulting in the loss of species diversity, life history diversity, and ultimately biodiversity. While the removal of in-stream barriers can improve desirable fish populations by restoring habitat connectivity, doing so can also threaten these populations by allowing invasive species to disperse and proliferate. Selective fish passage can restore habitat connectivity for desirable fishes while selectively blocking the movement of invasive fishes like the sea lamprey (Petromyzon marinus). While selective fish passage is effective in restoring connectivity to desirable fishes without allowing invasive fish to escape, it is costly and time consuming for management agencies to manually sort sea lamprey out from desirable fishes. These costs can be mitigated by having fish passively sort themselves. River discharge is a stimulus that may induce sorting, as it has been shown to attract sea lamprey and deter recreational fishes like salmonids and suckers. We tested if manipulating discharge through a two-chambered selective fish passageway could sort desirable fishes into one chamber, and sea lamprey into the other. A field experiment was conducted at Big Carp River near Sault Ste Marie, ON during the sea lamprey spawning run. The spawning run was divided into two-night blocks with high and low discharge treatments assigned to each night. Daily catches of sea lamprey and desirable fishes were quantified. Over 18 treatment blocks, it was found that the degree of passive sorting on nights of high discharge did not differ from nights of low discharge (discharge = -0.01, 95% CI [-0.44 - 0.42]). While discharge was found to be ineffective at inducing passive sorting, there is evidence of discharge impacting the total catches of fishes. Over the course of the experiment, total catches of sea lamprey were 159%

higher on nights of high discharge, whereas the total catches of desirable fishes were 164% higher on nights of low discharge. These results suggest that sea lamprey can be separated from desirable fishes prior to entrance into a fishway by using high discharge to attract sea lamprey to a separate entrance.

Changing forests and the bird species living within them

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Three-dimensional habitat structure provides the resources necessary for many birds to meet their life history and behavioural needs. Forest are dynamic environments, experiencing disturbances, subject to biological invasions, influenced by climate change, undergoing succession, and subject to other natural and anthropogenic changes. This study examines the effect of forest succession, i.e., the directional change in tree species composition and three-dimensional structure over time, on bird distributions. Of particular interest are Chiffchaff (Phylloscopus collybita) and Willow warbler (Phylloscopus trochilus), two breeding habitat specialists preferring mature forest and early successional forest, respectively. As forests undergo succession, the distribution of both species is expected to change as birds move to their preferred habitat. Changing forest structure can be examined using airborne laser scanning (ALS), a type of a remote sensing data that produces a threedimensional point cloud from which structural attributes can be derived. The goal of this study is to identify ALS-derived structural variables describing the habitat where Chiffchaff and Willow warbler occur, and then characterize and compare those three-dimensional spaces. This is accomplished using bird survey data collected annually during the breeding season across the Cambridgeshire (United Kingdom) landscape between 1999 and 2017, and ALS data collected in 2000, 2005, 2012, and 2015. Structural variables were extracted from ALS data for the full vertical profile and for three strata (<2m, 2-8m, >8m). The relationship between Chiffchaff and Willow warbler occurrence and ALS structural variables is modelled using random forests. Accuracy is assessed using the out-of-bag error estimate (OOB) and area under the receiver operating characteristic curve (AUC). Important variables identified by random forests are then projected onto the Cambridgeshire landscape to examine distribution changes over time. Accuracy for the Chiffchaff random forests model were reasonable with OOB=31.65% and AUC=0.75. The three most important variables for Chiffchaff are foliage height diversity, mean height >8m, and mean height <2m. Willow warbler accuracy was good with OOB=13.84% and AUC=0.94. The three most important variables for Willow warbler are mean height >8m, mean height <2m, and skewness <2m. Chiffchaff habitat is characterized by a higher foliage height diversity than Willow warbler, and a substantially greater mean height >8m. There was no

significant difference in mean height <2m, nor for skewness <2m. Projecting onto the landscape, changes in bird distribution are best observed in early successional forest patches. These are more dynamic spaces, actively undergoing succession with a greater rate of forest structural changes.

Describing turkey (*Meleagris gallopavo*) primary wing feather growth: implications for biomarker measurement

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It is now common to use feathers to non-invasively measure physiological biomarkers in both wild and domestic avian research. Biomarkers, such as glucocorticoid hormones, are believed to be deposited in the feather structure in a time-dependent manner throughout feather growth. If this is true, then the rate of deposition, and concentration measured in the feather, must be affected by feather growth rate and molting patterns. To make inferences about factors affecting the deposition of substances in the feather, it is important to know when the feathers appear and when their growth ceases. The objective of this study was to provide an updated account for the juvenile primary feather molting pattern and feather growth rates for domestic turkeys as part of a larger study investigating feather corticosterone as a novel welfare phenotype. The ten primary wing feathers (P1 - P10) of 48 female turkeys were measured weekly from week 1 (0 days of age) to week 20. Feathers were manually measured, and the presence or absence of each primary feather was recorded weekly. Generalized linear mixed models were used to investigate if feather growth differed between the primary feathers and to provide estimates for feather growth rate. The molting of the juvenile primary feathers followed a typical descending pattern starting with P1 (5 weeks of age) while P9 and P10 had not molted by the end of the study (20 weeks of age). Average feather growth rate was 2.4 cm/week, although there was a significant difference between the ten primary feathers (P<0.0001, 2.1 – 2.8 cm/week). Over time, the rate of feather growth was not constant; feather growth reached a peak soon after emergence and then declined until the feather molted. This study provides a critical update of the pattern of molting and feather growth in primary wing feathers of modern turkeys. This can have implications for the interpretation of physiological biomarkers in this species, such as the longitudinal deposition of glucocorticoids.

The effects of multiple stressors on the ecophysiology of Lampsilis siliquoidea: Effects and interactions among water temperature, velocity and suspended solid concentration

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Freshwater unionid mussels are considered ecosystem engineers in aquatic ecosystems, but they are highly endangered because of land-use changes that have altered their habitat and negatively impacted their ecophysiology. The environmental factors that affect mussels do not act alone, rather they interact, and are better understood using a multiple stressor approach. The goal of this study was to examine how changes in water temperature, turbidity (total suspended solids; TSS) and velocity affected the clearance rates (CR), oxygen consumption rates (OC), and resultant Scope for Growth (SFG) of Lampsilis siliquoidea in laboratory experiments. The CR, OC and SFG of L. siliquoidea increased with acclimation temperature and velocity, and decreased with TSS concentration and acute temperature exposure, although these responses were more variable when factors were combined. The primary factor affecting CR and OC varied with acclimation temperature, with warmer temperature and high TSS leading to strong declines. A worst-case scenario would involve a summer season where temperatures and TSS loads are above-average, and water velocities are either belowor above- average, with increased frequency of storm, flood, or drought events. These situations, which are unfortunately becoming more common through the consequences of climate change and land-use changes, would describe the worst possible effects on freshwater mussel feeding, respiration and scope for growth. This makes it imperative that protection plans account for these potential scenarios and protect unionid habitat so as to preserve not only species at risk, but also the freshwater systems they inhabit. The multistressor approach provided a more ecologically-relevant way to examine organisms in nature as well as insight into ways to protect and recover their population.

Urbanization and landscape evenness drive patterns in cavity-nesting bees and wasps from residential gardens

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Urbanization filters species and communities across landscapes, with some more affected than others. We explored patterns in bee and wasp diversity in relation to urban land cover to determine to what extent urbanization alters diversity and community composition. Bees and wasps are important pollinators and predators, respectively, contributing to resilient ecosystem functioning. Understanding the impacts of urbanization on bees and wasps will contribute to their conservation, habitat protection and ecosystem services that benefit people. We focused on cavity-nesting bees and wasps and used trap nests to sample them at 104 residential gardens across the GTA (Greater Toronto Area) from May to October 2019. This survey was in partnership with the David Suzuki Foundation's community science pollinator initiative, the BIMBY ('Bees in my Backyard') Project. Using open data sources available from the City of Toronto, three land cover types of were extracted for each garden: open green space, closed (forested) green space, and impervious surface cover. Total land cover diversity and evenness were also calculated per site. Bee and wasp richness, abundance, and community diversity were analysed in response to each of the land cover metrics within a 200 m and 500 m radius of the nest site using generalized linear models. We collected a total of 7471 brood cells belonging to 14 genera including 4278 brood cells from 6 bee genera, and 3193 brood cells from 8 wasp genera. We found that diversity for both taxa was best explained by a significant negative relationship with impervious surface cover at both radii (p=0.015 and p=0.017 at 200 m and 500 m, respectively). Impervious surface (negative) and land cover evenness (positive) were the best predictors of cavity-nesting bee and wasp abundance and richness. Bees had a stronger relationship with land cover evenness in residential gardens presumably due to higher floral availability. On the other hand, wasps were negatively impacted by impervious surface in all top models. Many wasps collect phytophagous arthropods as prey which we expect to be more abundant in urban forests and parks. Our findings illustrate the value of urban residential gardens as refuges for cavity-nesting bees

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and wasps, which were negatively impacted by urbanization. Considering the mosaic of different land cover types (landscape evenness), including urban forests and parks near to residential areas will support these taxa and their essential ecosystem services.

Short-term intensive warming shifts predator communities (Parasitiformes: Mesostigmata) in Boreal forest soils

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Increasing global temperatures from climate change represents one of the greatest threats to the natural world. Temperature increases will coincide with longer and more frequent short-term, extreme warming events (e.g., heat waves), particularly at northern latitudes. Habitats at northern latitudes, like Boreal forests, are predicted to have drastically new temperature regimes because of this. Boreal forest soils contain a hyperdiverse array of microarthropods and nematodes, creating a densely connected food web. Atop of the soil food web are mesostigmatid mites (Parasitiformes: that provide top-down control on other mites, collembolans Mesostigmata), nematodes. Although extensive research exists on the effect of climate warming on oribatid mite and collembolan communities, fewer studies have examined the effect of warming on Mesostigmata communities. We tested the effect of short-term (three months), intensive warming (+8°C) on Mesostigmata communities from the Boreal forest using experimental mesocosms. We collected forest-floor material from a Boreal forest near White River, Ontario, Canada (48°21' N, 84°20' W) and incubated it for three months at 12°C and 20°C to examine how warming affects mesostigmatid adult and juvenile abundance, species richness, diversity, evenness, community composition, and community-level biomass. We extracted microarthropods and nematodes from mesocosms and counted juvenile and adult Mesostigmata and identified adults to the species-level. In addition, we counted the other microarthropod taxa (oribatid, astigmatid and prostigmatid mites, and collembolans), along with nematodes within each mesocosm. In total, we counted 43,662 individuals of microarthropods and nematodes, of which 3,349 individuals (1,899 adults and 1,450 juveniles) were mesostigmatid mites, consisting of 14 mesostigmatid species. Mesostigmatid adult and juvenile abundances were far greater under warming, leading to greater community-level biomass that shifted community composition. In addition, astigmatid mite abundance were greater and prostigmatid mite abundance were lower under warming. Changes to Mesostigmata communities were driven by the

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greater abundances of parthenogenetic species, primarily *Veigaia mitis* (Berlese) under warming—a result that has been shown in oribatid communities, but not mesostigmatid communities, before. Overall, we found that warming shifted mesostigmatid mite communities in the Boreal forest, which has wide ranging implications for the soil food web.

A population genetics study of eastern flowering dogwood (*Cornus florida*) in southern Ontario

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Eastern flowering dogwood (Cornus florida L.) is an endangered tree in Canada, occurring only in the Carolinian forest of southern Ontario. Populations of C. florida in this region have experienced declines over the last several decades, largely because of ongoing habitat fragmentation and, more recently, following widespread infections by the fungal pathogen dogwood anthracnose (Discula destructiva). I assessed the genetic structure of Ontario C. florida populations using information from nuclear microsatellite loci generated from 572 trees across 21 sites in southern Ontario. Two genetic clusters C. florida populations may be explained by two post-glacial recolonization routes around the east and west coast of Lake Erie. Overall low genetic differentiation among sites and a low number of private alleles suggests ongoing gene flow, although spatial autocorrelation analysis suggests that dispersal is typically over short distances (~40 km). In general, levels of genetic diversity within sites were high and inbreeding was low. However, genetic diversity was overall lower in younger versus older trees, and in smaller versus larger populations; these findings raise concern about the sustainability of high within-population diversity. Overall, these insights obtained from these analyses provided insight into the genetic structure of C. florida populations and will improve our understanding of the potential future genetic risks to these vulnerable populations. These inferences will be important to incorporate into conservation strategies to ensure the persistence of this endangered tree in southern Ontario.

Freshwater fish diversity of the Barra del Colorado Wildlife Refuge, Costa Rica: Traditional and molecular approaches to biodiversity investigation

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As concerns over declines in global biodiversity continue, baseline measures of diversity become increasingly important for effective conservation planning and management. This is especially the case for diverse groups like Neotropical freshwater fishes. Morphology is conventionally used for specimen identification, but this can be inefficient when intra- and interspecific morphological variations overlap, and the challenge can be even greater in highly diverse areas such as Costa Rica. Previous assessments of the diversity of freshwater fishes in Costa Rica were completed using only conventional identification methods. No studies have been done to date to assess the diversity of freshwater fishes in Costa Rica using molecular techniques such as DNA barcoding. We collected 396 specimens in the Barra del Colorado Wildlife Refuge (BCWR) in Costa Rica during 4 expeditions from 2013-2019. We successfully amplified the Cytochrome Oxidase Subunit 1 (col) gene for 257 specimens. We also obtained morphological identifications from experts for 115 specimens. First, we wanted to test the ability of public databases to identify sequences from BCWR. We found that overall, these databases were able to identify specimens from well resolved, well represented groups, but fell short for comparatively more rare groups. In addition, for taxonomically challenging groups such as those found in the Neotropics, misidentifications were found to be the largest source of error. Due to these results, we resolved to creating a local reference library using an integrative approach. We combined sequences from expertly identified specimens, and critically evaluated public data. We tested the functionality of the library, using sequences from specimens not identified by experts in our collection. We found that our local reference library was more effective in identifying specimens. This project identifies lingering issues with using DNA barcoding for identification and conservation, and presents solutions to combat these issues in the form of curated, reliable, local libraries. As the first baseline measure of the species present in BCWR, this project provides critical information to be

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used by conservation managers in the effort to protect the neotropics. Finally, this study is the first done in Costa Rica to address species diversity of freshwater fishes using molecular techniques.

Modeling soil carbon cycling in boreal peatlands under future climate warming scenarios

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Little is known about how warming will affect carbon inputs and energy flux in peatlands, the largest terrestrial sink of carbon. At the level of the individual organism, the Metabolic Theory of Ecology (MTE) predicts that metabolic rate will increase with warming, which can lead to either an increase or a decrease in species' growth and reproduction capacity (i.e., population biomass). We investigated the effects of warming on the soil C cycle at the level of the individual and the population. We hypothesize that fluxes of energy between pools defined by a peatland soil food web will change under modelled warming conditions, affecting the estimated average metabolic rate of populations of individuals linked by trophic interactions and the estimated average biomass of trophic levels (nodes); we further predict that these changes in metabolic rate and biomass will have corresponding effects on energy cycling throughout the peatland soil food web. We estimated C flux for the entire soil community of a peatland site in Northern Ontario under three warming scenarios: 1) metabolic (M) increases under warming at the level of the individual, 2) changes in population biomass (B) under warming, and 3) metabolic plus population level changes in biomass (MB) under warming. We parameterized each scenario with empirical data from a field experiment called BRACE (Biological Response to A Changing Ecosystem); this site represents a typical boreal, sparsely forested, Sphagnum-dominated peatland. We then quantified and compared total C flux, and flux at each trophic level under warming scenarios at ambient, +2°C and +4°C. The effects of warming on modelled total system energy flux was greatest in the MB warming scenario and was least in the M warming scenario. In each model scenario, the effects of warming on the flux of a single interaction are greater for interactions that include more basal nodes from the soil food web. These findings suggest that warming may increase C cycling in Sphagnum-dominated peatland sites, potentially shifting peatlands from being carbon sinks to becoming carbon sources.

Inbreeding and inbreeding depression in invasive cattail hybrids ($Typha \times glauca$) and their progenitors (T. latifolia and T. angustifolia)

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In regions around the Laurentian Great Lakes, the two cattail species Typha latifolia and T. angustifolia regularly hybridize to form the hybrid cattail T. × glauca. Hybrid vigour in firstgeneration (F_1) $T_1 \times glauca$ has made this hybrid invasive in wetlands around the Great Lakes, although the longer-term impacts of $T. \times qlauca$ will also depend on the fitness of advancedgeneration hybrids relative to F1 hybrids and progenitor species. Reproduction may be key to the evolutionary trajectories of this hybrid complex: all Typha spp. can reproduce both sexually and clonally. Typha spp. are wind-pollinated, and sexual reproduction includes both outcrossing and self-fertilization. Self-fertilization can lead to inbreeding depression, however, the magnitude of inbreeding depression among cattail taxa has not been studied. Earlier studies have confirmed that F₁ hybrids have higher heterozygosity than either parental taxon, and we hypothesized that this might reduce the expression of inbreeding depression in the progeny of F1 hybrids. We tested this hypothesis using controlled hand-pollinations of hybrids and their parental species to generate both selfed and outcrossed seed families. We measured fitness as seed set and seed germination rate, and used these measurements to estimate the magnitude of (early-acting) inbreeding depression T. \times glauca, T. latifolia, and T. angustifolia. Our results provide insights into the fitness of hybrids and progenitors under different reproductive scenarios, and thus will help to predict the longer-term impacts of Typha spp. in wetlands around the Laurentian Great Lakes.

The contributions of a trematode parasite infectious stage to carbon cycling in a model freshwater system

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Parasites remain understudied members of most ecosystems, especially free-living infectious stages, such as the aquatic cercariae of trematodes (flatworms). Recent studies are shedding more light on their roles, particularly as prey for a diverse array of aquatic predators, but the possible fates of cercariae remain unclear. While this is critical to elucidate because cercariae represent a large potential source of energy and nutrients, determining the fate of cercariae-derived organic matter involves many logistical challenges. Previous studies utilized elemental and stable isotope analysis when examining host-parasite interactions, but none have used such approaches to track the movement of cercariae biomass within food webs. Here we report that *Plagiorchis* sp. cercariae were effectively labelled with ¹³C by introducing this compound in the food of their snail host. We then added ¹³Clabelled cercariae as a potential food source to experimental mesocosms containing a simplified model freshwater food web represented by diving beetles (Dytiscidae sp.), dragonfly larvae (Leucorrhinia intacta), oligochaete worms (Lumbriculus variegatus), and a zooplankton community dominated by *Daphnia pulex*. The oligochaetes had the highest ratio of ¹³C to ¹²C, suggesting benthic detritivores are substantial, but previously unrecognized, consumers of cercarial biomass. In an experiment where L. variegatus were fed mass equivalents of dead D. pulex or cercariae, growth was greater with the latter diet, supporting the importance of cercariae as food source for benthic organisms. Given the substantial cercariae biomass possible in natural settings, understanding their contributions to energy flow and nutrient cycling is important, along with developing methods to do SO.

Do existing constructed ponds on Pelee Island, Ontario match the habitat requirements of endangered ambystoma larvae?

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Global loss of wetlands has significantly reduced the habitat available for amphibians. Various organizations now regularly construct wetlands to provide areas for amphibian reproduction and larval development. To support wetland taxa, including federally-endangered salamanders, numerous ponds have been constructed in Southern Ontario. Yet, the degree to which these ponds provide suitable habitat conditions for reproduction and recruitment, and thereby contribute meaningfully to the conservation of these animals, remains unclear. We therefore examined natural and constructed ponds on Pelee Island to address three questions: 1) What environmental variables govern the presence of salamander larvae in ponds?, 2) What environmental variables predict relative abundance (CPUE) of salamander larvae?, and 3) Do constructed ponds match the habitat needs for salamander larvae as observed in natural ponds? We sampled 32 ponds on Pelee Island using a dip-netting approach of 80 sweeps / pond. Environmental variables sampled included: crayfish presence, substrate type, proximity to forest, pond water temperature, pond water depth, pond water surface area, canopy cover, and the presence or absence of submergent vegetation. Presence of larvae was associated with high canopy cover and crayfish burrow presence, whereas catch-per-unit-effort increased with the amount of leaf litter in the substrate and presence of submergent vegetation. This may suggest that aspects of the terrestrial habitat promote breeding in specific wetlands, whereas relative abundance of larvae is related more directly to factors that influence their survival. Constructed ponds had less canopy cover, less leaf litter in the substrate, warmer water, and fewer contained submergent vegetation. Larvae were caught in only 33% of constructed ponds, and catchper-unit-effort was ~4-10x lower than in natural ponds. The constructed ponds on Pelee Island therefore require additional restoration support or naturalization before they provide substantial conservation value to salamanders. While constructing new ponds should benefit amphibian populations over time, our results caution against the presumption that the loss of natural ponds can

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be offset by building new ponds. Constructed ponds often remain unsuitable for 15–20+ years, and even then may not adequately alleviate the loss of natural habitat. Protecting natural breeding sites therefore remains critical for amphibian conservation.

Population decline in a ground-nesting solitary squash bee (*Eucera pruinosa*) following exposure to a neonicotinoid insecticide treated crop (*Cucurbita pepo*)

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Insect pollinators are threatened by multiple environmental stressors, including pesticide exposure. Despite being important pollinators, risks to solitary ground-nesting bees are inadequately represented by pesticide risk assessments reliant almost exclusively on honeybee ecotoxicology. Here we evaluate the effects of realistic exposure via squash crops treated with systemic insecticides (Admire-imidacloprid soil application, FarMore FI400-thiamethoxam seedcoating, or Coragen-chlorantraniliprole foliar spray) for a ground-nesting bee species (Eucera pruinosa) in a three-year semi-field experiment. Hoary squash bees provide essential pollination services to pumpkin and squash crops and commonly nest within cropping areas increasing their risk of pesticide exposure from soil, nectar, and pollen. Squash bees exposed to a crop treated at planting with soil-applied imidacloprid initiated 85% fewer nests, left 5.3 times more pollen unharvested, and produced 89% fewer offspring than untreated controls. No measurable impacts on bees from exposure to squash treated with thiamethoxam as a seed-coating or foliage sprayed with chlorantraniliprole were found. Our results demonstrate important sublethal effects of fieldrealistic exposure to a soil-applied neonicotinoid (imidacloprid) on bee behaviour and reproductive success. Soil must be considered a potential route of pesticide exposure in risk assessments, and restrictions on soil-applied insecticides may be justified, to mitigate impacts on ground-nesting solitary bee populations and the crop pollination services they provide.

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The role of flight feathers on the ability of egg-laying hens to access elevated resources in complex housing environments

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Navigating through an environment is crucial for all living things, and for birds, this ability is largely dependent upon their wings. Wings are known for their role in flapping-flight but are also important for balancing, manoeuvring, and temperature regulation. For egg-laying chickens housed in aviaries, accessing essential resources requires coordinated wing-body movements to reach tiers of varying heights. The presence of intact flight feathers (FF) is necessary to maintain the wings' flapping-flight capabilities. Interestingly, laying hens in aviaries have greater rates of feather-pecking and are more likely to be missing wing feathers. To date, there are no studies investigating the importance of intact FFs on the ability of hens to access elevated resources. We investigated the impact of FF presence on the amount of time hens spend accessing elevated resources. To investigate this, we distributed 120adult laying hens (60 white-feathered strain; 60 brown-feathered strain) amongst 12 floor pens furnished with two platforms 70 cm above the ground, two feeders, and two nest boxes (one of each on the ground or atop a platform), and two perches. Hens were assigned to one of three treatments: FFs intact (control), bilateral primary FFs clipped (half clip), or bilateral primary + secondary FFs clipped (full clip). We recorded the amount of time (minutes) hens spent accessing feeders and nest boxes for 48-hour intervals every two weeks over an 8-week period with the use of an RFID system. We predicted that hens receiving either clipping treatment would spend less time accessing the elevated feeder and nest-box after treatments were applied. Statistical analysis was done separately for each strain to assess the effect of clipping status on behaviour using a repeated measures model. Results showed that two weeks after receiving the full clip treatment, white-feathered hens spent 41.8% less

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time at the elevated feeder (p <.0001) and 37.3% (p=0.0004) less time at the elevated nest box, and this decrease remained throughout the trial. A decrease in elevated feeder use was only seen in brown-feathered half clip hens due to the control and full clip groups not using this feeder before treatment application. However, elevated nest box usage decreased by 54.40% for the half clip (p <.0001) and 58.49% for the full clip (p <.0001) brown-feathered hens, and this decrease remained throughout the trial. These results highlight the importance of intact flight feathers for the ability of laying hens to navigate elevated space in complex housing systems.

Does hemolymph viscosity affect spider joint motion during vibration perception?

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Ectothermic animals cannot regulate their body temperature; they are subject to physiological changes with variations in external temperature. In the case of spiders, changes in temperature affect their leg kinematics, presumably through changes in hemolymph viscosity. However, spiders also use their legs to perceive vibrations. Therefore, mechanical interactions between the cuticle and its internal fluid channels might also affect vibration perception. Spiders perceive vibrations mainly at their joints: their limbs have mechanoreceptors called slit sensillae which form lyriform organs that appear predominantly at the joints. These sensory organs react to strains in their exoskeleton produced by self-generated motion or externally imposed vibrations. These strains are produced through changes in internal hemolymph pressure during locomotion and vibration perception. Assuming that spider hemolymph follows similar temperature dependent changes as other arthropods, it will be less viscous at higher temperatures and more viscous at lower temperatures. These changes in viscosity may alter the mechanical properties of the joint, particularly joint stiffness and damping. Stiffness, in mechanical terms, refers to an object's ability to resist deformation when subjected to an applied force. Damping refers to the attenuation of vibratory motion that is associated with a dissipation of energy. In this study, I developed two highly simplified 3D models of fluid-filled spider joints using the software COMSOL Multiphysics. I used these models to test whether the spider joint properties mentioned can change with temperature as a result of changes in internal fluid viscosity. The spider morphological data were obtained from Zentner, 2013 and cuticle properties from Hößl et al., 2007. The nature of the methodology used is deterministic: model creation depends on a series of partial differential equations that solve for both the fluid and solid behaviour. Therefore, these are highly idealized models of the biological question at hand and it is important to note that such a system is subject to a great deal of variation in the natural world. For example, there is both morphological and material variation between spider species. The study at hand provides preliminary evidence that

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reductions in viscosity at higher temperatures hinder the perception of high frequency vibrations in *Phrixotrichus roseus*. Therefore, these results highlight the importance of optimal temperature habitats for this species in maintaining their perceptual abilities. These are the first models of their kind and future work should focus on increasing model complexity and accounting for variation in the system.

What did the cleaner fish say to the salmon? Communication from a facultative cleaner wrasse facilitates interaction with infected Atlantic salmon

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Cleaner fishes remove and consume ectoparasites from heterospecific client fishes to the mutual benefit of both parties, with different species of cleaner fish classed as either dedicated or facultative depending on the extent of mutualism recorded in its diet and behaviour. The behaviour of dedicated cleaners has been well-documented, and species such as bluestreak cleaner wrasse (Labroides dimidiatus) show sophisticated communication with clients through conspicuous poses and tactile stimulation to aid parasite removal. Contrariwise, the behavioural interaction between facultative cleaners and clients is relatively unknown. This presents an obstacle for salmon farmers who use facultative cleaners to control parasitic lice in sea-cages, as better understanding how these species interact would help inform stock selection and breeding programs. We investigated whether cunner wrasse (Tautogolabrus adspersus) communicated with potential clients by recording how this facultative cleaner interacted with non-infected and lice-infected Atlantic salmon (Salmo salar) over repeated trials. Video analysis found cunners displayed posing and body-contact behaviours significantly more frequently when interacting with lice-infected salmon rather than non-infected salmon. Increased expression of these behavioural cues resulted in significantly closer proximity between cunner and salmon, and significantly higher levels of mutualistic interaction including inspection for and removal of parasites. We also found that larger cunner interacted more frequently with salmon than did smaller cunner, though there was a high degree of individual variation. Our findings imply cunner wrasse can communicate with salmon to aid parasite removal, which challenges preconceptions that only dedicated cleaners show sophisticated interaction with clients. These newly described behaviours provide traits of interest for further development in cleaner fish aquaculture across eastern Canada.

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Geographic patterns of genetic diversity in Darwin wasps (Ichneumonidae, Hymenoptera)

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It is well known that a greater variety of organisms is found in the tropics compared with temperate and polar regions. The study of large-scale patterns of intraspecific genetic diversity might show biogeographic patterns congruent with those of species diversity due to diversification processes acting along a micro- to macroevolution continuum. This research aims to investigate global patterns of genetic diversity in Darwin wasps (Ichneumonidae), a poorly studied family of the order Hymenoptera that is among the shortlist of taxa showing an inverse latitudinal diversity gradient, as the species richness is higher at higher latitudes than in the tropics based on available morphological data. This parasitoid wasp family is among the most species-rich taxa on the planet. Nevertheless, it is one of the groups for which our knowledge most severely lags behind their enormous diversity. It is estimated that less than a quarter of their true richness has been described, representing 25,000 species. A substantial proportion of the species inhabiting tropical latitudes remain undescribed. In that regard, the present study used an extensive COI barcode sequence database and employed counts of molecular operational taxonomic units as a surrogate for species counts, accounting for a more significant amount of information in estimating species richness in the tropics, considering species not yet described as well as cases of morphologically cryptic yet evolutionarily distinct lineages. Using a mined dataset of 94,098 georeferenced COI sequences for 6963 species units, we examined the global distribution of nucleotide diversity at two levels of spatial resolution: an equal-area grid with a cell size of 69,967.8 km² and latitudinal bands of 10°. We found that intraspecific genetic diversity in ichneumonids is heterogeneously distributed across the globe. The mean nucleotide diversity values are higher in the temperate regions, where there is more data available. Investigating the causes of this pattern with further rarefaction analysis provided additional evidence regarding the sampling effect. Statistical analysis for spatial correlation

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and lineal models to explore the relationship between nucleotide diversity per cell and environmental, geographic, and sampling factors supports our results. Investigating macroevolutionary patterns integrating analyses at genes, species, and community levels represent a novel approach in biodiversity studies, providing clues about the potential drivers of biodiversity patterns. Understanding the factors determining the distribution of biodiversity across the planet constitutes a key area in evolutionary biology and is of relevance considering climate change and its effect on species extinction.

Do females prefer colour-enhanced males in a drab species? A test of the sensory bias model in medaka (*Oryzias latipes*)

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Understanding how mating preferences evolve has long been a focal area of research. The sensory bias model proposes that mate choice preferences arise first in a non-mating context, as a by-product of natural selection acting on a female's perceptual system. Recent research has shown that many species of fishes, from across a large clade including poeciliids, goodeids, and medaka, have a bias for long wavelength (LW) colours (orange, yellow, red) in a non-mating context. Several species that do not have LW-coloured ornaments, apparently because they have been lost secondarily, retain this latent bias for LW colours. Here, we predicted that female Oryzias latipes (Japanese medaka), a drab species with a latent preference for LW colours, would show a mate choice preference for males with an artificial secondary sexual trait—a coloured stripe added to their flank. We tested this preference for LW colours first in a non-mating context by presenting females with a series of coloured disc and scoring their preference. We then examined female mating preferences in a mating context using free swim mating trials. Finally, we looked to see if individuals had similar preferences across these two behavioural contexts. We confirmed that females were more responsive to red (Z = -2.067, P = 0.039) and orange (Z = -2.583, P = 0.010) coloured objects in a non-mating context than to other colours, and that females were less resistant towards males with a LW-coloured stripe than to those enhanced with a non-LW stripe ($F_{1,19} = 12.373$, P = 0.0004). We also found that, for many females, responses towards specific LW colours were consistent across these non-mating and mating contexts ($r_{(18)}$ = 0.47, P = 0.036). Therefore, our results provide further support for the sensory bias model and suggest that females demonstrate consistent responses to LW colours across different behavioural contexts.

The evolution of reproductive accessory glands across fishes

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Reproductive accessory glands are organs involved in reproduction but not directly in the production or release of gametes. In fish, the function of these glands has only been described in a handful of species, where they appear to play diverse and crucial roles in reproduction. To date, the selective factors leading to the evolution of accessory glands in fishes remain a mystery with two leading alternative hypotheses — they evolved in response to pressures from sperm competition and/or from parental care. In this study, we tested these hypotheses and explored the evolutionary past of accessory glands. To do so, we conducted a systematic literature survey on ray-finned fishes, documenting the presence or absence of male reproductive accessory glands, as well as information regarding parental care, spawning behaviour, and mating system, for 618 species, representing 267 families. We found accessory glands in 26 different families and 116 species, with the majority of species found in three clades: the blennies, the gobies, and the catfish. We then reconstructed the evolutionary history of reproductive accessory glands using stochastic character mapping and found that accessory glands were gained 14-18 times and lost 7-11 times in ray-finned fishes. By using a Bayesian analysis of correlation between discrete binary traits and comparing transition rate estimates, we show that reproductive accessory glands are strongly correlated with, and are more likely to appear in, lineages with male parental care, pair spawning, and females mating with only one male. The strong association between accessory glands and male parental care supports the hypothesis that these glands arose as a means to enhance parental care activities. The associations with pair spawning and female monogamy provide no evidence for the sperm competition hypothesis. This study represents the first large-scale phylogenetic comparative analysis of accessory glands across fishes and provides novel insights into the evolution of an important and complex aspect of reproduction.

Sex-specific selection in hermaphroditic and dioecious populations of a clonal plant species

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Modular growth in plants means that fitness will often scale with size and size can vary greatly among individuals, particularly in age-structured populations. Clonal growth contributes to variation in plant size but how it affects mating success is not well understood. The outward expansion of clones might increase the size of mating neighbourhoods with benefits for mating success. However, clonal expansion might also interfere with mating success if it increases the probability of self-pollination, and if so, this would weaken the association between plant (clone) size and sexual fitness. These contrasting effects between clone size and sexual fitness are in turn expected to be influenced by the sexual system (whether plants are hermaphroditic, female, or male). In particular, by removing the possibility of self-interference among the parts of a clone, plants in dioecious populations (i.e., populations of unisexual females and males) should have a stronger association between mating and reproductive success than plants in monoecious populations (i.e., populations of hermaphrodites). This association between mating and reproductive success indicates selection on mating success and is measured by the so-called Bateman gradient. I examined patterns of mating and reproductive success in a hermaphroditic (monoecious) and a dioecious population of the clonal plant broadleaf arrowhead (Sagittaria latifolia). The segregation of alleles at seven microsatellite loci was used to identify clone families and infer mating and reproductive success. I compared the association between mating and reproductive success in both populations using Bateman gradients (β_{ss}). In both populations, we found strong linear associations between mating and reproductive success for both sexes (and both sex functions for hermaphrodites) that was driven by underlying variation in clone size - larger clones had higher mating and reproductive success than smaller clones. However, this association between mating and reproductive success was stronger for females (and the female function of hermaphrodites) than for males (and the male function of hermaphrodites). The strong association between mating and reproductive success indicates that sexual selection is operating in these populations and our results indicate that this selection is driven by variation in clone size.

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Because of trade-offs between the size and number of clonal shoots, such patterns of selection might have important effects on the evolution of plant life histories.

Whole genome sequencing reveals the evolutionary history of an enigmatic seabird family

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The Stercorariidae (skuas and jaegers) are a family of pelagic seabirds that range across the oceans of the world and breed at high latitudes in the Arctic and Antarctic. The evolutionary relationships amongst this family have been controversial, and discordance between mitochondrial and morphological characters have led to the suggestion that one of the species — the Pomarine Jaeger (Stercorarius pomarinus) or the Great Skua (S. skua) — originated through hybridization between divergent lineages. To test for a hybrid origin of these species and to resolve the enigmatic skua phylogeny, we sequenced the whole genomes of all seven Stercorariid species. We used a multispecies coalescent framework to reconstruct the Stercorariid phylogeny and performed a series of genome-wide tests for introgression between lineages. We find that discordance between the mitochondrial and nuclear phylogeny of Stercorariidae is caused by introgression of the mitochondrial genome from S. pomarinus into S. skua, but that this introgression event is accompanied by very little nuclear introgression. We also document additional introgression events involving most members of Stercorariidae, including introgression between lineages that were approximately 3.6 million years diverged at the time of hybridization. In addition, we uncover an unexpected signature of shared ancestry between a species that breeds in the northern hemisphere and a species that breeds in the southern hemisphere, suggesting a complicated biogeographical history of the group involving multiple trans-equatorial dispersal events between the Arctic and Antarctic. While we find that hybrid speciation has not likely contributed to the radiation of this seabird family, introgression has been a widespread feature throughout the evolution of this group and may have provided an avenue for adaptive introgression of traits between lineages.

The genetic basis of sex determination in *Catostomus* fishes

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Gonochorism, or the existence of separate sexes, is common among eukaryotic organisms. Yet, the underlying mechanisms of sex determination are poorly understood and highly variable. Sex determination systems that cause a skewed sex ratio or differ across closely related taxa may eventually lead to reproductive isolation. In hybridizing species, sex determination mechanisms may promote or impede reproductive isolation depending on whether mechanisms are similar between species. However, due to variability in sex determination mechanisms, it is difficult to draw conclusions on its impact on hybridization outcome. This is especially seen in fish, where sex determination is highly variable and relatively undescribed across taxonomic groups. In Catostomus suckers, contemporary hybridization is variable and extensive, and sex determination mechanisms are unknown. Catostomus suckers are a species rich genus of freshwater fish and are part of the Catostomidae family that experienced a whole genome duplication event. Hybridization outcomes between Catostomus species are quite variable and can vary spatially. We aim to describe the genetic basis of sex determination in *Catostomus* suckers to understand the impact of sex determination on reproductive isolation. We used genomic data to identify regions of the genome associated with sex using a genome wide association study (GWAS) and the detection of sex specific loci. The GWAS was used to identify sex associated loci that are in both sexes with different genotypes while the detection of sex specific loci is used to identify loci that are in one sex but not the other. In the future, we aim to determine differences in sex determination across species and locations. Preliminary results show that there is a significant region of the genome associating with sex. The results are currently indicating that it could be one significant region such as a sex chromosome or master sex determining region. It could be also be polygenic with this significant region being the only region shared among species, thus a shared basis of sex determination. We will be continuing this analysis by determining the regions of the genome associated by sex in each species. The outcome of this study will provide

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insight on the underlying mechanisms of sex determination in *Catostomus* suckers and will provide knowledge on how sex determination interacts with reproductive isolation.

Outcrossing rates in an experimentally admixed population of self-compatible and self-incompatible Arabidopsis lyrata

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The transition to self-compatibility from self-incompatibility is often associated with high rates of selffertilization, which can restrict gene flow among populations and cause reproductive isolation of selfcompatible (SC) lineages. Secondary contact between SC and self-incompatible (SI) lineages might re-establish gene flow if SC lineages remain capable of outcrossing. By contrast, if intrinsic features of SC plants reinforce high rates of self-fertilization this could maintain evolutionary divergence between SC and SI lineages. Arabidopsis lyrata subsp. lyrata is characterized by multiple origins of self-compatibility and high rates of self-fertilization in SC-dominated populations. Our objective was to test whether these high rates of selfing by SC plants have intrinsic or extrinsic causes. We estimated outcrossing rates and patterns of pollinator movement for 38 SC and 40 SI maternal parents sampled from an experimentally admixed array of 1512 plants sourced from five SC and five SI populations grown under uniform density. We found that, although plants from SI populations had higher outcrossing rates (mean tm = 0.81 ± 0.05 SE) than plants from SC populations (mean tm = $0.57 \pm$ 0.06 SE), outcrossing rates among SC plants were substantially higher than previous estimates from natural populations. Patterns of pollinator movement appeared to contribute to lower outcrossing rates for SC plants; 40% of floral visits were followed by visits to other flowers on the same plant. The relatively high rates of outcrossing for SC plants under standardized conditions indicate that selfing rates in natural SC populations of A. lyrata are facultative and not driven by intrinsic features of SC plants. We conclude that SC lineages of A. lyrata remain capable of outcrossing and are therefore not likely reproductively isolated.

A universal relationship between femur architecture and bone mineral density across *Anolis* lizards

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"Many-to-one mapping" is a key concept in ecomorphological theory that describes scenarios in which different morphological structures can perform the same function. Surprisingly though, there has been little attention to whether the architectural design and material properties of structures commonly exhibit many-to-one mapping patterns. For instance, the stiffness and strength of long bones (e.g., femur, humerus) depend on their bone mineral density (BMD). In addition though, long bone cross-section shape also affects these characteristics, with hollow designs conferring greater stiffness and strength. These alternative ways to achieve an optimal functional performance are limited, however, by the mechanical principles of different structures and by ecological factors affecting the development and life history of organisms. In this context, alternative strategies arising from the combination of architectural design and material properties provide a potential evolutionary solution to such limits when optimizing a functional attribute. Using Anolis, an ecologically and morphologically diverse radiation of lizards, we tested whether diverse species exhibit a restricted combination of architectural design and material properties in the femur, or whether a range of combinations defined by a universal compensatory relationship is observed. Additionally, we tested if the use of particular combinations could be predicted by variables such as body size and insularity. We performed CT scans on multiple individuals from over 100 Anolis species and measured average BMD and a cross-section shape index representing degree of hollowness for the shaft of the femur. We found a strong negative relationship between femur BMD and hollowness in both males and females, suggesting that these two determinants of long-bone strength are alternatively deployed across the Anolis radiation. We also found that BMD and the shape index were negatively and positively related to body size, respectively. More hollow femora may be adaptive at larger sizes as they represent a minimal mass solution for bone strength. Mainland lizards tend to have femora with thinner walls than island species but, contrary to predictions from life history theory, we found no

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differences in BMD between regions. Our results indicate that *Anolis* femora are built following consistent rules defined by a universal relationship between material properties and architectural design, and point to body size as a possible driver of this widespread pattern.

Hybridization varies geographically in *Abronia umbellata* (Nyctaginaceae)

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A long-standing goal in evolutionary ecology is determining the sources of genetic variation required for adaptation in natural populations. One source of genetic variation may be through hybridization, but very few studies have quantified the impact of hybridization across a species range. Abronia umbellata is a coastal dune endemic that ranges from San Quintin, Baja California, Mexico to Coos Bay, Oregon, USA that co-occurs and potentially hybridizes with two other coastal Abronia species, A. latifolia and A. maritima, which themselves are parapatric. Abronia umbellata exhibits striking differentiation in its mating system with obligately outcrossing A. umbellata var. umbellata populations occurring south of San Francisco and predominantly selfing A. umbellata var. breviflora populations occurring in the north. In this study, we quantified geographic variation in hybrid occurrence frequency across the range of A. umbellata. Additionally, using morphology from 840 individuals and sequence variation at five single-copy genes assayed for 261 individuals from 37 populations, we determined if introgression, required for the transfer of interspecific alleles, was occurring, and if so, if it played a part in the origin of A. umbellata var. breviflora. Exhaustive searches detected hybrid phenotypes in 43.4% of populations where A. umbellata co-occurred with a congener but these hybrids only contributed to hybrid occurrence frequency greater than zero in 18.9% of populations and 2.4% of 695 plots and only in populations sympatric with A. maritima. NewHybrids and DAPC analyses reveal that introgression is likely occurring almost anywhere where A. umbellata var. umbellata hybridizes with another species, but likely did not contribute to the origin of selfing A. umbellata var. breviflora, as they are genetically similar to outcrossing populations of A. umbellata and not intermediate between proposed parental species. Overall, the findings from our study show that hybridization has the potential to be a source of genetic variation for natural outcrossing populations, including populations at the southern edge, but likely not selfing populations as selfing may be acting as a reproductive barrier preventing opportunities for hybridization.

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T Determinants of performance in a novel object recognition task in pumpkinseed sunfish (Lepomis gibbosus)

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Individuals within species often exhibit consistent differences in activity and exploratory behaviours which may contribute to a "personality," or behavioural syndrome. Behavioural syndromes may introduce variation in cognitive abilities, including learning, as bold individuals may be more likely to investigate novelty than shy individuals. Here, we test for evidence of learning and behavioural syndrome in field conditions to determine if individual differences in behaviour have an effect on learning in pumpkinseed sunfish (Lepomis gibbosus). Nesting male sunfish were subject to novel object recognition tests and recorded to analyze behaviour throughout trials. Discrimination ratios were calculated using time spent on the novel side of the nest and number of approaches toward the novel object to quantify learning. Behavioural variables including latency to return to the nest after disturbance (LTR), latency to begin interacting with the objects (LTI), and the total time spent investigating objects (TTI) were used to determine evidence for consistent behaviour across contexts indicative of a behavioural syndrome. We found evidence of variation in learning where some animals were better able to recognize novelty than others. Further, we found strong correlations across behavioural contexts including LTI and TTI, indicating evidence for behavioural syndrome. Finally, we found evidence that this behavioural syndrome explains learning variation, where animals that take less time to begin investigating objects and spend more total time investigating objects are more likely to recognize novelty. We conclude that individual differences in latency behaviour suggestive of a weak behavioural syndrome may influence the cognitive recognition capacity of nest-quarding male pumpkinseeds under natural field conditions. As our study is the first to evaluate fish behaviour in a field setting it may provide further insight into the relationship between behaviour, cognition, and variation within these factors in a natural environment.

Singing snappers - Hatchling vocalizations are associated with beneficial social interactions in subterranean nests of a freshwater turtle

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Social behaviour in non-avian reptiles is largely underestimated, and until recently, turtles were thought to be voiceless. Despite this belief, recent work suggests that freshwater turtles communicate using vocalizations, and theory suggests that this behaviour may underlie complex social behaviours. For instance, hatchling vocalizations among turtles may be important for hatchling communication in early life, perhaps to encourage hatching synchrony and coordinate group emergence from subterranean nests (the 'Social Facilitation Hypothesis'). Using the broadly distributed Snapping Turtle (Chelydra serpentina) as a model organism, we examined whether vocalizations function in hatchling communication. First, we characterized the vocal repertoire of hatchling turtles by recording the audio of a turtle nest and examined vocalizations produced within three distinct hatching periods (pre-pipping, pipping, and emergence). Second, we examined whether the presence of siblings during hatching and nest emergence increases fitness (a basic prerequisite of the Social Facilitation Hypothesis) by manipulating egg burial depth (shallow or deep) and sociality (presence or absence of siblings) in a 2 x 2 factorial design. Finally, we experimentally tested a component of the Social Facilitation Hypothesis by examining whether vocalizations facilitate hatching synchrony, playing vocalizations to late-stage embryos, and evaluating whether vocalizations modify hatch timing. First, we demonstrate that C. serpentina hatchlings have a vocal repertoire: we detected 261 vocalizations of 5 different types in a simulated nest environment. Most vocalizations occurred in the 24 hrs following egg pipping, where more complex vocalization types were observed after hatching. Next, we demonstrate that hatchlings benefit energetically from emerging with siblings, where eggs in the presence of siblings hatched 1.6 days earlier and lost 0.5q less weight. This study is the first to demonstrate that snapping turtle hatchlings can modify development timing within the egg and confirms that siblings reduce energy expenditure during

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nest emergence. However, contrary to a leading hypothesis that vocalizations cue hatching synchrony, our results show that hatchlings did not modify hatch timing with respect to vocalization playback. The present study raises the possibility that vocalizations do not synchronize hatching, but that hatchling social behaviour still plays a role in nest emergence. We provide incremental support to the Social Facilitation Hypothesis while contributing to a growing literature on the adaptive significance of reptile sociality. By studying a lineage that greatly differs from well-studied vertebrates, this work generates fresh insight into factors that drive the origin and persistence of social behaviour in vertebrates.

Should I stay or should I go? Consensus and leadership in thermal decision making

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Environmental heterogeneity exists even on the smallest scale, meaning that when determining where to go, individuals have many choices to make. To remain part of a group, however, groupliving individuals must compromise on where to spend their time. For example, individuals may have to compromise on their choice of preferred temperature, which has been shown to vary among individuals within a species and to correspond to an individual's optimal temperature for physiological processes. To better understand how individual variation influences group thermal decision-making, we studied collective decision making in social, group-living redside dace (Clinostomus elongatus). We had three competing hypotheses about group thermal decisionmaking. The first was that thermal group decisions are shaped by the individuals in a group, and each of their temperature preferences (averaged). The second was that that group thermal decisions are dictated by the largest individual in a group. The third was that group thermal decisions are dictated by the boldest individual in a group. A shuttlebox was used to determine individual temperature preference and a trio's group temperature preference. An exit test was used to measure boldness. Fish were first tested as individuals, and then in trios, and individual preferences were compared to group preferences. While none of our hypotheses were supported, we found that group temperature preferences had less variance than individual temperature preferences. This may indicate that factors aside from size or boldness, such as social dynamics, may instead be at play in shaping thermal group-decision making.

Within- and among-individual variation in the Connecticut Warbler's extended song type

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The Connecticut Warbler (Oporornis agilis) is one of the least studied passerines in North America. This species is secretive in nature and utilizes low-elevation habitats within boreal forests. Due to their relatively inaccessible environment, their vocal behaviour has not been well studied. Using autonomous recordings made in the Northwestern Ontario boreal forest, we examined this species' vocal repertoire. We report a novel but rarely used, second song type: an extended song that covers a broader frequency range and is significantly longer and more variable than the species' primary song. The extended song is similar in composition to the Ovenbird's (Seiurus aurocapilla) flight song; It includes an introductory segment, several notes from its primary song, and a terminal segment. We predicted we would find variation within and among individuals in song characteristics and diel separation of song type use as is observed in Ovenbirds. First, we found that song length and syllable versatility index varied within and among individuals. We also found that primary songs peaked at dawn and declined through the day while extended songs peaked in evening, suggesting temporal separation. Our work highlights the value of environmental recordings created for survey purposes so that we may answer questions about the vocal behaviour of difficult to study species, in addition to expanding our knowledge of natural history. While the vocal repertoires of most North American passerines are considered to be well characterized, we demonstrate that novel vocalizations are still waiting to be discovered, particularly for boreal species.

Disentangling effects of building height and floral display on green roof pollinator communities

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Green roofs are increasingly common in cities and are promoted as opportunities for pollinator conservation by extending foraging and nesting habitat onto building rooftops. However, green roofs are vertically isolated from the ground, and present harsh environmental conditions that affect opportunities for diverse plant growth. As a result, a single drought tolerant non-native plant group, Sedum, are often dominantly planted on extensive green roofs, which are the most widely implemented green roof type due to their lightweight, shallow substrates and low expense. Subsequently, the contribution to habitat for a range of pollinators, including bees, flies, and wasps, might be limited on Sedum-dominant green roofs, but could be improved if additional drought tolerant plants are included. The purpose of this study was to investigate how building height and floral display on Sedum-dominant extensive green roofs shaped visiting pollinator communities. Pollinators (bees, flies, and wasps) were surveyed from five plant species (Sedum acre, S. album, S. kamtschaticum, S. spurium and the native, Rudbeckia hirta), during timed observations on eight replicated green roof arrays (six placed on buildings with varying height (6 - 20 meters) and two at ground level) between June and September 2019. We identified 31 species of pollinators and found a significant negative relationship with building height for species richness and abundance for each pollinator group. Sedum and Rudbeckia flowers showed distinct blooming patterns on our green roofs and so we partitioned our analysis into early (Sedum dominated) (June – August), and late (Rudbeckia flowering) (August – September) blooming periods. Percent Sedum display during the early bloom was significantly positively correlated with bee species richness and abundance, but no relationship was observed during the late period. The inclusion of R. hirta onto Sedum-dominant green roofs lengthened the bloom period and supported a significantly different pollinator community than Sedum. We show that even simplistic Sedum-dominated green roof designs can

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support pollinators due to their high blooming abundances, however distinct pollinator communities (especially bees) benefit from green roofs that include non-Sedum, native flowering plants whose bloom does not overlap with Sedum (i.e., Rudbeckia). Implementing green roofs that encourage pollinator habitat should focus on low-rise buildings, high bloom amounts, and diverse flowering communities with species of complementary (non-overlapping) blooming periods.

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Studies on Macromycetes of Pakistan

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Fungal diversity in many regions of the world has been poorly explored and the geographic distribution of most species or species groups is unclear. Our study tests the hypothesis for a lesser explored region, Pakistan, that climate, ecological niches and lifestyle rather than geographical proximity is a major driver to organismal distribution on Earth. Pakistan covers a wide altitudinal range from sea level (Arabian sea) to the second highest peak of the world, K2. The variety of ecoregions from Himalayan alpine grasslands and subtropical pine forests to deserts and xeric shrublands promotes a great deal of fungal diversity that is yet to be comprehensively documented. In this work we compile a compendium of macromycetes ('Fungi visible to a naked eye") reported from Pakistan in the literature and the NCBI (Genbank) database, and from collections we made recently. DNA sequences data are being used to reassess the taxonomy of many species, help in the discovery of new species, and infer their biogeographic relationships. To date, we have recorded 610 species corresponding to 205 genera (Agaricomycotina) that encompasses all major orders and families (the estimated number of known macromycetes species worldwide is about 15,000, but many more exist). We found that many Pakistan macrofungal sequences deposited in Genbank have not been identified to species level or are possibly misidentified: they may represent new species. For instance, we will present a newly discovered truffle genus & species of potential economic importance, Ahmadea dalanensis. Inference of biogeographic relationships using the ITS rDNA barcode marker indicate that, generally, ectomycorrhizal taxa (ECM) are more restricted geographically than saprophytic taxa (SAP). ECM are significantly more abundant in the northern regions and show pan-Himalayan and European relationships ("temperate climatic range"), whereas many species groups of SAP tend to be more widely distributed globally.

Testing bioinformatics approaches for building large phylogenetic trees by placing DNA barcode sequences

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Building large and comprehensive phylogenetic trees is valuable for diverse studies, including biodiversity, biomonitoring, community ecology, and evolutionary biology. High-throughput sequencers, combined with DNA barcoding field campaigns throughout the world, are providing unprecedented data for biological studies. There is a great opportunity to build larger trees than ever before, yielding new biological insights, but there is a gap in knowledge regarding the performance of existing bioinformatics tools. As well, it remains unknown how we may best combine the strengths of large-scale DNA barcoding campaigns (many species, few genes) with phylogenomics studies (few species, many genes) to increase phylogenetic accuracy and species coverage. To test the effect of sampling completeness of the backbone tree upon phylogenetic accuracy, I used multi-gene DNA sequence data retrieved from "The Fish Tree of Life" dataset. I first built a large phylogenetic tree using a data set comprised of DNA sequences from 27 genes for 12K fish species. Keeping this phylogenetic tree aside as the reference tree, the full fish species dataset was then randomly sampled at varying levels of species sampling (10 times each including 80%, 60%, 40%, and 20% of species) to build multi-gene trees at each level of completeness. Finally, the fish species omitted during each cycle of random sampling were added back onto the backbone phylogeny using DNA barcode data alone (a fragment of the cytochrome C oxidase subunit I gene, COI) with the aid of the RAxML-EPA software. Metrics of phylogenetic congruence were used to measure phylogenetic accuracy, as judged against the reference tree built using the full multi-gene dataset built using all species. Topological accuracy strongly related to the backbone completeness while CADM, the concordance among distance matrices, exhibited no difference in accuracy with increasing sampling completeness. Therefore, it can be concluded that the different methods/ metrics (topology-based, distance matrices-based) generate contrasting trends. Hence, striving for topological accuracy through increasing sampling completeness of multi-gene datasets is likely important when the purpose of future research is taxonomic revision. On the other hand, distance

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matrices may be relatively accurately constructed based upon fewer multi-gene data points and are relevant when the goal of the research is to calculate large-scale tree properties and evolutionary diversity among taxa and geographic regions. The next steps for this research are to investigate the impact of stratified sampling, such as building backbone trees using representatives of all families or genera, upon phylogenetic accuracy.

Root trait plasticity in response to contrasting phosphorus environments and its consequences for plant performance

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There is significant variation in plant root functional traits such as root diameter, root branching, specific root length (SRL) and root tissue density across (RTD) plant species, families, and growth forms in nature. There is no consensus on the mechanisms driving the diversification of root traits. One hypothesis is that the variation in plant root traits is directly associated with variation in soil nutrients, particularly essential and limiting nutrients such as phosphorus (P). Adaptations like increased root biomass, root depth and development of finer root characteristics, are often associated with low P soil because these traits facilitate P acquisition. Studies examining root traits plasticity in response to P availability has previously focused mostly on root depth and biomass, with very few studies examining root morphological traits; and no study done on natural plant populations. If P limitation triggers the development of finer root traits, low P conditions should lead to expression of higher SRL, higher root branching, and thinner root diameter compared to plants under high P conditions. To test these hypotheses, we planted 34 distinct naturally inbreeding Medicago truncatula (annual Mediterranean legume) populations and grew them in University of Guelph phytotron under controlled conditions, we used a randomized complete block design with four temporal blocks. Plants were subjected to high P (30mg/kg soil) and low P (3mg/kg soil) treatments. Plants were grown for 8 weeks, root and shoot biomass were measured after harvest to determine plant growth, root biomass allocation and root:shoot ratio. Multiple root subsamples were taken from each root system, they were scanned and analysed for root functional traits including SRL, root diameter, root branching and root tissue density. Preliminary analyses indicated that plants across all experimental population had increased growth under high P compared to those under low P; plants grown under low P generally had higher root: shoot ratio due to higher biomass allocation to roots, though the extent varied depending on population. While all root functional traits varied greatly among populations under both high and low P; differing P availability did not affect the expression of root diameter and root

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tissue density, however, under low P, plants expressed higher SRL, and lower branching compared to those under high P. These results suggest that while low P availability does increase root development through higher biomass allocation, it does not uniformly lead to the expression of finer characteristics for all root functional traits.

Genetic diversity and its implications for restoration of the endangered American chestnut

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Knowledge of the magnitude and geographic patterns of genetic diversity is instrumental for recovery of endangered tree species whose persistence and expansion are limited by genetic variation. One such species is American chestnut (Castanea dentata), which has experienced a dramatic reduction in population size in North America associated with the spread of chestnut blight. To examine the role of genetic diversity on population dynamics and recovery, we conducted a population genetic assessment of native American chestnut populations in the understudied northern range in Canada and along a gradient towards the center of the U.S. range. Leaf tissue from 13 natural populations in Canada (N=7) and northern U.S. (N=6) were genetically characterized using 16 microsatellite loci and compared to a sample of reference trees of C. dentata and three other Castanea species. Genetic diversity and population structure were assessed within and among populations to determine population connectivity and the presence of admixture with other Castanea spp.. Populations throughout the range displayed high genetic diversity and significant inbreeding, with no significant difference in diversity between those at the center or edge of the range. We found evidence of interspecific hybridization in some Canadian populations, although the second species was not identifiable, and that Canadian populations clustered away from US populations according to population genetic structure. Mantel tests revealed significant isolation by distance, likely due to the effect of Lake Erie acting as a barrier to gene flow between Canadian and US populations. American chestnut appears to have retained sufficient genetic diversity following population bottleneck, which may facilitate adaptation to future climatic and environmental change.

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Evidence for hybrid breakdown in the cattail (*Typha*) hybrid swarm

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Hybridization leads to the mixing of genetically distinct lineages, and in some cases produces hybrids that are successful invaders. Hybrid success can be driven by heterosis, which is increased hybrid fitness arising from heterozygosity and novel gene combinations. Heterosis typically peaks in firstgeneration hybrids (F1s), and in some hybrid zones, advanced-generation hybrids (F2+) can exhibit lower fitness than F1s. This decrease in fitness is called hybrid breakdown, which can occur from the uncoupling of co-adapted gene complexes and increased homozygosity. The overall incidence of hybrid breakdown remains poorly understood, particularly in plant hybrid zones. The Laurentian Great Lakes (LGL) region is the site of a widespread cattail (Typha spp.) hybrid zone that comprises three taxa: Typha latifolia, which is native; Typha angustifolia, which was introduced from Europe; and their F1 hybrid Typha × glauca, which is invasive and negatively impacts wetlands. In the LGL hybrid zone, F1 hybrids display heterosis and displace parental species via competition and introgression. However, surveys of molecular-genetic variation indicate a paucity of advanced-generation hybrids and backcrosses to parental species, a pattern consistent with hybrid breakdown. To better understand these patterns, I compared the fitness of backcrossed and advanced-generation (F2) hybrids to that of F1s and native T. latifolia. I measured and compared seed set, germination rate, plant height, and above-ground biomass. I found evidence for hybrid breakdown among F2s and backcrossed hybrids - these plants had reduced germination rates, plant heights, and aboveground biomass compared to F1s. The expression of hybrid breakdown in the F2s and backcrossed hybrids may help explain why F1 hybrids remain more common than later generation hybrids in the *Typha* LGL hybrid zone.

Patterns of diversity in stream macroinvertebrate communities in the Sydenham River watershed (SW Ontario)

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Stream macroinvertebrates are commonly employed bioindicators for stream assessments, as their tolerances to pollution and other stressors are well studied. However, invertebrate diversity metrics might assess streams at different, often finer scales than that of human disturbance, especially in agriculture landscapes. Our research seeks to better understand how water quality, sediment and land-use impacts stream biodiversity in the Sydenham River watershed, Southwestern Ontario. More specifically, this study asks: does macroinvertebrate diversity vary predictably with environmental factors at the microhabitat or reach scales? Here we present biodiversity data from a watershed survey of the Sydenham River and its tributaries conducted in Fall of 2020. Invertebrates were sampled at randomly selected and existing monitoring sites following Ontario Benthic Biomonitoring Network protocols. Water quality, sediment and land-use data were collected using a modified Ontario Stream Assessment Protocol. Data were analysed using a suite of exploratory statistics, including ordination and other community analyses, to determine which factors best explained variance in invertebrate richness and biodiversity indices across microhabitat and reach scales. Watershed assessment and restoration face challenges in overcoming barriers of scale as while restoration actions take place locally, previous publications suggest that coordinated actions across a landscape may be required to ensure ecosystem recovery. The findings of this research have the potential to inform local understanding of ecosystem health and help accelerate more coordinated restoration and stewardship practices in the region.

The effect of hydrodynamics on the feeding of a freshwater grazer (*Daphnia magna*): the influence of particle size and shape

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The transfer of energy through the consumption of phytoplankton producers by zooplankton grazers is one of the most important interactions in aquatic ecosystems. There are many factors influencing these interactions such as the physical characteristics of the algae food – size and shape in particular. Further, physical interactions between grazer and algae are dictated by hydrodynamic forces at low Reynolds number (ratio of inertial to viscous forces), which define these interactions, and a unimodal relationship between grazer clearance rates (CR) and turbulence is expected. The goal of this study is to determine the effect of the size and shape of algae on the relationship between CR and turbulence. A rotational chamber system, in which different levels of vorticity and acceleration are generated to simulate increasing turbulence was used to examine the clearance rate of Daphnia magna. The clearance rate of Daphnia magna was examined separately for two algae species; the small, spherical Chlorella vulgaris and the larger, elongated and colonial Scenedesmus quadricauda. It is likely that the clearance rate will follow the unimodal trend for all treatments but will be suppressed for the larger algal species under high vorticity and acceleration, and this will be examined empirically. Turbulence in a lake is dictated by physical parameters of the lake and the atmospheric conditions, which are subjected to the influence of climate change, such as increased frequency and severity of storms, chronic increases in advective properties due to water level decrease or drought, or average wind speed increase. The results of this study can help further the understanding of grazer feeding interactions and provide insight on the potential implications for energy transfer at this important trophic level as hydrodynamic conditions fluctuate.

City Living: The influence of the urban environment on stress and early rates of neurogenesis in eastern grey squirrels (*Sciurus carolinensis*)

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As the fastest growing habitat type on the planet, urban environments encroach upon existing ecosystems and habitats at a pace never seen before, yet the severity and implications of these alterations are largely unexplored. One means through which we may gain insight on the impact of urbanization on wildlife is through examining the hypothalamic-pituitary-adrenal (HPA) axis, a mechanism that connects organisms with their environment via the stress response and glucocorticoid (GC) production. Furthermore, the multi-generational impact of living in an urban setting may be revealed through the examination of maternal effects, and how a mother's stress, in response to urban stimuli, shapes offspring neurological development, specifically neurogenesis. Eastern grey squirrels (Sciurus carolinensis) are abundant throughout both urban and non-urban habitats, making them a fitting representative population, not only for their success in these environments, but for their reliance on neurogenesis, a process also found in humans, as a means of survival. Using the University of Guelph campus as our urban site and rare research reserve in Cambridge as our natural site, this study applies a comparative approach to investigate potential trade-offs of an urban lifestyle in grey squirrels. Through physiological methods including hormone challenges, and immunohistology tissue staining, this study examines the stress of mothers and offspring, and its impact on the rates of neurogenesis in juveniles from both habitat types. If the urban environment imposes a suite of stressors that stimulate the HPA axis to increase GC levels, early-life brain development may be impacted, yielding downstream consequences for behaviour and fitness. Knowledge regarding the impact of urban habitats gained by this research may aid in the design of more accurate and meaningful conservation strategies as well as reinforce the necessity of urban forests for wildlife within this ever-growing landscape.

Needle in a haystack: Using experimental nutrigenomics to identify and characterize unique nutritional phenotypes in *Daphnia pulex*

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catrionajones@trentu.ca; Environmental and Life Sciences Graduate Program, Trent University Nutrition is at the center of interactions between organisms and their environment and affects the movement of energy/nutrients through an ecosystem. Decades of anthropogenic nutrient loading, acidification, and deforestation have altered nutrient dynamics in freshwater ecosystems across the industrialized world. It is therefore important that we have the tools necessary to track and monitor nutrients in the environment and their effects on food web dynamics. While long-term monitoring efforts have successfully tracked and monitored nutrient shifts at an ecosystem level, the effects of these shifts on consumers are harder to measure. Long-term monitoring also requires years of consistent data collection in order to build up a dataset robust enough to detect change. At shorter time-scales, lab-based growth assays are used to study the nutrition of aquatic animals and have greatly advanced our knowledge on the effects of poor nutrition in consumers. However, these experiments rely on using animals with known reaction norms and fed on known quantities and qualities of food. These experiments are largely impossible in situ which means that our ability to track and measure nutrition in the field and in animals with an unknown nutritional history is limited. The result is a disconnect between our knowledge on individual animal nutrition and wider ecological processes. One possible solution for bridging this gap is to identify nutritional indicators capable of differentiating multiple types of nutrient stress in wild populations. Broad physiological traits (such as growth rate and reproduction) have been found to be too general in their responses to poor nutrition to differentiate among multiple types of nutritional stress. However, advances in molecular techniques have allowed us to investigate nutritional limitation at the transcriptomic level, revealing highly nutrient specific responses. Further development of nutritional indicators would not only allow us to directly measure nutrition in consumers, but they would also allow us to identify and track change at an ecosystem level without the need for years of measurements. I am using next-generation RNA sequencing technology to explore differential gene expression in *Daphnia* pulex exposed to 5 ecologically relevant nutritionally limited diets in addition to a nutritionally

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replete diet; low phosphorus, low calcium, low nitrogen, low carbon and cyanobacteria. I am using machine learning algorithms on the sequence data to identify the indicator genes with the highest predictive power.

Scientific and educational benefits of using citizen science as experiential learning for undergraduate students

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eporte02@uoquelph.ca; Department of Integrative Biology, University of Guelph The explosive growth of citizen science projects (CSPs) has permanently changed the scientific landscape through unprecedented knowledge advancement enabled by the creation of enormous datasets, particularly in ecology and conservation. It has also provoked serious questions that challenge the integrity of data generated by untrained citizen scientists. Current research takes an inefficient, resource intensive downstream approach (removing or correcting for problematic data), and there is limited guidance on how to address data problems. A better investment would be creative upstream solutions that use the extensive resources already available. Universities are well positioned to address this in their role as knowledge creators and trainers of researchers and professionals. Scientific evidence points to experiential learning, which is known to enhance transferable and discipline-specific skills and knowledge gain, as a potential way to uphold scientific and educational excellence. Unfortunately, experiential learning is underused in undergraduate science curriculum because of barriers like class size, curriculum restrictions, resource limitations and faculty resistance. This does the science community and general public a disservice by robbing students of high-quality learning experiences and limiting their future success. The learning outcomes of CSPs and experiential learning are remarkably similar, but few studies examine the role of CSPs as a powerful and innovative education tool to train undergraduates on robust field ecology research methods and data integrity, and no one has quantified the scientific or educational benefits. We have created the Squirrel Life CSP to provide experiential learning opportunities to students and community members. Participants go on walks to observe squirrels, which are diurnal, charismatic, and easy to identify. Observations will be used in a long-term monitoring program to compare urban and suburban squirrel densities and behaviours in Guelph, ON. We will compare the data produced by participants with different levels of skill and knowledge to determine how much experience is needed to deliver high quality data. We will conduct surveys and interviews to understand the effect of involvement on participants.

Defining the core microbiome: Common trends in microbial ecology

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racinek@uoguelph.ca; Department of Integrative Biology, University of Guelph Organism, soil, and water-associated microbiomes can be composed of hundreds of unique microbial taxa, all playing different roles within their community. Within the field of microbial ecology, the term "core microbiome" was previously used to identify taxa that were the most abundant that also provided a functional role for the host. Using more recent adjustments to this belief that began to include rare taxa, Risely (2020) proposed five general definitions of a core microbiome: the common core; the temporal core; the ecological core; the functional core; and the host-adapted core. This study involves a literature search through studies focusing on microbiomes, with the goal of finding if there are common characteristics respective to each core. These characteristics include the field of study (medical, ecology, agricultural, and industrial), the study system/host of focus, and a variety of different statistical methods. Using the literature cited in Risely, we were able to form a list of keywords commonly found in the majority of the papers, however only the most relevant terms (relating to microbiomes or microbial communities directly) were used. The keywords were placed into a new search using R, yielding a total of 479 relevant papers from the PubMed database published between 2018 and 2020. General information (title, authors, goal of the study, etc.) and information related to the characteristics of interest (field of study, study system/host, and statistical analyses) will be extracted from a random set of 125 papers. We will be performing RxK (chi square) tests to examine if there is any correlational relationship between the core microbiome and the characteristics used in each study. This will be visualized with bar graphs, and the direction of interaction will be provided by Bonferroni tests. Currently, there is an overwhelming dominant option within some characteristics such as the field of study, whereas other characteristics such as the study system contain a wide variety. We expect that the common core and functional core will be used most frequently in most fields of study, because of how the core microbiome was believed to consist only of abundant and functional taxa. We do not expect much correlation between the study system/host, however the medical field will most likely correlate with the host-adapted core because of an increase of interest in survival and fitness.

Identifying suitable habitat and movement corridors for endangered salamanders on Pelee Island

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graemesmith@trentu.ca; Environmental & Life Sciences Graduate Program, Trent University Habitat loss has caused severe decline in amphibian populations globally. Pelee Island, Ontario is home to at-risk populations of small-mouthed Salamanders (A. texanum) and Unisexual Ambystoma (Small-mouthed Salamander dependent population), whose populations have declined following dramatic changes to the landscape since the late 1800s. Surviving populations on the island would be at greater risk if impermeable habitat between breeding sites results in low connectivity among remaining patches of suitable habitat. We therefore conducted intensive field surveys to document terrestrial areas occupied by adult and newly-metamorphosed juvenile salamanders. This data was then used to assess salamander habitat suitability (MaxEnt) and connectivity (Circuitscape) across the island. Salamander habitat suitability models confirmed that terrestrial capture locations (n=469) were most strongly influenced by proximity to suitable breeding sites, but also found strong effects related to land cover type. Compared to sympatric unisexuals, Small-mouthed Salamanders had a narrower range of suitable habitat, were found closer to breeding sites, and were more closely tied to deciduous forest, wetland habitat, and clay soils. Our evaluation of potential connectivity among breeding sites found that there was low likelihood of salamanders successfully dispersing among sites. This indicates that salamander populations on Pelee Island are largely isolated from one another and have low probability of genetic exchange. These findings should prompt conservation groups to prioritize the protection and enhancement of habitat between isolated populations to create dispersal corridors. This could be achieved by restoring deciduous forest and forested wetland habitat, and by constructing new wetlands in strategic locations between existing breeding sites. These endeavors would also benefit other rare species on Pelee Island.

Using transcriptomics to investigate whether asexuality facilitates range expansion in the clonal wetland plant Decodon verticillatus

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All species have limits to their geographic distributions, and these limits are thought to reflect a failure of adaptation to conditions beyond the range. The adaptive capacity of populations at range limits could be influenced by the amount and distribution of genetic variation, which in turn is affected by variability in reproductive system. Shifts from sexual reproduction to clonal asexual reproduction at range limits has been observed in a wide variety of plants, but whether this shift impedes or promotes range expansion is largely unknown. Asexuality might hinder range expansion by reducing genetic diversity within edge populations, thereby impeding response to selection for traits that could allow persistence beyond the range. Alternatively, asexuality could promote range expansion if restricted recombination allows the build-up of multi-locus genotypes with high fitness at the range edge. These contrasting expectations suggest two alternative hypotheses: (1) the "asexual expansion" hypothesis, under which asexual populations should be well-established, longlived and adapted to range-edge conditions; (2) the "asexual constraint" hypothesis, under which asexual edge populations should consist of short-lived genotypes recently derived and dispersed from populations within the range. Decodon verticillatus (Lythraceae) is a tristylous, emergent, wetland plant native to eastern North America that reproduces sexually in the southern portion of its range but shifts to asexual reproduction at its northern range limit. Previous studies suggest that this shift to asexuality has facilitated range expansion: asexuality appears to have arisen independently multiple times at the range limit and is associated with better survival in colder climates. We will be more directly testing the asexual expansion hypothesis through genome-wide analysis of genetic variation. We sequenced RNA from one individual from 12 asexual populations at the northern range limit and 12 sexual populations throughout the range with Illumina RNAseq and performed de novo transcriptome assembly. We will use this reference transcriptome to identify single nucleotide polymorphisms (SNPs) and look for signatures of long-term asexuality, including increased heterozygosity and a greater proportion of deleterious mutations. This study will help

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further our understanding of the factors that impose range limits, which is becoming increasingly important to better anticipate the capacity of species to adapt and potentially shift their ranges in response to anthropogenic environmental changes.

Hybrid genome assembly of the cattail Typha latifolia

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Cattails (Typha spp.) comprise a genus of aquatic macrophytes that are essential components of wetlands. In recent decades Typha have become more widespread and abundant across the globe. Typha latifolia and Typha angustifolia, two of the most widespread cattail species, interbreed in some regions of sympatry to produce the hybrid *Typha* x *glauca*. In regions near the Great Lakes and St. Lawrence Seaway, T. x glauca displays heterosis, allowing it to outcompete and displace both parental species while reducing ecosystem diversity and altering nutrient cycles. Interestingly, hybrids are uncommon in other regions where the parental species are sympatric including Europe, Canada's Maritime provinces, and China. The goal of this study was to begin creating a high-quality set of molecular Typha resources through whole genome sequencing to address evolutionary and ecological questions. A high-quality genome of *T. latifolia* was assembled *de novo* using high coverage Illumina short reads and longer PacBio reads. The total number of scaffolds was 1189, with an N50 of 8.706 MB. We detected 96% of the Liliopsida universal single-copy orthologs present in our assembly, making the genome suitable for functional annotation. Species-specific markers can be found by aligning other *Typha* species to this reference genome, and then used to differentiate hybrid classes. An annotated reference genome will provide the extensive genetic data needed for the identification of potentially adaptive genes that may explain why this hybrid is more successful in certain environments.

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Machine learning to classify trout hybrids

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apitura@uoguelph.ca; Department of Integrative Biology, University of Guelph Human-mediated species introductions can result in hybridization between closely related native and non-native species. In the United States, hybridization with non-native rainbow trout threatens the persistence of native Yellowstone cutthroat trout. Yellowstone cutthroat x rainbow trout hybridization is a conservation concern, but hybrids have varying phenotypic outcomes and can be difficult to identify without expensive, time-consuming genetic analysis. Juveniles are especially difficult to identify phenotypically, as the phenotypes that differentiate adults are not yet developed. A more cost-effective and quicker identification strategy may be found in machine learning. Machine learning models have successfully been developed to classify wildlife camera trap images (99-100% accuracy), and we aim to determine if a machine learning model can also be trained to classify images of adult and juvenile trout as parental or hybrids. Initial model training with genotyped juvenile trout resulted in a low successful identification rate (60-67% accuracy), likely caused by a small training dataset. We have increased the number of juvenile training images and added adult images, which are larger and have more defined features than juveniles. We expect the model will have higher success with the larger juvenile training dataset and the more detailed adult images.

Mutations and reorganization in bacterial genomes

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DNA is a powerful tool that can be used to examine an organism's lifestyle. Some organisms, such as bacteria, undergo many complicated processes to help organize their genetic content. Horizontal gene transfer (HGT), reorganization, and mutations have allowed bacteria to efficiently access and manipulate their DNA. Scientists typically focus on each of these processes individually, leaving uncertainty as to how mutations, HGT and reorganization interact to shape the evolution of bacterial genomes. In this work, we focus on assessing mutations in the context of genomic reorganization. To do this, we look at the evolutionary history of mutations and reorganization in ancestral bacteria. We found that incorporating reorganization into our analysis changes the distribution of mutations within bacterial genomes. DNA mutations give us a glimpse into an organism's evolutionary history. However, all aspects of DNA organization — or reorganization — need to be incorporated to accurately interpret mutations.

Investigating non-random death and selection on flowering time in seedbanks

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Measuring the rate and direction of adaptive evolution in response to global change is essential to ensure longevity of populations, species, and ecosystems. Resurrection experiments are a valuable tool for this task; ancestral propagules, such as seeds, are kept in storage banks and revived and grown alongside descendent generations in a common environment. However, even under the best conditions, long-term storage over years or decades causes mortality of a fraction of propagules. If storage acts as a selective sieve favouring survival of certain individuals, and probability of survival is genetically correlated with a trait of interest, resultant estimates of evolution may be distorted. Here, we test whether seed ageing in storage results in non-random survival in Brassica rapa, and in turn, how this can impose selection on adult flowering time. In the parental generation, comprised of early- and late-flowering genotype lines, we recorded the beginning of each plant's flowering period and made various measurements of fitness. We have also quantified the time at which each seed was created on a plant and estimated of the ratio of early- and late-flowering alleles in the pollen pool in 5-day intervals. We performed germination trials at various temperatures and treatment durations to determine the conditions that induce 50% mortality, thereby mimicking the physiological effects of propagule ageing during long-term storage. We are growing the F1 generation, half of which were artificially aged as seeds. We compared survival rates of seeds from the first flowers made by individual maternal plants (which have first access to maternal resources) to those from her last flowers, to determine whether seeds that experience priority effects of maternal provisioning have greater resilience against ageing. These last flowers are also more likely to be pollinated by genetically late-flowering donors. If the poorer condition of last-produced seeds causes storage death, genes from late flowering fathers will be disproportionately lost. Thus, agerelated decline in maternal allocation to seeds can impose selection on flowering time through pollen donors, falsely shifting flowering time earlier. The results of this project will help inform methodology for seedbanks and resurrection experiments, such as *Project Baseline*.

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Phenotypic selection on mating system and floral traits in mixed-mating columbine

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Because plants are sessile, they depend on biotic and/or abiotic vectors to transfer pollen from the male pollen-producing anthers to the female pollen-receiving stigmas. As a result, plant mating systems evolve through selection on the floral traits that influence how much pollen is transferred from anthers to stigmas within flowers (self-pollination) vs. between flowers on different individuals (outcrossing). Thus, mating systems are influenced by the traits that dictate the relative abundance of self-versus outcrossed pollen on stigmas. Spatial separation between anthers and stigmas within flowers (herkogamy) is expected to regulate self-pollination yet there are few estimates of how natural selectin acts on this trait. Aquilegia canadensis (columbine, Ranunculaceae) is a short-lived herbaceous plant of rocky outcrops throughout eastern North America that makes seed through both self-fertilization which is influenced by herkogamy, and outcrossing, which is likely influenced by the plant's floral display size (flower number and size). Selfing provides reproductive assurance in natural populations of columbine, whereas outcrossing appears to produce much fitter offspring, and there is a trade-off between these two components of the mating system. We, therefore, predicted correlational selection between herkogamy and display size: selection would favour reduced herkogamy among individuals with small floral displays (to enhance reproductive assurance) and increased herkogamy among individuals with large floral displays (to reduce selfing when outcrossing is likely). We tested this prediction using multivariate linear regression to estimate phenotypic selection through seeds/fruit and seeds/plant on floral traits and plant size for 1015 plants from nine populations of A. canadensis at the Queen's University Biological Station. We found that plant size was universally favoured, through selection for display height in all nine populations and selection for number of basal leaves in four of the populations. Yet, selection for floral morphology was weak. We found no directional selection for herkogamy or floral size and detected correlational selection between herkogamy and floral display size in only one population.

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Furthermore, the correlational selection we detected favoured individuals with large floral displays and reduced herkogamy, opposite to what we had predicted. Therefore, our results do not support our original hypothesis.

One shell shape to rule them all? The effects of hydrodynamic forces on the shape of freshwater mussels

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sabetime@uoguelph.ca; Department of Integrative Biology, University of Guelph Unionid mussels are a diverse group that play a pivotal part in aquatic ecosystems. They provide a number of ecosystem services including the improvement of water quality through their suspension feeding. The interaction of water flow with mussel shape also affects the ecophysiology of freshwater mussels in terms of access to the water column for suspension feeding and for sexual reproduction. Unfortunately, hydrodynamic forces can disrupt this access through the dislodgement of freshwater mussels from the benthos. This presentation focuses on the role of hydrodynamics in the evolution and diversity of shell shapes of unionid mussels. Specifically, it is hypothesized that unionids have evolved in response to the hydrodynamic forces of lift and drag as well as shear stress in the benthos while carrying out their feeding and reproductive activities. It is likely that unionid with elongated and circular shell shapes differ in their response to the hydrodynamic forces. It is predicted that mussels with elongated shell shapes will experience less force than nonelongated shell shapes while accessing resources higher in the water column. These predictions are examined using computational fluid dynamic modeling, direct measurement in a flow chamber using force transducers and inferences from measurements in the field in southwestern Ontario. This research will provide a boarder understanding of the effects of hydrodynamic forces on organisms living in rivers, as well as the evolutionary history of unionids, which date back to the Triassic Period. In addition, further knowledge on past aquatic ecosystems can assist with developing conservation plans for endangered bivalves as well as other aquatic organisms. Data from computational fluid dynamics modelling and analysis, and measurements in a flow chamber are currently being collected. It is expected that lift and drag data will be correlated based on these measurements and analysis.

Evaluating the efficacy of an intervention on reducing Catostomus sucker hybridization in the Upper Colorado River Basin

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icampb37@uoguelph.ca; Department of Integrative Biology, University of Guelph Non-native species are considered a leading threat to biodiversity. They pose risks to native populations through competition¹, predation², disease³, and hybridization⁴. Often driven by anthropogenic influences, native and non-native species can sometimes hybridize to the detriment of the imperiled native species⁵. Catostomus fishes in the Upper Colorado River Basin are an example of this. Recent evidence has shown extensive hybridization between the non-native white suckers (C. commersonii) and the native flannelmouth and bluehead suckers (C. latipinnis and C. discobolus)^{6,7}. This system provides a suitable model for testing the efficacy of an intervention to reduce the abundance of non-native species and their hybrids. For this study we are implementing a Resistance Board Weir (RBW) across Roubideau Creek, a tributary of the Gunnison River Basin, to restrict non-native fish access to spawning habitat. Conducted over four years, the study will gather genetic data from larval species, pre- and post-implementation of the RBW. The larval fish will be used for DNA extractions, which will then be sent for sequencing after extractions and library preparation are complete. The raw sequencing data that is returned will be filtered and assembled. Using bioinformatic approaches, such as the hierarchical Bayesian model entropy^{8,9}, ancestry of the larval fish will be estimated. This ancestral genetic evidence will allow us to determine the efficacy of a RBW at controlling non-native species. It is expected that white suckers and their hybrids abundance will decrease significantly during years of controlled access. Additionally, we will determine larval ancestry trends across the study location to better understand species spawning preferences. Based on advice from Colorado Parks and Wildlife fisheries biologists, who have extensive experience working on this system, it is predicted that more native larval suckers will be found upstream, and more hybrids and non-native species will be found downstream within the spawning habitat (K. Thompson, personal communication, 2020). This work will contribute novel information to our understanding of how effectively conservation efforts using fish barriers can reduce non-native species and prevent hybridization.

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Measuring thermal tolerance over a small ecological gradient

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 Ants play a substantial role in our ecosystems and changes in temperature that affects ants could

potentially have a cascading effect throughout terrestrial ecosystems. We know that ants are thermophiles, with a thermal maximum typically exceeding 40 degrees Celsius. We also know that the thermal tolerance range of ants is heavily dependent upon their location. While numerous studies have estimated the thermal tolerance of ant species in tropical areas, there are fewer studies that look at the thermal tolerance of ants north of the United States. My research focused on the two most common ant species found in a natural heritage woodlot on the University of Guelph campus (the Dairy Bush) Solenopsis molesta (field) and Aphanoegaster rudis (forest). I wanted to better understand the critical thermal maximum values for each species and whether that tolerance changes throughout the active season (May – October). Forests are known to be cooler in temperature compared to fields, due to the canopy created by the trees which reduces the amount of heat that comes into the forest. Previous research has shown that ant species may be adapted to their thermal environment. Diamond et al, 2018 found increased cold and decreased heat tolerance in the coldest rearing environments, but increased heat and decreased cold tolerance in the warm rearing environments for acorn ants (Temnothorax curvispinosus). If the critical thermal maximum of Solenopsis molesta and Aphaenogaster rudis are created by the predominant maximum temperatures of the environment they are found within, then Solenopsis will have a higher thermal tolerance than Aphaenogaster. Ants species collected in the field were brought back to the lab to conduct the experiment. Thermal tolerance was measured by heating a container of water, while ants were placed in tubes in the floating rack. The temperature was increased gradually until the ant species spasmed uncontrollably, indicating that they have reached their CTmax. It was found that the species that live in the field (Solenopsis molesta) had a higher CTmax value than that of the species that lived in the forest (Aphaenogaster rudis). Proving our hypothesis to be true. This

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indicates that as temperatures continue to rise, species more adapted to forest like temperatures will have to increase the thermal tolerance or they will risk the chance of extinction.

Assessing long-term patterns in male territorial behaviour in eastern carpenter bees (*Xylocopa virginica*)

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Territoriality is displayed in various male bees and wasps that establish and defend potential spaces for reproduction, including males of the eastern carpenter bee (Xylocopa v. virginica) found throughout the eastern United States and southern Ontario. Males emerge before females in spring and usually hover in spaces (i.e. territories) near the nesting site while waiting for females to emerge. During the breeding season, males spend most of their time holding and defending territories by chasing away competitors, with the goal of acquiring mates and passing their genes to the next generation. Since these behaviours are energetically costly, males eventually wear themselves out and disappear from the nesting site. Thus, a male's reproductive fitness depends on his ability to hold and defend territories long enough to mate before disappearing. One of my research objectives is to investigate long-term patterns in territoriality and social interactions among males, such as the relationship between body size and territorial behaviour. My analyses are based on the hypothesis that a male's size may influence his ability to hold and defend territories, ultimately impacting his reproductive fitness. Thus, large males should hover at the nesting site longer and chase other males more frequently than small males, giving large males a selective advantage at acquiring mates. During May to July 2020, I caught, measured and individually marked X. v. virginica males emerging from nests throughout the Glenridge Quarry Naturalization Site (GQNS) in St. Catharines, Ontario. Using the unique colour combinations painted on each male's thorax, I recorded hovering behaviour and male-male chase interactions observed at the nesting site throughout the breeding season. I plan to investigate and compare patterns in hover rates and male-male chase interactions across multiple years, by comparing the same data from 2016 through 2020. These comparisons will demonstrate whether there are changes in patterns of male interactions and territorial behaviour across years and the potential selective factors behind this annual variation, providing further insight into the evolution of social behaviour and territoriality in X. v. virginica. Genotyping analyses will be essential in the future to assess paternity levels across

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years and investigate relationships of body size and territoriality on reproductive fitness in *X. v. virginica* males.

Long-term vs. transient plant responses to warming and nitrogen addition in a temperate old field

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Climate warming and atmospheric nitrogen (N) deposition are two global change factors expected to have strong effects on plant productivity and species composition in northern temperate ecosystems over the next century. While analyzing the physiological responses of individual plants to temperature variation and nutrient availability under controlled conditions provides important mechanistic information, there also is a need to investigate responses to these factors in the field, where greater contextual relevance is present. However, such field experiments are typically constrained by temporal scale, and the question arises as to what extent short-term findings can be extrapolated to the longer term. We hypothesized that lags in plant species composition responses to treatments and treatment effects on litter accumulation result in a divergence of long-term versus short-term responses in productivity. As well, long-term N addition should decrease nitrogen resorption efficiency. To test our hypothesis, we compared the short-term (1 year) vs. long-term (14 year) effects of warming (via overhead heaters) and N fertilizer addition on plant productivity, leaf N content, relative species abundances and litter accumulation in a field experiment conducted in a temperate old field. Litter subsamples were collected from each plot in the spring to measure litter accumulation. Spectral data (normalized difference vegetation index) were used to examine the treatment effects on the timing of plant green-up and senescence. The aboveground biomasses of the dominant grass species, *Poa pratensis* and *Bromus inermis*, were destructively sampled at peak biomass, and aboveground biomass estimates for the forb species were obtained non-destructively based on percent cover measurements. Total belowground plant biomass estimates were obtained from root cores. During the destructive harvest, leaf subsamples (green tissue) of the two dominant grasses were collected and additional samples were collected in the fall (senesced tissue) for tissue N analysis and nutrient resorption efficiency estimations. Furthermore, plot litter samples were collected in the fall and placed in nylon mesh litter bags, which will be recollected this spring to examine litter decomposition rates based on the treatment effects. To date, our biomass results show there were few differences in treatment responses among the short- and long-term plots. We

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accredit this result to the continued competitive dominance of the two highly abundant non-native grass species. Our results suggest an important role of initial species composition in potentially delaying the emergence of long-term treatment effects in global change field experiments.

Does seasonality influence the foraging efficiency of eastern carpenter bees leading changes in maternal investment?

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Seasonal weather patterns influence the availability of pollen and nectar resources for pollinators, like bees. Bees adjust their size and number of offspring according to pollen availability. Previous evidence suggests smaller, fewer offspring when pollen availability is lower and larger, more offspring when pollen availability is higher. When pollen is hard to find, bees forage longer to make up for the lack of pollen. When pollen is easier to find, bees spend less time foraging. So, in years with high pollen availability we predict: 1 more foraging trips per day, 2 shorter foraging trips, 3 longer total time spent foraging per day or per offspring therefore, more pollen provisioned, and 4 handling time does not change depending on pollen availability. Niagara experienced a drought in 2016, which represents are year with low pollen availability, during the first year of a multi-year study (2016-Present). We aim to determine whether those poor weather conditions altered foraging behaviour thereby lowering maternal investment leading to smaller, and fewer bees in the Eastern carpenter bee, Xylocopa virginica. We observed bees in the Spring of 2016-2019 during their provisioning season and recorded the time of departures and arrivals of bees at nest entrances. To capture bees, we used simple plastic cup traps with a hole cut in the bottom and cellophane secured with an elastic band on the open side. We marked each bee with enamel paints each year to track individual bee behaviour. We also removed a tarsal sample for genotyping, measured their head width, inter-tegular width, and costal vein length. We hope that the results of this study will show the mechanism, foraging time, that bees adjust due to pollen availability, which should be indirectly related to the size and number of offspring.

Pollen capture and inflorescence architecture in a windpollinated plant

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Plants are sessile organisms, and therefore outcrossing species must rely on outside vectors to disperse their pollen. Pollination success in wind-pollinated plants depends on the capture of pollen from the airstream. Wind-pollinated plants have small flowers with long, exerted stigmas that protrude into the airstream to maximize pollen capture under aerodynamic constraints by minimizing interference of other parts of the flower. The long or feathery stigmas are typically receptive along their entire length. In some wind-pollinated species, it has been documented that stigmas continue elongating after initial anthesis, but it is unclear whether this depends on successful pollen capture and fertilization. Furthermore, inflorescence architecture may also affect pollen capture. In this project, we are investigating pollination in Amaranthus tuberculatus, a dioecious wind-pollinated annual. Specifically, we are testing whether plants with longer stigmas capture more pollen, and the effect of inflorescence architecture on pollen capture. We predict that (1) stigmas will continue elongating if they are not pollinated and longer stigmas will capture more pollen, and (2) unbranched plants will have more flowers per cluster than plants with branches as an alternative strategy to capture pollen. In August 2020, we conducted an outdoor array experiment, where each day 40 male plants were placed around a smaller ring of 15 female plants. Each plant was exposed for 8 hours to natural wind pollen for 12 days. We placed a subset of flowers in ethanol to assess stigma length and pollen capture, and the rest of the flowers were allowed to set seed. Plants that did not have branches were more likely to have denser flower clusters on the main stem compared to plants that had branches, indicating alternate inflorescence architecture designs. Surprisingly, pollen capture was very low, despite high seed set, in part suggesting that very few pollen grains are necessary for fertilization and seed set. We are following this experiment with a growth chamber study to test the role of stigma elongation for pollen capture and the impact of inflorescence architecture on flower number. This research represents novel approaches to understanding floral and inflorescence variation in wind-pollinated plants.

Delayed pollination extends floral longevity at a cost to seed set in an Eastern North American wildflower

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Pollinator declines are predicted to decrease the fitness of insect-pollinated plants because nonselfing species require pollen transfer between flowers to reproduce. However, plants may respond to pollinator declines by plastically adjusting their floral traits to increase the opportunity for pollination. One such trait is floral longevity. Increased longevity can enable plants to prolong the opportunity for fertilization under pollinator declines, but this trade-off may be costly because longer-lived flowers increase the carbon and water requirements of seed production. To determine if longevity is plastic and if the plastic response is costly, we measured floral longevity and seed set in female and hermaphroditic Lobelia siphilitica flowers. We used a paired-design approach by marking two flowers per plant, with one flower hand-pollinated on day-1 of the female phase, and the other hand-pollinated on day-5 of the female phase. To measure longevity, we tracked the number of days from when the flower opened until it wilted. We also measured seed set to determine the cost of delayed pollination in flowers that were pollinated on day-1 versus day-5. Lastly, we calculated the difference in seed set between day-1 and day-5 flowers for each sex to determine if delayed pollination was costlier in hermaphrodites than females. We predicted that delayed fertilization would be costlier in hermaphrodites because of their larger corolla which require more resources to maintain. Flowers on both female and hermaphroditic plants responded to delayed pollination by extending floral longevity, resulting in lower seed set. Longevity was an average of 1.7 days shorter in flowers that were pollinated on day-1 compared to day-5, which supports the hypothesis that pollination timing effects longevity. Additionally, flowers pollinated on day-1 produced 35% more seeds than flowers pollinated on day-5, demonstrating that delayed pollination reduces seed set. However, there was no difference in seed set between the sexes which demonstrates that the larger corolla for hermaphrodite plants are not a significant resource sink compared to females during prolonged longevity. Overall, our results suggest that plants can respond plastically to pollinator declines by extending longevity. The ability to extend longevity prolongs the opportunity for fertilization, but at a cost to seed set. These findings demonstrate that Poster Presentations, Ecology

plants capable of plasticity are active participants in the plant-pollinator relationship, and that we need to study plasticity in floral traits in order to understand the response of plants to pollinator declines.

Applying ecosystem-based adaptation to protect biodiversity of avian species in coastal communities in the greater Niagara region

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Communities located in the coastal zone are increasingly vulnerable to climate change. The effects of climate change may push coastal ecosystems to undergo irreversible changes. This is especially true for shorebirds as it results in the loss of biodiversity and resource-rich areas to rest, refuel and breed. To protect these species, it is critical to conduct more research related to nature-based solutions. Through a scoping review of scientific literature, this paper evaluated 85 articles and included a summary of various sustainable ecosystem-based adaptation strategies, including living shorelines and beach nourishment. These strategies were evaluated under the eight core principles of nature-based solutions in order to determine the efficiency of protecting shorebird biodiversity in the Greater Niagara Region. All adaptation strategies were examined through a social, economic and environmental lens and future improvements were suggested to increase the efficiency of these strategies. This research suggested the best strategy would be a combination of living shorelines and beach nourishment. Forest cover and managed realignment/retreat would also be beneficial in some areas, depending on geographical location and characteristics. To maximize the efficiency of sustainability of these adaptation strategies, Integrated Coastal Zone Management (ICZM) could be implemented to ensure the involvement of all stakeholders and levels of government.

Fungal endophyte discovery, characterization and function in various host grasses

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It is well understood that there exists a crucial relationship between the soil microbial community and plants. This can greatly improve plant nutrient acquisition, abiotic stress tolerance and some biotic stress resistance. Co-evolution has led to some micro-organisms from the rhizo- and phyllosphere becoming incorporated within plant tissues, and these microbes are known as endophytes. The interaction between plant and endophyte is of much interest because although mutualistic, secondary metabolites produced by endophytes can cause pathogenic outcomes, detrimental to the host. There is much focus on root endophytes, and/or endophytes in general, however, seed-borne endophytes are unique, as this point of the life-cycle represents the beginning of a life-long relationship between symbiotic partners. Clearly, the vertical transmission of beneficial endophytes perpetuates the symbiosis across generations and enhances symbiont fitness. Here, we investigate whether endophytes belonging to one host species can be transferred for the benefit of another host species in the same family. To this effect, the aim of this study is to isolate, characterize, and inoculate selected endophytes into various grass species to determine their individual effect on host plant growth. More than 400 fungal endophyte strains were isolated from seeds that were harvested from 24 Andropogon gerardii (big bluestem) plants grown in a low-input system. We used DNA sequencing to assign 111 isolate morphotypes to 11 genera. Isolates were screened for nitrogen fixation, phosphate solubilisation, and cellulase and protease production. Selected candidates will undergo further characterization and a plant assay trial to determine which will be used in greenhouse growth experiments with the host grasses. Biomass, proteomics, and metabolomics measurements will provide a comprehensive analysis of microbial-plant interactions. By discovering potential plant-growth-enhancing endophyte(s) on multiple hosts, we hope to further the understanding of endophytic symbioses towards novel ecological and agronomic applications.

Discovering endophytes from *Andropogon gerardii* for economic and agronomic applications

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We need better methods of producing crops, whether they are for food or fuels, in order to sustain the growing global population without expanding the amount of area under cultivation. One sustainable alternative to explore involves utilizing plant species containing microbes that could increase yields. Endophytes are non-pathogenic bacteria or fungal microbes that provide benefits for improving plant performance. Andropogon gerardii (Big bluestem) is a perennial grass with recognized potential for biomass production known to host endophytes, and the species has been selectively bred for performance in low-input systems. However, limited information exists about the identity and function of such endophytes. Here, we addressed the following questions: 1) What are the plant-growth promoting benefits and antimicrobial resistant properties of endophytes identified in A. gerardii, and 2) how do individual endophytes affect the growth of their host plants? Seeds from 24 individual A. gerardii plants were provided from Resource Efficient Agricultural Production Canada. Bacteria and yeasts were isolated into pure cultures from aboveground tissue, and they were identified using DNA barcoding. In addition, a series of biochemical and phenotypic assays were performed in vitro to determine how the endophytes can use nutrients and efficiently perform within the plant host. We found a total of 14 bacterial and seven yeast endophyte isolates, which were successfully identified using the 16S rRNA and ITS gene regions, respectively. Our data show that several yeast endophytes exhibit positive enzymatic production associated with the use of inorganic nutrients. Additionally, several yeast endophytes demonstrated resistance towards fungal and bacterial pathogens in antagonistic assays. Bacterial endophytes are currently undergoing similar tests to determine their substrate use and antimicrobial effects. Ultimately, once all in vitro tests have been completed, the best-performing endophytes will undergo in planta experiments to test whether they can promote host plant growth. These results will have the potential to demonstrate positive plant-microbial interactions provided by endophytes as well as contribute to future agronomic applications.

Studying the bee-cosystem: using community science and DNA barcoding to investigate cavity-nesting bee and wasp distribution and interactions across Canada

Sage F. Handler*, Dirk Steinke, Nigel E. Raine

shandler@uoguelph.ca; School of Environmental Sciences, University of Guelph Landscape urbanization often has negative effects on species' survival and community structure by replacing natural, green habitats with impervious surfaces. This habitat loss and fragmentation harms many species and their interactions, some of which provide beneficial ecosystem services to both natural and human ecosystems. Plant-pollinator, predator-prey, and host-parasitoid interactions provide essential services including pollination and pest control but are difficult to study as they are extremely complex. In cavity-nesting Hymenoptera, including solitary bees and wasps, all three interaction types can be observed as bees visit flowers, wasps interact with their prey, and both are hosts for parasitoids. The importance of pollinator populations, both to food production and wild ecosystem pollination, is well-known. Bees are essential pollinators of many fruit, vegetable, nut, and animal feedstock crops and sustain the diversity of terrestrial plant ecosystems. Natural predators such as wasps are increasingly being used in integrated pest management to control agricultural pests, reducing the need for pesticides. They are also good indicators of habitat health as they are sensitive to resource losses. To further explore the effects of landscape on these essential interactions, we are using data collected from the Bees@Schools community science program in 2019 and 2020. Each year, nest boxes were distributed to elementary and high schools across Canada and installed on school or teachers' home properties in May. At the end of the summer, nest boxes were returned to the University of Guelph, and all occupants and their food sources were identified using DNA metabarcoding. With these identities, we will examine the interactions between bees, wasps, and pollen in interaction networks and their relationships with their environment using landscape analysis. Results from our project will provide policy makers with current cavity-nesting hymenopteran distributions and interactions in Canada to aid management decisions in supporting their populations and the essential ecosystem services they provide.

Comparing total RNA sequencing and metagenomics pipelines for multi-domain taxonomic profiling: implications for ecological assessments

Christopher A. Hempel*, Julia Harvie, Jose S. Hleap, Natalie Wright, Sarah J. Adamowicz, Dirk Steinke

hempelc@uoguelph.ca; Department of Integrative Biology, University of Guelph Ecological assessments are necessary to evaluate the status of our deteriorating ecosystems, however, assessment methods traditionally omit most microbes because unicellular organisms are challenging to identify. This omission is not ideal, as microbes might be better indicators for changes in environmental conditions than taxa traditionally used. DNA- and RNA-based techniques are increasingly applied for ecological assessments to overcome this challenge but require more testing and optimization. In this study, we compare metagenomics and total RNA sequencing (total RNA-Seq) for their taxonomic profiling performance for microbial communities. We applied both techniques on two sample sets, 1) a commercially available microbial mock community consisting of eight bacterial and two eukaryotic species, and 2) a display tank water sample. We processed the data using 1,532 bioinformatics pipelines and evaluated each workflow, i.e., the combination of sample type (metagenomics or total RNA-Seq) and pipeline, in terms of their accuracy and precision. This poster will showcase preliminary results and highlight differences in workflow performances. A recommended workflow to maximize taxonomic profiling accuracy of microbial communities will also be presented.

Germination differences between forage crop and nonforage species

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Forage crops are plant species that are cultivated in fields for the purpose of consumption by livestock. These species have been widely distributed and are broadly naturalized in many plant communities. Forage crop species have been chosen based on specifically desirable characteristics and have likely undergone human-directed selection that has further altered their character. Since these species are generally planted from commercial seed, we expect that forages have been selected for higher seed germination and lower seed dormancy. We tested whether forages differ from non-forages in their germination traits using three experiments. We first compared percent germination and time to germination between forage and non-forage species in a greenhouse experiment, and then repeated this in a second experiment in field conditions. These experiments tested whether forage species germinated more readily, and more quickly than other species that they grow with in grasslands. Thirdly, to test whether human selection on forage species has contributed to differences in percent germination rates and time to germination, we conducted a growth chamber germination experiment comparing germination between forage species to nonforage congeners. In both greenhouse and field conditions, the average percent germination of forage species was significantly higher than that of non-forage species. Additionally, we found that forage species had significantly higher germination percentages and faster germination times than did non-forage congeners, providing evidence consistent with forage species having undergone human selection for high germination rates and low seed dormancy. Our results indicate that forage crop species germinate at significantly higher percentages, and significantly faster, than the nonforage species that they regularly co-occur with in hay fields and abandoned agricultural fields. Furthermore, our results show that while forage crops may have initially been chosen because they possessed specific germination traits, they also appear to have undergone human selection to germinate more readily and faster. These findings are significant because the germination performance of these species appears to have possibly resulted in their disproportionate use in at least some kinds of ecological growth experiments that begin with germination. For example, forage Poster Presentations, Ecology

crop species are overrepresented in species pairwise competition studies. Such an overrepresentation may produce a biased understanding of many basic ecological processes. Future work should investigate other differences among forage and non-forage species, as well as their impact on the current state of ecological theory.

Arctic biodiversity and colonization: Past, present and future

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The Arctic has been shaped by glaciation and post-glacial colonization and is again undergoing significant changes. As the climate shifts, so will Arctic communities and species compositions. One way to predict future colonization is through past successful colonization or dispersal. The goals of this study are to: 1) determine realized dispersal rates and test whether species with similar traits have similar realized dispersal rates, 2) determine which traits are associated with Arctic animal species and use these to predict future trends in Arctic composition and colonization, 3) create efficient, reproducible, reusable pipelines that will be made publicly available for future research. This is a large-scale study including a diverse set of animal groups with varying traits (e.g. habitat, diet, dispersal ability, breeding system). I hypothesize that realized dispersal rates and Arctic species composition will be influenced by the biological traits of organisms. Data will be pulled from the Barcode of Life Systems (BOLD), Global Biodiversity Information Facility (GBIF), as well as taxonspecific literature and publicly available trait databases. Population genetic structure measures (e.g., F_{ST}) will be calculated as an indicator of realized dispersal rates, and analysis of variance will be used to determine whether these rates are similar across species with different traits. I will determine whether certain traits are more associated with Arctic taxa and then use these traits, realized dispersal rates, and climatic data to predict where species are likely to be found and what kinds of species are likely to move north in the future. This project is currently in its early stages, but the poster will include my plans for methods, expected results and some data exploration figures and summary statistics, such as the number of species present in my Arctic study region and the proportion of different traits present in the population. This study aims to provide insight into the factors affecting species composition in the Arctic and realized dispersal, to predict future Arctic colonization and biodiversity patterns, and to create efficient bioinformatic pipelines.

No stomach, no problem: an integrated morphomolecular approach to assessing the diet diversity and composition of a cleaner wrasse, *Tautogolabrus adspersus*, among coastal, nearshore regions of Atlantic Canada

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moirc@uoguelph.ca; Department of Integrative Biology, University of Guelph High biodiversity in coastal marine ecosystems allow for the formation of complex and often inconspicuous trophic arrangements between consumer and prey taxa. For marine invertivores, the true composition and diversity of such arrangements are often poorly reflected in their observable diet. Feeding characteristics of the cunner, with presumably high digestion rates linked to the absence of a true stomach, further obscure the efficacy of traditional diet assessments. This study aimed to address several knowledge gaps including: (i) prey composition and diversity in the diets of T. adspersus along latitudinally-defined regions with context to biogeographical changes in prey community composition, and (ii) to evaluate the efficacy of an integrated morpho-molecular approach for diet assessments of the stomachless cunner wrasse. We used morphological and mitochondrial COI sequence data of 110 whole diets from 10 sample sites among four latitudinallydefined regions along the exposed-coast of Atlantic Canada. Common prey taxa include the invasive sea vase tunicate, blue mussel, acorn barnacles, and several malacostracan species. Distinct patterns in prey composition among four sampled regions were found to be significant through morphological assessment. However, beta dispersion of assigned prey taxa from multi-marker COI sequences demonstrated vast similarities among regions, with just ~5% of taxa disparity explained by region. Our analysis provides considerable advancements with regards to the inconspicuous diet of a temperate marine wrasse within highly diverse coastal communities and contributes to a rapidly growing field of ecologically significant approaches in assessing highly complex trophic interactions.

Characterization of cytokinins in cover-cropped soils

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ushettigeperera@trentu.ca; Environmental and Life Sciences Graduate Program, Trent University Cytokinins (CKs) are a group of naturally occurring hormones produced in trace amounts by plants and other living organisms, including microorganisms (archaea, bacteria and fungi), nematodes, insects, mammals. CKs are chemical compounds with an adenine ring and a side chain at the N⁶ position that determines their biological activity. CKs produced by living organisms can be released to the soil environment via root exudates, degradation of organic matter, and microbial secretions, indicating soils may be CK-rich environments. Studies related to the occurrence of hormones in environmental samples have been long neglected because of the complexity of hormone isolation from complex environmental matrices like soils, and the highly sensitive analytical techniques required to identify trace levels of hormones. In Ontario, many farmers have adopted the use of cover crops within their cash crop rotations; cover crops are grown for a variety of reasons, including soil erosion control, nutrient retention, and soil health promotion. In agricultural soils where living roots are kept in field via use of cover crops in non-growing seasons, there may be changes in the relative abundances of CKs, which may have downstream impacts on cash crop yields. Therefore, characterizing the impacts of cover crops on CKs in agricultural soils is of critical importance to better understand how our agricultural management may impact these small molecules, which act as growth regulators and signalling molecules in the plant-microbe-soil continuum. We extracted CKs from bulk and rhizosphere soils using HLB cartridges after growth of cereal rye (Secale cereal) and tillage radish (Raphanus sativus), two cover crops often used by Ontario farmers - over three months in a greenhouse pot experiment using two agricultural field soils. Cytokinin profiles were identified using high-performance liquid chromatography – highresolution tandem mass spectrometry. Two groups of CKs, Riboside-CKs and 2-Methylthiol-CKs, were detected in all soil samples, while freebase-CKs were detected only in tillage radish-grown soils. CK levels in control soils (no living roots) were below detection limits. These findings suggest that cover crops positively affect the occurrence of CKs in soil samples, indicating a potential indirect mechanism contributing to improved soil health and crop yields in agricultural production systems utilizing cover crops.

A deadly hiding spot: A unique case of plant carnivory in Algonquin Provincial Park

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asemenuk@uoquelph.ca; Department of Integrative Biology, University of Guelph Juveniles of pond-breeding amphibians must face many threats to their survival as they embark from their natal lake to the forest on their first day of terrestrial life. While these have included predation by species of birds and mammals, this list of threats was recently expanded to include a carnivorous plant, the Northern pitcher plant (Sarracenia purpurea). In my thesis work over the past three summers, I have documented that a significant proportion of the emerging spotted salamander (Ambystoma maculatum) juveniles are captured by these carnivorous plants in Algonquin Provincial Park. Pitcher plants, principally known to consume arthropods to gain nutrients, are now recognized to be claiming at least 9% of the new salamander generation each year. In my thesis, I am investigating whether salamanders are attracted to these pitcher traps, what factors lead to their death, and if their nutrient contribution to the plant is, in fact, beneficial. To date, I have shown that salamanders are more likely to enter pitcher leaves that face the salamanders' natal lake. This suggests the animals are following the most direct route between the lake and their eventual adult home – the adjacent forest. My preliminary nutrient analyses have identified a new aquatic-based carbon signature in plants that have consumed salamanders, indicating a change in plant tissues may be occurring at the molecular level. To continue researching this trend and its implications on the plant population, I will continue to conduct stable isotope analyses of pitcher leaves that have consumed salamanders to determine if there is a characteristic trace of this unique vertebrate carnivory.

Temporal dynamics of freshwater mussel larvae

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ssmodis@uoquelph.ca; Department of Integrative Biology, University of Guelph Native freshwater mussels have a complex reproductive cycle involving larval parasitism on a vertebrate (usually fish) host that has contributed to their decline. Gravid female mussels release their parasitic larvae, known as glochidia, into the water column and successful attachment onto a host is critical for continuing development into juveniles. Freshwater mussels often live in multispecies 'mussel beds' (e.g., 25 sp. in sites in the Sydenham River, Ontario) where many of the mussels have been reported to use the same fish species as hosts. Given the coupling of mussels and host fishes, the purpose of this study is to gain a better understanding of the early life history and ecology of mussels to aid in their conservation. We are examining the temporal dynamics of glochidia in the water column and the potential for partitioning of their 'host' environment with the timing of glochidial release among species. We hypothesized that glochidia from different mussel species are released at different times to minimize potential competition for host fish. Glochidia were continuously sampled for 2-h discrete periods over ten 24-h sampling days (120 x 2-h samples) in the Sydenham River from late August to September. This is a period when a large number of mussel species are known to be gravid. Glochidia abundance and identification to the species level was undertaken using morphometrics based on the known shapes and sizes of mussel species in the Sydenham River. The results will inform us when gravid females release their glochidia during the day and can be used to examine whether females try to match the diel activity of fish, increasing the chance of successful parasitism. Greater insight into the host-parasite relationship will be achieved, and potential threats and mechanisms leading to mussel declines can be revealed. This study will help improve our scientific understanding of mussel reproduction and ecology to supplement appropriate recovery, conservation and management plans.

Does installation of low-head barrier dams have long term effects on downstream fish prevalence species, richness, and diversity?

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adsolomon@algomau.ca; Department of Biology & Environmental Sciences, Algoma University Invasive species are one of the greatest threats to North America's natural wildlife and ecosystems. Efforts to control the advance of sea lamprey (Petromyzon marinus) into the Great Lakes includes the installation of low-head barrier dams in rivers and streams to prevent the sea lamprey from migrating upstream to locate spawning grounds. Damming of streams also affects non-target species, with that in mind we undertook a study to determine the effects of these dams on native fish populations. This study utilized a dataset collected by Fisheries and Oceans Canada (DFO) consisting of trapping data on the downstream face (i.e., fish moving upstream) of temporary weirs and low-head barrier dams for over 30 years. We focused on three streams where data was available before and after installation of the dam; Graham Creek, Shelter Valley Creek and Carp River. Two different analyses were undertaken, a comparison of catch per unit effort (CPUE) for rainbow trout (Oncorhynchus mykiss) and white sucker (Catostomus commersonii) pre- and post-installation of the dam; and an analysis of species diversity, CPUE for all species caught, and two measures of diversity index for each of the three streams in the years post installation of the dam. While some of the data indicates the dams may have had an effect on fish populations, it appears it is minimal. By understanding how dams influence local populations of fish, we can help educate decisions about the use of low-head barrier dams in sea lamprey control.

Short-term vs. long-term effects of climate warming and nitrogen deposition on soil carbon fractions and extracellular enzyme activity

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Climate warming and atmospheric nitrogen deposition are two influential global change drivers anticipated to have strong effects on the productivity and nutrient cycling of northern temperate grasslands over the next century. Previous studies investigating the effects of climate warming and nitrogen additions on soil carbon and microbial activity in grass-dominated systems have typically been performed over a short time period (< 5 years). In contrast, long-term experiments (>10 years) have been uncommon but may reveal important cumulative effects. The objective of our research is to compare the short vs. long-term effects of warming and nitrogen deposition on soil carbon fractions and extracellular enzyme activities in a field experiment located at a grassdominated site in London, Ontario. An array of 1 m² plots have been receiving either warming or nitrogen fertilization treatments for 15 years, with overhead infrared heaters used to administer warming (2-3 °C increase at the soil surface) and nitrogen has been applied at a rate of 6 g per year, consistent with predicted nitrogen deposition in the study region by 2050. New warming and nitrogen addition plots were established in 2019, to directly compare short-term effects with longterm effects. Soil samples are currently undergoing fractionation by density using sodium polytungstate to isolate the heavy and light fractions of soil organic matter. These organic matter fractions will be then be analyzed using fourier-transform infrared spectroscopy (FTIR) prior to the conference. In addition, soil extracellular enzyme activities will be assayed in the summer of 2021. This study will provide insight into how well the results of short-term global change studies of soil carbon and microbial function can be extrapolated to the longer term.

A microbial tale of domestication, invasion and conservation: on the gut bacteria of European and American minks in Western Europe

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Specific research in wildlife has explored the influence of the host environment and genotype on the gut microbiota, however many questions remain on those community dynamics, especially related to differences between invasive and native species. The critically endangered European mink is threatened by the invasion of the American mink into its natural habitat in Western France, and we explored the differences in microbiota and genetic diversity between these two species to test for phylosymbiosis. Phylosymbiosis occurs when host phylogenetic relatedness correlates with similarities in gut microbial communities between populations. We investigated the gut microbial community using 16S rRNA metabarcoding of European and American minks in a controlled environment (captive breeding compounds and fur farms) to account for the impact of the environment on gut bacterial composition. We compared these communities to the gut microbiota of both mink species in the wild across multiple habitats. We also investigated population genetic structure among mink populations using microsatellite markers. We found differences in the microbiome community between wild and captive individuals for both species in alpha and betadiversity. Captive individuals had lower alpha diversity than wild mink, with more extreme differences in American mink compared to European mink. Despite captivity being more influential than species identity in terms of gut microbial richness, greater differences between captive and feral American minks may have a historical explanation; the American mink is invasive in Europe and highly domesticated compared to the native critically endangered European mink. However, the beta-diversity and differential abundance analysis showed that American minks from a longestablished population exhibited gut bacterial composition closer to the wild European mink compared to more recently established wild American mink populations. This same American mink population was observed to be the most divergent genetically compared to other American and European mink populations, but not the most diverse compared to farmed conspecifics. No

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phylosymbiosis signal was thus detected because the genetic relationship between hosts did not reflect gut microbial communities' relationships. These results could also be explained by dietary shifts in both species in areas where their gut microbes were closer, potentially based on population densities of riparian carnivorous mammals, rather than genetic relatedness. We therefore advocate for multispecies monitoring in the future to gain a better understanding of the microbial ecology of these mustelids. This exploratory study contributes to the scarce literature currently available on the dynamics between gut microbiota and mammal invasion.

Does urbanization disrupt seasonal changes in the gut microbiome in a wild mammal?

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The impact of herbivory on plant height distributions

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Evidence clearly indicates that the growth of plant species in natural communities is limited by competition with neighbouring plants. Furthermore, experimental evidence suggests that possessing a large body size provides a competitive advantage, in which case we would expect to see larger species limit the diversity and abundance of smaller species in plant communities. However, we find that small species are generally more numerous and diverse than larger species in natural communities. Exactly how small species survive competition with larger species is not entirely clear. In this project, we investigated whether herbivores, which are known to influence vegetation structure and composition, can help explain the coexistence of large and small plant species within competitive plant communities. We tested whether plot-level measures of plant species height (e.g., mean plant height, mean nearest neighbour distance, range and skew) differed between an herbivore exclusion (caged) and control treatment. We also considered two types of census data in our analyses, where non-reproductive species were either included (full) or excluded (flowering), to determine whether including non-flowering data can significantly impact assessments of community structure and composition. We found that herbivores influence the mean nearest neighbour distance in height, with differences being larger in caged than in control plots. This suggests that small mammal herbivores may disrupt the competitive processes facilitating the coexistence between large and small plant species. Comparisons of the two census types show that flowering species are typically larger in size, whereas smaller species often fail to reach the flowering stage. These results suggest that smaller plant species are more transient, their presence supported by regular dispersal into hostile competitive environments. Our findings demonstrate that herbivores play a role in shaping the species size distribution of plant communities, and that examining which species are reproducing within plots can provide important insight into community dynamics, in this case regarding how small species survive competition with larger species.

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Investigating how spider morphology impacts vibration perception

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Many spiders rely primarily on vibrations to obtain information about their surroundings, such as prey in a web, predators walking by, a male plucking a web in courtship or from environmental sources such as wind and rain. Slit sensillae appear like cracks in the exoskeleton and are the organs used to sense web-vibrations. Sensillae are condensed at the joints and organised into lyriform organs which are excited by joint bending. Therefore, we hypothesize that the lengths of leg segments and the positions of joints will determine how vibrations travel through the body and affect perception. It has been shown that even subtle internal changes in leg positioning such as seen in postural changes can alter perception greatly, so here I predict that variations in leg length may affect vibration perception as well. To investigate how varying leg length may alter vibration perception, I took a modeling approach. The power of a modeling approach is that it allows us to identify what specific aspect of morphology impacts vibration perception by altering the model in ways that we cannot do with real biological organisms. Three species were selected in order of increasing leg length: the European garden spider, the black widow and the cellar spider. Multibody models of all three were made and used to study the effect of morphology on joint bending spectra. Comparison of the joint bending velocity across the species will be used to investigate how, if at all, morphology impacts vibration perception. Currently, I am analyzing the output of the three models by visualizing the leg segment velocities, joint bending velocities, and overall movement of the spider at different naturally relevant frequencies. I am also using cross correlation comparisons to compare the joint bending velocities of each pair of spiders to determine how similar or different the joint bending is between each spider. Prior to the meeting I will finish my analyses for all three models and have generated the final figures for my undergraduate thesis. The results of this study will provide insight to how the body of the spider affects its perception by filtering incoming vibrational signals, thus how brain and body are connected through embodied cognition.

Measuring cognitive flexibility in a wild living fish with Alternative Reproductive Tactics

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Investigating and uncovering the underlying mechanisms and evolution of cognition in animals has been furthered by research on numerical abilities, social behaviour, and personalities in fishes. To date, research on fish cognition has been conducted mainly on domesticated or lab-based strains of a few freshwater fish species. My current research aims to examine fish cognition in the context of behavioural plasticity/flexibility in Symphodus ocellatus, a wild living marine fish with three alternative male reproductive tactics (ART's); Nesting Males (NM), Satellite Males (SAT), and Sneaker Males (SN). ART's in this species are thought to occur as part of the male life history pathway rather than by genetic determination. We have been developing a series of lab-based behavioral assays while stationed in the Mediterranean Sea at the research facility, STARESO, during the yearly spawning season (mid-May to mid-June) of S. ocellatus. The measurements from the various assays can then be associated with underwater observations of the social behaviours which occur during the spawning cycle of S. ocellatus under natural conditions. When conducting a lab-based forced choice assay for familiar versus unfamiliar conspecific females, preliminary results indicate that, while time spent with each female was not significantly different between alternative male phenotypes, the activity level and number of side switches by Satellite Males did differ significantly. Ongoing analyses will compare individual performance on the lab-based assays with their behavior at actively spawning nests under natural conditions observed in the wild.

An investigation of reinstatement in the fire-bellied toad (Bombina orientalis)

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Recovery from extinction effects occur when a previously learned behavioural response returns after it has been extinguished by repeated presentation of an event without reinforcement. Recovery can be spontaneous or triggered by features of the context (i.e. renewal) or the reinforcer (i.e. reinstatement) that were used to establish the original learning. Recovery from extinction effects have been shown in mammals, birds, and fish. Thus, they appear to be conserved in vertebrates; however, they have yet to be investigated in amphibians. We investigated if reinstatement occurs in the fire-bellied toad (Bombina orientalis) using a prey catching conditioning task. First, toads underwent one week of prey catching conditioning by feeding a cricket reinforcer following prey catching attempts directed at a cricket stimulus displayed on a computer screen. Once the latency to reach five prey catching attempts had significantly decreased, the toads were submitted to three weeks of extinction training, where prey catching responses were no longer reinforced with crickets. Extinction increased prey catching latencies to levels seen at the beginning of prey catching conditioning. Following extinction, reinstatement was assessed by feeding half of the toads one cricket prior to a single prey catching trial, the other half of the toads were not fed and served as controls. If reinstatement occurred, it would decrease prey catching latency in toads that were prefed a cricket compared with controls. No difference in prey catching performance was seen between the reinstatement and control toads, which suggests that reinstatement is absent in B. orientalis. Our finding suggests that recovery from extinction effects is not universally present in vertebrates. Future work should evaluate which environmental and life history factors favour the presence of recovery from extinction effects.

Analyses of the daily pattern vocalization of *Sciurus* carolinensis, *Tamiasciurus hudsonicus* and *Tamias* striatus (Family: Sciuridae) in an urban forest patch in Northern Ontario

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Quantifying vocal communication is a useful tool for understanding diel activity patterns of species, and it have been studied in a diverse group of animals. For example, many birds, mammals, anurans, and insects have a peak in vocalization during the twilight hours at dawn and/or dusk. In sciurids, seasonal and diel patterns of acoustic communication are not well studied. For example, most studies focus on alarm calling and social species (i.e., ground squirrels). I used automated recorders and spectrographic images to analyse the vocalization rate of Northern Ontario sciurids (eastern gray squirrel: Sciurus carolinensis, eastern chipmunk: Tamias striatus and the American red squirrel: Tamiasciurus hudsonicus) at five sites during eight weeks of fall (14 September – 6 November) in a 13 hectares urban forest patch, in Sault Ste. Marie, Ontario. Supplemental food was provided at recording stations to test how changing resource abundance influenced vocalization rates. We analyzed recordings two days before and two days after food was added for three cycles of food addition separated by two weeks (12 days total). We counted calls during the day, average call rate at different times of day, and average bout length. We also recorded daily temperature and precipitation. Data analysis is in progress; however, we will compare call rates before and after food addition and determine the diel pattern of vocal activity for each species and examine whether there is a seasonal change in call rate during fall. By the time of our meeting, I intend to have finished all statistical analyses, discussion, and conclusion. This study is the first to analyze daily vocalization patterns of populations of S. carolinensis, T. hudsonicus and T. striatus in Northern Ontario, and thus, our results will contribute with more knowledge in the Sciuridae family, by better understanding their vocal behavioral patterns with respect to food cues and may result in new research guestions about this family in the future.

The effects of probiotic therapy on the stress coping styles of triploid juvenile farmed Chinook salmon (Oncorhynchus tshawytscha)

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stlouisb@uwindsor.ca; Department of Integrative Biology, University of Windsor In recent years aquaculture production has more than doubled, becoming an increasingly important and substantial source of meat for consumption worldwide. Triploid salmon – fish with three sets of chromosomes – are regularly produced and used in aquaculture as they are functionally sterile and therefore avoid the decline in flesh quality associated with sexual maturation, but also cannot outbreed in the wild should they escape. However, triploid fish exhibit many behavioural, physiological, and immune drawbacks which reduce their survival and quality in aquaculture environments. Our research examines the effects of probiotic treatment on triploid behaviour and stress responses of juvenile Chinook salmon (Oncorhynhcus tshawytscha), with the ultimate goal of increasing aquaculture-based yield of these fish. In this study, crosses were developed in a 3x3 and 3x2 design, with each family's eggs split so that one half remained diploid (2N) and the other underwent triploidization (3N). At the alevin (exogenously feeding) stage, fish were reared under regular- or probiotic-supplemented feed resulting in four treatment groups: 2N probiotic/regular feed and 3N probiotic/regular feed. Juveniles were tested eight months postfertilization and individually assayed for behaviours in response to an open-field test, novel object, predator, and conspecific stimuli to test for activity, exploration, predator responsiveness, and sociality, respectively. Plasma was then collected for analysis of stress-induced cortisol concentrations; and two subsets of fish were assayed immediately upon tank removal or after a 1.5-minute hypoxia challenge to obtain baseline and maximum cortisol, respectively. We predict triploid fish treated with probiotics will exhibit an increase in a 'proactive' coping style: increased activity, boldness, and sociality behaviours, which will be similar to that of diploid fish on regular feed. We also predict these individuals will exhibit reduced stress-induced, maximum, and baseline cortisol concentrations when interacting with a potential stressor. Currently, behavioural data has been analyzed and results are being statistically interpreted. Preliminary results have shown that the triploid and diploid individuals exhibit differences in their behaviour, indicating that the triploidization process was

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successful, however the probiotic treatment does not appear to have an influence on behavioural output. At the time of the conference, all behavioural analyses should be complete, and cortisol assays should be run and analyzed, with results interpreted. Results from this study will be discussed in light of the use of probiotics and allow for an increasing yield of triploid salmon in aquaculture farms.

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Investigating the allometric diversity of Anolis skulls

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The dimensions of morphological traits often covary with size—a pattern known as allometry. In many taxa, a large proportion of interspecific shape variation is allometric, suggesting that size may influence the evolution of phenotypic shape diversity (i.e., disparity). Specifically, disparity has been hypothesized to be more constrained at body size extremes. For a given body plan, there are two possible sources of size-related constraints. First, intrinsic developmental constraints may limit the production of variation at size extremes. Species can achieve very large or small size via changes in ontogenetic timing over evolutionary time, which may also affect the developmental pathways of other traits, potentially limiting their variation. Second, the environment may limit what is functionally possible for an organism at a particular size and with a certain bauplan. Extremely large and small species may have fewer prey options or microhabitats than mid-sized species, resulting in ecological constraints on morphology. To date, hypotheses that address how extreme size may shape macroevolutionary disparity have received little empirical investigation. To better understand the role of allometry in adaptive radiations, I am studying size and shape evolution across the lizard genus Anolis. Anoles vary greatly in size and have independently and repeatedly evolved similar ecology-associated morphologies across the Greater Antilles. I seek to answer whether allometry can explain patterns of macroevolutionary diversity in anoline skull shape. I predict that mid-sized Anolis species will exhibit greater cranial shape disparity than very small or very large species. I am quantifying anole skull size and shape in 3D using microcomputed tomography (microCT). I will place 53 landmarks on each specimen and use geometric morphometric methods to quantify size and scale-free shape for nearly 200 Anolis species. To test for a relationship between body size and skull shape diversity, I will organize my data into size-class bins and will calculate within-bin morphological disparity using the R packages geomorph and dispRity. I will then compare disparity between size classes using a rarefaction approach to account for sampling differences across bins.

Genetic sources of variation in unreduced gamete production in a mixed-ploidy plant

Sarah DeVries*, Brian C. Husband, Paul Kron

sdevri04@uoguelph.ca; Department of Integrative Biology, University of Guelph Unreduced gametes are the primary mechanism of the formation of polyploids - organisms that possess more than two complete sets of chromosomes per nucleus. Although the meiotic processes that give rise to unreduced gametes are well known, the contribution of environmental and genetic sources of variation in the production of unreduced gametes is mostly untested. In this study, we measured variation in unreduced gamete production in sperm and egg cells in diploid fireweed (Chamerion angustifolium) plants from three low and three high elevation populations in a common greenhouse environment. Diploid plants in each population were pollinated reciprocally with a set of reference tetraploids. Seeds were collected from mature fruit and using flow cytometry we estimated the frequency of tetraploid seeds, which indicate the presence of unreduced pollen (tetraploid mothers x diploid pollen) and eggs (tetraploid pollen x diploid mothers), respectively, in diploid plants. Analyses remain in progress and are projected to be complete by May, in time for the upcoming OE3C meeting. The results from this study will allow us to estimate the magnitude of variation in unreduced gamete frequency within and among populations, and from this, the extent to which this variation is genetically based. Moreover, this study will provide insight into whether unreduced gamete production varies between the sperm and egg. Advancing our current understanding of polyploid formation will allow us to more broadly understand a process which can contribute to evolution and speciation.

How evolutionary psychology helps explain personality

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Simrit Dhillon*

Personality and its evolutionary routes is a fairly new concept, with evolutionary psychology itself only being a recent field of study. However, evolutionary psychology has played a significant role in personality research for the past two decades. The common underlying question that faces this body of research is trying to understand how evolution explains, or helps explain, personality. One possibility is the heritability of personality traits, for which evolutionary psychology provides the possibility of clearer understanding. In fact, various studies have stated that the correlation between personality traits and heritability is approximately 0.40 to 0.50, without genetic variation or environmental influences. Thus, it is necessary for a concrete understanding of both personality and evolutionary psychology, to grasp their connection. Personality is a pattern of characteristics that differs between each person, made up by their thoughts, feelings, and behaviours; from this, it is important to discover what these differences are, as well as how they collectively develop into an individual's personality. Personality traits are also strongly affected by genes, although the how and why is yet to come from evolutionary psychology. Evolutionary psychology refers to the continual development of our biological mechanisms in accordance with our psychology (e.g. our thoughts come from the repeated evolution of our neural networks, etc.). Differences are viewed as possessing different mechanisms, and all psychological theories are viewed to have roots in the inclusion of internal psychological mechanisms in human beings. To attempt to answer the aforementioned question, various research articles will be studied to compose a literature review in

current point in time, notes are being made based on the research articles, alongside notes on two

the form of a poster presentation was put underway. To do this, four literature reviews were pulled

from Google Scholar and Pub Med, to determine some relevant and consistent findings. At this

that will likely be made by the event involves the creation of the poster with the compiled research.

A genetic basis for sex determination in creek chub (Semotilus atromaculatus)

Amanda Meuser*, Cassandre Pyne, Elizabeth Mandeville

ameuser@uoguelph.ca; Department of Molecular and Cellular Biology, University of Guelph Sexual reproduction is almost universal in the animal kingdom; thus, each species with separate sexes must have a method of assigning male or female sex. The XX/XY heteromorphic sex chromosome system in mammals is the most well-known system of sex determination, but there is wide variation across other clades, including the ZW/ZZ system in birds, homomorphic sex chromosomes, polygenic sex determination, and environmental sex determination. In fish, this system is widely variable across species and even closely related species can have very different mechanisms for sex determination. However, genetic sex determination is known to be more common than environmental in fish and all characterized minnows of the Cyprinidae family use a genetic mechanism, thus common creek chub (Semotilus atromaculatis) fishes are hypothesized to follow suit. Recent advancements in sequencing and computing power have made investigations into genetic sex determination more feasible. This research project will aim to identify a genetic basis for sex determination in the common creek chub, using GEMMA for GWAS (genome-wide association study) and the new software RADSex to analyze genotyping-by-sequencing (GBS) data on 79 creek chub of known sex sampled from streams in southern Ontario. GWAS analyses are aimed at identifying statistical associations between genotypes and a phenotype of interest, which in this case is sex. RADsex takes a different approach, identifying loci only found in one sex. Preliminary results suggest that large heteromorphic sex chromosomes are unlikely; ongoing analyses will examine the potential for homomorphic or polygenic sex determination systems.

Investigating the effects of urbanization on polyploid plant distribution

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Human-induced changes to ecosystems and the climate are a serious problem for plant biodiversity, and also for human food security, crop productivity, and overall human survival. In particular, urban areas are rapidly expanding, but the effects of urbanization on the ecology and evolution of plant species are largely unknown. Several recent reviews have highlighted the role of environmental stress in promoting widespread polyploidy, a macromutation resulting in somatic cells with 3 or more whole chromosome sets, in the evolutionary history of the flowering plants. Here, we propose that the drastic biotic and abiotic changes associated with urbanization may similarly drive presentday ecological and evolutionary divergence between polyploid and diploid species. For example, polyploidy is associated with changes in physiology and morphology, such as larger cell sizes, that may confer broader ecological tolerance and allow polyploids to better adapt to urban life. The idea that polyploids may be better suited to human-disturbed environments is gaining popularity, but studies have yet to measure the effects of urbanization on polyploids versus diploids. Our study is the first to investigate the potential relationship between urbanization and polyploidy in plants. We are analyzing polyploid and diploid species distributions across 52 urban and natural parks in the Greater Toronto Area (GTA), using biological inventory datasets made available to the public by the Toronto and Region Conservation Authority. We will use this data to assess how urbanization influences differences in diploid vs. polyploid species occurrence using phylogenetically corrected ANOVA analyses. This research will provide valuable insight on fundamental questions in polyploid evolution that are still unanswered, such as the reasons for continued polyploid occurrence and success. Additionally, our findings will have implications for conservation, urban planning, and the management of invasive species in urban areas.

Does mutualism result in ecological success?

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Mutualisms are defined to be beneficial to both participating species. This definition is useful in understanding interactions in shorter timescales, but the long-term effects of mutualisms are still to be understood. In plant-mutualist systems, the ability of a plant species to colonise new habitats can be viewed as a measure of its ecological success. The benefits provided by a mutualist may increase the host's chance of survival in a novel environment compared to a host that does not partner with that mutualist. On the other hand, obligate dependence on a mutualist may prevent the host from colonizing new ranges, if the mutualist is not present in those ranges. It is therefore important to investigate the effects of different mutualists on hosts to understand if they impact host ecological success differently. Legumes (plants in the family Fabaceae) are well-known for their mutualistic interactions with rhizobia, which form nodules and fix atmospheric nitrogen in return for carbon from the plant host. In addition, the majority (approximately 22%) of known species possessing extra-floral nectaries (EFNs-- nectar-producing structures present on non-floral parts of the plant which attract ants, and are considered a form of biological protection against herbivores) are also legumes. Legumes are thus a great system to compare the effects of different mutualisms on range expansion. In this study, I compared the effects on legume range size of participating in a) nitrogen fixation mutualisms with rhizobia and b) protection mutualisms with ants by producing EFNs using a PGLS analysis for over 1000 legume species. I found that being a fixer did not significantly affect range size but producing EFNs had a significant positive effect. This result combined with what is already known about fixers and legume range sizes suggests that different mutualisms do indeed affect the ecological success of their hosts differently. The biology of why these interactions may affect plant ecological fitness differently will also be discussed.

Investigating correlated evolution of sexual and asexual reproduction in *Mimulus guttatus*

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For many plants, sex is optional. Many species have the ability to asexually reproduce (for example, via stolons or corms) and sexually reproduce via flowers and seed. The question, then, is why some invest resources into both, and whether a trade-off exists when investing in traits associated with one mode or the other. It is likely that a trade-off between asexual and sexual reproduction does occur, and that it is maintained by a correlated suite of underlying traits. By imposing artificial selection on a population of Mimulus guttatus in the greenhouse, our research aims to identify how investing in asexual reproduction through stolons affects the evolution of correlated sexual traits, such as flowering time, flower number, and male/female investment. The ability of populations to respond to selection depends on the underlying additive genetic variance in the population. To predict the eventual response of this population to four generations of truncation selection, we first grew ~900 plants (36 maternal families, with 24 half sibs) from field collected seed in a common greenhouse environment. We used quantitative genetic analyses to investigate additive genetic variance, heritability, and genetic correlations among vegetative and reproductive traits measured throughout the life cycle. The suite of traits includes germination timing, leaf size, stem size, flowering time, flower size, pollen number, ovule number, stolon number and stolon size. This first analysis will provide a framework for understanding and predicting the evolutionary trajectory of this population as it evolves in response to artificial selection. The study will test a classic, yet unresolved, evolutionary question and provides novel insight about the joint evolution of sex and sex allocation.

Using bioinformatics tools to predict and collect the highly similar duplicates in eukaryotic genomes

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Gene duplication as a strategy to adapt to various environmental conditions has been documented in a wide range of species. Zhang et al., for example, argued that hundreds of highly similar duplicate genes (HSDs) are aiding the survival of an Antarctic green alga via gene dosage. However, the numbers of HSDs in other eukaryotic genomes are largely unknown, and computational methods for identifying them can be time-consuming and labor-intensive. Here, we present an automated online tool (HSDFinder) for identifying HSDs in eukaryotic genomes and with high accuracy and reliability using Pfam domains and KEGG pathways. HSDFinder can analyze unannotated genome sequences by integrating data from InterProScan and KEGG databases. To compare HSDs among different species, we offer an online heatmap plotting option to visualize the results in different KEGG pathway functional categories. These predicted HSDs results are documented in HSDatabase, which has collected total of 28, 214 HSDs in 15 eukaryotes so far. There are many features in HSDatabase including, but not limited to the statistics of HSDs number, gene copy number and length, and the protein function domains. What's more, a BLAST search option is provided for users to conveniently explore potential homologous sequences of interest. HSDFinder and HSDatabase aim to become useful platforms for identification and comprehensive analysis of HSDs in the eukaryotic genomes, which can deepen the insights into how gene duplications can impact adaptation. The web server is freely available at http://hsdfinder.com.

OE3C 2021 Plenary Speakers



Behavior is a Motor and Brake for Evolution | Dr. Martha Muñoz

Martha Muñoz is Assistant Professor of Ecology and Evolutionary Biology at Yale University. Her research focuses on conceptually and empirically synthesizing pattern and process in evolution. To uncover the mechanisms that cause evolution to accelerate or stall, she investigates how animals currently interact with their environments, and how those interactions scale up to repeatable patterns across deep evolutionary time. She then applies her discoveries to major

contemporary issues like global climate change and bioinspired engineering. Prior to joining Yale, she served for two years as an Assistant Professor of Biological Sciences at Virginia Tech. Prior to joining Virginia Polytechnic Institute and State University, she conducted postdoctoral research at Duke University and at the Australian National University and was a William J. Fulbright research fellow at the National Museum of Natural Sciences in Madrid. She received her Ph.D. from Harvard University in 2014, and her B.A. from Boston University in 2007. You can find out more about Dr. Muñoz's work here.

Braiding Indigenous and Western Knowledge Systems in Environmental Science in a Good Way | Dr. Jesse Popp

Dr. Jesse Popp is an emerging scholar, Chair in Indigenous Environmental Science and Assistant Professor in the School of Environmental Sciences at the University of Guelph, and member of Wiikwemkoong Unceded Territory, and strives to



promote inclusive science that embraces multiple ways of knowing while on her journey of learning and sharing. Her research and teaching weaves Indigenous and Western ways of knowing to contribute to the advancement of environmental and ecological science. Dr. Popp recognizes that the number of declining species across the globe are increasing, jeopardizing ecological and cultural integrity. Dr. Popp's interdisciplinary research uses a two-eyed seeing approach to investigate the causes and consequences of wildlife population fluctuations in ecosystems and to Indigenous people's ways of life, contributing to conservation, sustainability, and the progression of the natural sciences in the spirit of reconciliation. You can find out more about Dr. Popp's work <a href="https://example.com/here-examp

The Ecology of Fear | Dr. Liana Zanette



Dr. Zanette works on fear; fear of predators, and the impact it has in nature. She has worked on a variety of wildlife, from birds to lions, in many ecosystems in Canada and abroad including the USA, South Africa, Eswatini, Uganda and Germany. Dr. Zanette and her lab have revealed that fear is a powerful force that can affect wildlife populations and stabilize (or de-stabilize) ecosystems. Her work has appeared on radio, television and in print, including Quirks and Quarks on CBC, NewsHour on the BBC WorldService, the Nature of Things, PBS NOVA, The Atlantic, Washington Post, The Guardian. Dr. Zanette is a Professor in the Biology Department at Western University where she teaches Conservation Biology and Population Ecology. She was a Killam PostDoctoral Fellow

at UBC, after receiving her PhD from the University of New England in Australia, her MSc from Queen's and BSc from the University of Toronto. You can find out more about Dr. Zanette's work here.

Dr. Jake Veasey

Dr. Veasey is a behavioural ecologist specializing in the interface between animal welfare science and conservation biology with a particular interest in species at risk of extinction in the wild and prone to welfare challenges in captivity. He has an undergraduate degree from the University of London in Zoology, a Masters Degree in Applied Animal Behaviour and Animal Welfare Science from the University of Edinburgh and a PhD in Behavioural Ecology from the University of Glasgow. Dr. Veasey has advised governments and NGOs across four continents on species conservation and animal welfare policy and management. He has two decades of executive leadership experience running some of the biggest wildlife facilities in



Europe and North America and regularly consults to zoos and sanctuaries on strategy, business development and conservation and animal welfare programming, producing development plans for some of the biggest and fastest growing wildlife based visitor attractions around the world. Most recently, he has developed methodologies to objectively assess welfare priorities for captive animals. You can find out more about Dr. Veasey's work here.

OE3C 2021 Planning Committee

OE3C is a student-run, student-focused conference celebrating the very best ecological, ethological, and evolutionary research being done in Ontario and surrounding areas. The University of Guelph organizing committee is very proud to host the 51sh annual OE3C online in 2021!

Committee Chair

Alicia Halhed

Alicia completed her undergraduate degree at Trent University in 2019, majoring in Forensic Science with a minor in biology. She is currently a Bioinformatics MSc student at the University of Guelph studying microbial ecology in the Department of Integrative Biology. Her thesis research focuses on the metacommunity dynamics of microbiomes from two host species – the Canada Jay and the North American Red Squirrel.



Committee Members



Michelle Lavery

Michelle is a PhD Candidate in Integrative Biology, studying how degree of preference might change the effects of environment enrichment on the welfare, cognition, and brain development of laboratory zebrafish with <u>Dr. Georgia Mason</u>. She has previously completed an MSc in Biology at the University of New Brunswick, where she studied the winter ecology of Atlantic salmon. She's currently running this website and handling <u>OE3C's twitter</u> account!

Aileen Maclellan

<u>Aileen</u> completed a BSc in Animal Biology at the University of Guelph, before beginning an MSc with <u>Dr. Georgia</u>

<u>Mason</u> examining the effects of standard, barren housing the behaviour of laboratory mice. This laid the groundwork for her PhD, where she is investigating whether the stress of standard cages causes states consistent with clinical depression, and whether environmentally enriched cages protect mice from such effects.





Cassandre Pyne

Cassandre is an MSc student in Integrative Biology studying in Dr. Mandeville's lab. She completed her BSc in Biology and Computing at Queen's University. Her research focuses on investigating the genetic basis of sex determination in *Catostomus* fish.

Sage Handler

<u>Sage</u> (she/her) is an MSc student in the School of Environmental Sciences studying a group of native bees all across Canada. She is currently coordinating the <u>Bees@Schools Program</u>, a community science initiative involving students in native bee research.





Jessica Nelson

Jessi is a Masters student in Dr. Shoshanah Jacob's lab. They have an Hon. Bachelor's degree in Marine and Freshwater Biology from Guelph and completed an undergrad thesis project comparing and modelling the proportion of sea lamprey trapped at a hydroelectric dam across three commonly used tagging methods. For their present MSc study, Jessi has shifted their attention to PhD students and will be evaluating how previous graduate degrees contribute to the mental health and early career success of doctoral graduates in Canada. As a former presenter at OE3C in 2015, Jessi is very excited to be part of the committee for 2021.

Kerry Schutten

Kerry is a PhD student in the department of Pathobiology studying the health impacts of plastic pollution on wildlife in Ontario with Dr. Claire Jardine. She is also a graduate of the Ontario Veterinary College, and a practicing veterinarian. She has previously completed an M.Sc. in the department of Geography focusing on the impacts of climate change on sugar maple-dominated forests in Ontario.





Sara Burilo

Sara is a MSc student in the School of Environmental Sciences working with Ryan Prosser and Paul Sibley. Her project is looking to compare the influences of different landscape characteristics on water quality in the Grand River watershed. She graduated from the University of Waterloo with a degree in Honours Environmental Studies. Her undergraduate thesis focused on the consistency of earthworm consumption patterns in different feeding experiments and was recently published in Pedobiologia – Journal of Soil Ecology.

Faculty Advisor



Georgia Mason

Dr. Mason is a behavioural biologist who moved from Oxford to Canada in 2004 to take up a Canada Research Chair in Animal Welfare at the <u>University of Guelph</u>. In May 2020, she became Director of the <u>Campbell Centre for the Study of Animal Welfare</u>. She and her fabulous lab are interested in how animals adapt (or otherwise) to barren, unstimulating captive housing conditions. In other words, if animals are healthy, well-provisioned and protected from physical harm, is that all that they need for good welfare? Or do they still need to be able to perform activities like social interaction, foraging, or ranging? She also studies sentience, environmental enrichment, and states like 'boredom', to name a few.

Abstract Review

The OE3C 2021 planning committee would like to extend thanks to the members of the University of Guelph community who assisted with the review of abstract submissions. We would like to acknowledge the following individuals for their assistance in abstract review.

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