CSZ 2023 Abstracts

All abstracts (oral and poster) are listed in alphabetical order of the presenting author's (*) last name.

Testing the Waters: the Choice of Euthanasia Techniques can Affect Experimental Results in Aquatic Behavioural Studies

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Abstract: With the use of animal models in research comes the responsibility of treating them ethically. While general anaesthetics are used to reduce pain, they have the potential to interfere with research results. This is a concern in behavioural ecology, particularly in aquatic systems where organisms use chemicals as a form of communication. One well-studied source of chemical information are alarm cues – compounds released from injured individuals that mediate antipredator responses in nearby conspecifics and facilitate learned predator recognition when paired with novel predator cues. To determine whether chemical euthanasia interferes with the ecological functioning of alarm cues, we first exposed woodfrog tadpoles, *Lithobates sylvaticus*, to alarm cues prepared using different euthanasia, preparation and use methods (physical versus chemical euthanasia; mortar and pestle homogenization versus tissue homogenizer; cues used fresh versus frozen versus aged) and compared the intensity of their antipredator responses. Second, we exposed tadpoles to a novel predator odour paired with alarm cues obtained using either physical or chemical euthanasia techniques to elicit learning, and subsequently compared their learned responses. We found that tadpoles exposed to alarm cues prepared with a chemical euthanizing agent (MS222) displayed a weakened antipredator response that was not significantly different from our water (negative) control. Similarly, tadpoles were unable to learn novel predator odours as a threat when alarm cues were prepared with MS222. In contrast, alarm cues obtained using a physical euthanasia method yielded the highest responses regardless of preparation and use methods, with the exception of cues aged 24 h, which became non-functional. Our results confirmed the potential for chemical euthanasia methods to interfere with the functioning of ecologically relevant chemicals. Therefore, a single euthanasia method cannot be applied to all experimental situations – options are needed so researchers can select a method that provides adequate animal welfare without interfering with results.

Risk or Reward? Behavioural Response of Crayfish to Alarm Cues When fed a Cannibalistic Diet

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Abstract: Cannibalism is a complex behaviour involving two parts, first the killing of a conspecific followed by consumption. When this behaviour is expressed, it is accompanied by other changes within the organism, including their interpretation of positive and negative cues. In aquatic organisms, a common risk cue are alarm cues – chemical compounds released upon mechanical damage to an individual. Since this is an accurate indicator of predation risk, it triggers an antipredator response when detected by conspecifics. However, when individuals are cannibalistic, this cue can become a positive foraging cue. Therefore, an individuals' behavioural response to conflicting cues is likely dependent on their previous life-history experience, environment, and current intrinsic properties, such as diet. To examine this risk and reward assessment under a cannibalistic-simulated diet, northern crayfish (*Faxonius virilis*) were fed either a crayfish carcass or fish meat always in the presence of alarm cues or a water control. After this positive (food) and negative (alarm cues) pairing period, they were tested for their response to the risk cues. Individuals exposed to alarm cues had a decrease in their antipredator response during feeding over time. However, when tested for their response to alarm cues without the food cue, crayfish previously fed carcasses in the absence of risk cues were the only group to exhibit a decreased antipredator response. These results suggest that individuals do not fully habituate to important risk cues, like alarm cues. Since the consequences of ignoring a predation cue are high (injury or death), animals cannot afford to make an error when interpreting these cues, resulting in retained fear responses being favored over habituation, even on cannibalistic diets.

Proinflammatory Cytokine Immune Response and High Serum Antioxidative Enzyme Activities Predominate in Cattle with High RoTat1.2 Variable Surface Glycoprotein Gene Copy Numbers of *Trypanosoma evansi*

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Abstract: The main aim of the study was to determine the cytokine, oxidant and antioxidant profiles of cattle with high RoTat1.2VSG gene copy numbers. Blood samples were collected from 90 crossbred Kedah-Kelantan x Brahman cattle and 40 Bali cattle. The selection of cattle for evaluation of serum concentration of cytokines (IL-12, GM-CSF, IFN-γ, IL-10, IL-4) and oxidant/antioxidants (MDA, SOD and GPx) was performed based on PCR detection of the protozoa. Quantitation of RoTat1.2 VSG gene was achieved by quantitative polymerase chain reaction (qPCR) analysis. A curve of dissociation was generated to verify the specificity of the amplification. The cattle were assigned into two groups

namely: cattle with high RoTat1.2VSG gene copy number and clinically healthy cattle (CHC). Quantification of bovine cytokines and serum antioxidant activities was by standard ELISA procedures. The detection rate of *Trypanosoma evansi* was 4/130 (3.08%;95 CI 1.20–7.64%). The number of Trypanosoma parasites quantified from the cattle blood samples were between 40,396,41.43 – 65,07798.94 GC/µL. High GM-CSF:IL-10 and GM-CSF:IL-4, and low IFN-γ:IL-10 were recorded in the *T. evansi* infected cattle when compared to the clinically healthy cattle. A slight increase in IL-12:IL-4 was also recorded in the *T. evansi* infected cattle when compared to the CHC. The MDA level was significantly higher in serum (10-fold) from *T. evansi,* infected cattle compared to serum from clinically healthy cattle ($P < 0.05$). A 2.1-fold increase in the serum SOD activity in *T. evansi* infected cattle was recorded compared to that of the CHC. Furthermore, 2.4-fold increase in serum GPx activity was found in *T. evansi* infected cattle relative to clinically healthy cattle ($P<0.05$). Therefore, Pro-inflammatory cytokine immune response characterized by high serum GM-CSF:IL-10 and GM-CSF:IL-4 ratio, and high malondialdehyde level alongside increased antioxidant enzyme activities were consistent findings in natural *T. evansi* infected cattle with high RoTat1.2 VSG GC numbers.

The Physiology Behind Socially-Mediated Responses to Warming Temperatures in an Isogenic Amphibious Fish

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Abstract: An animal's social environment can significantly affect how they function. For fish, we know that social context affects both their stress response and the response to environmentally relevant high temperatures. We have early data suggesting that the social environment may affect how a fish perceives or senses warm temperature. Temperature perception is facilitated through transient receptor potential (TRP) ion channels. In fishes, a change in temperature perception could have dramatic consequences on how they cope with climate warming given that average sea surface temperatures are predicted to continue to increase. While socially-mediated thermal risks have been identified, the physiological mechanisms underlying them have not. To test this, I used the mangrove rivulus fish to test three hypotheses: 1) socially mediated thermal responses are a direct result of a change in physiological state, specifically, an increase in physiological stress.; 2) an animal's physiological stress state (e.g. cortisol) directly influences thermal behavior; 3) socially mediated thermal responses are a result of changes in TRPV1 and this is mediated by cortisol. I tested these hypotheses by measuring cortisol of fish placed in dyadic contests or groups of three. Cortisol levels were significantly higher in socially experienced fish compared to control isolated fish. Following this, I focused on dyadic contest fish and recorded 1 hour of social interaction to determine dominant or subordinate social status. I then took these fish and measured their emersion response to capsaicin, a transient receptor potential vanilloid subtype 1 (TRPV1) agonist. Submissive fish need significantly higher capsaicin concentrations to elicit an emersion response, suggesting that social status affects how fish perceive temperature. Overall, my research will provide an understanding of how social behavior affects thermal sensation in fish and will aid in predicting animal responses to increased environmental temperatures.

Validation of Mitochondrial Density Markers in Ectothermic Species

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Abstract: Mitochondria are key contributors of cellular energy production and have been extensively studied in numerous fields. Particularly in ectothermic species, changes in environmental conditions can be reflected in mitochondria, which are known for their high level of sensitivity and plasticity. These can result in either a functional change within the mitochondrion itself, a variation in total density within a tissue, or even a combination of the two. Therefore, experimental data must be presented with an adequate measure of mitochondrial density in order to be interpretable. One of the most effective ways to quantify mitochondrial density is through transmission electron microscopy. However, due to its lack of simplicity, affordability and availability, other potential markers of mitochondrial density have been explored. Commonly, quantifications of mitochondrial protein content, enzyme activity, and mitochondrial DNA copy number are employed as estimates of mitochondrial density. The most frequently used estimates include measurements of citrate synthase, complex I (NADH-oxidoreductase), and complex IV (cytochrome c oxidase) activity but results vary greatly between studies. Indeed, mitochondrial plasticity, especially in ectotherms, may partly explain the discrepancies between studies. Here, I propose to conduct an extensive comparative study to identify universally applicable markers of mitochondrial density. To do so, I will be exploring more than 15 potential markers - some traditional, some non-traditional - in various tissues from fruit flies (*Drosophila melanogaster*), Colorado potato beetles (*Leptinotarsa decemlineata*), fish (*Salvelinus alpinus*) and mussels (*Mytilus edulis*), each acclimated to cold and warm temperatures. This poster will focus on the conceptualisation of the experimental design, with the presentation of some preliminary results.

Worming Their way into the Boreal: Earthworm Invasion via Bait Abandonment in the Saskatchewan Southern Boreal Forest

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Abstract: Understanding the methods of introduction is critical for management of invasive species. No research has yet examined the introduction of exotic earthworms into the boreal forest in Saskatchewan; however, it is believed that bait abandonment by anglers and dispersal by vehicles are the primary introduction mechanisms in other parts of Canada. To test whether bait abandonment has contributed to earthworm dispersal in Saskatchewan, I conducted earthworm sampling near boat launches and remote shores at two lakes in the southern boreal forest of Saskatchewan. Earthworm density was significantly higher at boat launches than at remote shores with *Apporectodea* representing the majority of earthworms sampled. These results mark the first direct observation of exotic earthworms in Saskatchewan's boreal forest and suggest that bait abandonment has contributed to earthworm invasion in this region.

However, it is still unclear whether *Apporectodea* spp. are commonly used as bait in Saskatchewan and future research is needed to assess the relative contributions of bait abandonment and vehicle transport to earthworm invasion.

Variation in the Kinematics of Black Widow Spider Legs During Locomotion

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Abstract: Black widow spider's legs are multifunctional and serve locomotory, as well as sensory functions. Highly sensitive strain sensors at the joints enable the spider to sense a variety of vibrational stimuli. Typically, spiders are thought to use their front legs mainly for sensory function, and hind legs are thought to be primarily locomotor in nature. However, leg kinematics – which would be indicators of these differences – have not yet been described for most species. In this study, adult female black widow spiders were tracked in 3D as they walked across their webs. The kinematics and movement patterns of defined points along the leg and body were captured. I will use these data to investigate whether the front and hind legs show differences in ranges and periodicity of movement, indicating differences in their function.

The Role of Water pH on Acid Base Compensation During Hypercapnia in White Sturgeon (*Acipenser transmontanus*)

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Abstract: Elevated CO_2 (hypercapnia) in fishes induces a respiratory acidosis, which is compensated by branchial acid-base relevant ion transfer. Compensation is thought to be limited by factors such as magnitude of the acid-base challenge, available counter ions, and pH of the surrounding water. The role of water pH in moderating or exacerbating this compensation is difficult to approach in fish that rely entirely on extracellular pH regulation (pH_e), as when it fails, mortality may occur quickly. Some fishes however, tolerate acidification beyond what can be compensated for by pH_e using preferential intracellular pH (pH_i) regulation (PPR), where pH_i is regulated despite sustained reduction of pH_e. Species capable of this, including the white sturgeon (*Acipenser transmontanus*), allow us to examine the effect of water pH on acid-base compensation during hypercapnia. To determine how water pH alters the rate and magnitude of acid-base compensation during hypercapnia in white sturgeon, we exposed sturgeon to 48 hours of CO_2 tensions below and above the limit for pH_e compensation while manipulating water pH to determine potential mediating and exacerbating effects. Water pH manipulation had significant effects on the pH_e compensatory response. Complete pH_e compensation was observed in fish exposed to untreated water equilibrated with 1 kPa CO_2, but reducing water pH to 5.2 prevented complete compensation. When sturgeon were exposed to alkalized water equilibrated with 4 kPa CO_2, (i.e., pH of 6.3 compared to 5.7 for water with 4 kPa CO_2), pH compensation was more rapid. Not surprisingly, pH_e compensation rates had no effect on pH_i regulation. Our findings demonstrate that water pH can aid or abet pH_e compensatory rates at a

range of hypercapnic challenges, and so may have contributed to the origin of PPR as a primary strategy for some fish species to protect critical tissues largely independent of environmental conditions.

Should I Stay or Should I go? Impacts of Biotic and Abiotic Factors on Nest Site Choice in a Singing Toadfish.

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Abstract: Where to raise young is an important decision that can have profound effects on the development, growth, and subsequent survival and reproduction of offspring. Parental phenotypes and even the phenotypic differences among offspring can result in some environments suiting particular parents and offspring better than others. Using a series of laboratory and field experiments with the Pacific toadfish, *Porichthys notatus,* we show how females can maximize reproductive benefits by targeting specific nest sites according to their egg and body sizes. These toadfish breed along an intertidal elevation gradient and down into shallow subtidal waters. We found that larger toadfish mothers produced larger eggs that hatched into larger young. Mothers laying these larger eggs also selected to lay their eggs in nests at higher tidal elevations where it is warmest. Eggs raised in similarly warm conditions in the laboratory grew into the largest juveniles with the highest survival rates. Juveniles that were raised in warm water (mimicking conditions in the high intertidal zone) were also bolder and exhibited faster swimming speeds when escaping from a simulated predator. Taken together, these results suggest variation in propagule and female size can support divergent nest site choices among females, with individual variation in individual size supporting niche partitioning and expanding the range of environmental conditions that constitute suitable nesting sites.

Impact of Cyanobacteria on Ecosystem Function

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Abstract: Research concerning cyanobacteria has increasingly focused on the health implications and causal factors promoting cyanobacterial blooms. However, there is another important aspect of cyanobacterial blooms which receives less attention. Recent studies have provided indirect evidence that pelagic food web function is affected when cyanobacteria are abundant in lakes. This may be due to a combination of factors, such as their inedibility, poor food quality, or their production of cyanotoxins. With the use of a radiotracer, we measured pelagic phosphorus cycling in a variety of lakes from the Canadian Prairie Region and beyond (i.e., Lake Erie and Lake of the Woods). These lakes provided a broad range in cyanobacterial biomass (0 to 75% cyanobacterial biomass). Phosphate turnover, planktonic

regeneration, zooplankton composition and most important, planktonic food web turnover will be presented as a function of cyanobacterial biomass to determine if food webs dominated by cyanobacteria do indeed have reduced cycling efficiencies.

The fat's in the Fire: Investigating the Regulatory Role of Mitochondrial fat Sensitivity in Shivering Muscle

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Abstract: High altitude is challenging environment to live in due to the negative relationship between elevation, and PO2 and temperature. Deer mice (Peromyscus maniculatus) overcome these environmental hurdles through prolonged thermogenesis, during which they sustain an aerobic metabolism as high, or higher, than running VO2max. Unlike during exercise, deer mice power extended periods of intense thermogenesis by primarily using lipids as fuel. Currently, the mechanisms behind high rates of lipid oxidation during thermogenesis remain unclear. Recent investigations into lipid oxidation inhibition during exercise revealed that carnitine palmitoyl-transferase 1 (CPT-1), a lipid transport enzyme on the outer mitochondrial membrane, loses sensitivity to its substrate, L-carnitine, during exercise. This phenomenon is a potential regulatory step for the fuel use shift from lipids to carbohydrates during high intensity exercise, as seen across all mammalian taxa. However, it is currently unknown whether this loss in L-carnitine sensitivity from CPT-1 occurs during the high aerobic metabolism observed during thermogenesis. I found that CPT-1 increases its sensitivity to L-carnitine in cold hypoxia-acclimated mice from high altitude populations, compared to thermoneutral-acclimated mice. Furthermore, these same mice display increased mitochondrial sensitivity to ADP. I will continue to investigate mitochondrial fat sensitivity by studying CPT-1 activity levels and use protein quantification experiments to corroborate my CPT-1 sensitivity results.

DNA Barcoding Reveals one Invasive, and Additional Previously Undescribed Species of Unionids

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Abstract: Three groups of morphologically distinct freshwater mussels (Family: Unionidae) were observed in an aquaculture facility near Cañas, Guanacaste, Costa Rica. Fourteen individual DNA samples were extracted and sequenced in the Stewart lab at Acadia University, using universal cytochrome oxidase subunit I primers, revealing invasive species *Sinanodonta woodiana*, and two unionid species that share ~93% and ~89% of similarity to native species *Psorula profunda* and *Anodontites trigonus*, respectively. Recent developments in Mesoamerican unionid phylogenetics suggest that the *P. profunda* relative is possibly among genus *Nephronaias*, which is undescribed on molecular databases such as GenBank. To confirm identification, phylogenetic trees were constructed using

MEGA7.0.26 and MrBayes3.2.7. Dioecious freshwater mussels typically exhibit mitochondrial DNA inheritance apart from conventional Strict Maternal Inheritance across metazoa: Doubly Uniparental Inheritance (DUI) propagates male-inherited mitochondria in male germ cells, and female-inherited mitochondria in male/female somatic cells, and female germ cells. DUI was detected in putative *Nephronaias sp.* using primers that only amplify male-only mitochondrial genomes within polymerase chain reactions. This research discusses ecological and economic implications of *S. woodiana* establishment in Guanacaste and surrounding regions, as its success can be attributed to the unusual glochidial life stage of unionids, and how phylogenetics, DUI, and invasive species within freshwater mussels are all critical aspects to consider in ecological conservation – an understudied, underfunded, and underappreciated field in Mesoamerica.

Assessing Thresholds of Thermal Tolerance in Bull Trout (*Salvelinus confluentus*)

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Abstract: Bull trout are a char species whose native range in Western North America consists mainly of cold-water streams. Warming due to climate change has reduced their habitat range, resulting in population declines and classification as a threatened species in Canada. Rising water temperatures lead to activation of thermal stress responses in fish, followed by increasingly critical sublethal thresholds, until the lethal threshold is reached. Characterization of these key thermal thresholds is important in predicting fish health as environmental temperatures increase, and can inform recovery strategies. Juvenile bull trout were acclimated to a range of environmental temperatures (6 to 21 °C), and several endpoints were assessed to determine thermal thresholds. The capacity for acclimation in terms of plasticity of agitation temperatures and critical thermal maxima were determined. Bull trout demonstrated a limited ability to increase CTmax with increasing acclimation temperature beyond 18°C, which was accompanied by reductions in growth and survival. Transcript abundances were assessed in gill, muscle, and liver tissue using a salmonid STP (stress-response transcriptional profiling) chip targeted to thermal stress, developed through GEN-FISH (Genomic Network for Fish Identification, Stress and Health). The transcriptional responses in these peripheral tissues revealed additional sublethal thermal thresholds in bull trout. Altogether, these data provide insight into the range of thermal thresholds and the health risks associated with a warming climate in this threatened freshwater fish species.

Crowd-Sourced Observations of a Polyphagous Moth Reveal Evidence of Allochronic Speciation Varying Along a Latitudinal Gradient

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Abstract: Allochronic speciation, where reproductive isolation between populations of a species is facilitated by a difference in reproductive timing, depends on abiotic factors such as seasonality and biotic factors such as diapause intensity. These factors are strongly influenced by latitudinal trends in climate, so we hypothesized that there is a relationship between latitude and divergence among populations separated by life history timing. *Hyphantria cunea* (the fall webworm), a lepidopteran defoliator with red and black colour morphs, is hypothesized to be undergoing an incipient allochronic speciation. However, given their broad geographic range, the strength of allochronic speciation may vary across latitude. We annotated 11,674 crowd-sourced observations of fall webworm to model geographic distribution, phenology, and differences in colour phenotype between morphs across North America. We found that red and black morph life history timing differs across North America, and the phenology of morphs diverges more in warmer climates at lower latitudes. We also found some evidence that the colour phenotype of morphs also diverges at lower latitudes, suggesting reduced gene flow between colour morphs. Our results demonstrate that seasonality in lower latitudes may increase the strength of allochronic speciation in insects, and that the strength of sympatric speciation can vary along a latitudinal gradient. This has implications for our understanding of broad-scale speciation events and trends in global biodiversity.

Using 3D Models to Train Deep Learning Classification Models: A Case Study in Canadian Carnivore Skulls

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Abstract: Specimen classification is a common and sometimes time-consuming task in zoological research. Recent advances in deep learning artificial intelligence have made automated classification of animals from images possible. While automated classification has many practical benefits for scientists, deep learning models typically require large amounts of training data in the form of annotated images to achieve high classification accuracy. For some species, it may be difficult or impossible to collect enough individual specimens to properly train a deep learning model if each specimen is only imaged once. This has led some researchers to explore methods whereby individuals are photographed many times from different angles. Such methods can create image datasets >100 times larger than the number of individual specimens. As an alternative to this approach, we introduce the use of white-light 3D scanning to generate large 2D image databases for deep learning model training. In this case study, we scanned the skulls of 16 Canadian carnivore species (30 specimens each) from the Canadian Museum of Nature mammal collection. To create our training dataset, each scan was rendered as a 2D image from 92 different angles, resulting in training dataset size of 44,160 images from 480 individuals. To test the deep learning model, we photographed an additional 10 specimens from each species from 92 angles. Photographs were used in the testing dataset rather than renders from 3D models to better simulate real-life use cases. Overall, the use of 3D scanning to generate large image datasets has several advantages over traditional photography. Using 3D models, image generation is more reproduceable and precise. Additionally, 3D models can be easily modified (e.g. adding or removing structures), which furthers the potential of 3D scanning for image generation.

From genes to physiology: Impact of fluctuating environments during early development

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Abstract: Average mean temperature and thermal variability are both expected to increase in aquatic environments. However, relatively little is known about the impacts of fluctuating temperatures on early development in fishes. Therefore, the objective of our study was to compare the effects of constant and fluctuating temperatures on embryonic, larval, and juvenile phenotypes from whole-animal physiology to underlying molecular mechanisms. To address this objective, we used *Fundulus heteroclitus*, a species of topminnow that inhabits intertidal salt marshes along the Atlantic coast of North America which experience daily temperature fluctuations due to the tidal cycle. We incubated embryos at four different temperature regimes, with the same mean temperature, but differing extents of fluctuation: 26±0°C, 26±3°C, 26±5°C, 26±7°C. We measured larval survival, growth, and morphometrics (length, weight, and yolk-sac volume) and changes in key regulatory genes involved in methylation, growth, and muscle development. Once hatched, larvae were raised at a common mean temperature of 26°C to test for the persistent effects of developmental plasticity. We found that larvae incubated at greater fluctuations (26±7°C) had decreased survival but were longer and had a larger yolk-sac at the time of hatch compared to larvae that developed at a constant temperature. Temperature fluctuation also significantly altered gene expression at both the embryonic and larval stages. Lastly, we found evidence of developmental plasticity, with small temperature fluctuations during development resulting in beneficial plasticity in juveniles (increased thermal tolerance; measured as agitation temperature), whereas larger fluctuations resulted in maladaptive plasticity (lower thermal tolerance, measured as CTMax). Together, these data demonstrate that the extent of temperature fluctuation during early development has both short and long-term effects on the phenotype of these fish.

Rapid Recovery from Loss of Equilibrium Induced by Hypoxia, Warming, or Exercise in Rainbow Darter (*Etheostoma caeruleum*)

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Abstract: Animals routinely experience physiological disruptions due to environmental (hypoxia, warming) and/or internal (exhaustive exercise) stressors, and there is extensive literature describing the mechanisms and extent of these disruptions. How animals recover from these stressors has generally received less attention, particularly in the context of how recovery from one stressor may differ from recovery from another stressor. To investigate this, we used rainbow darter, a small-bodied fish often used as a sentinel species for pollution in their native range in the Grand River watershed in southern Ontario. Rainbow darters cope with a variety of environmental fluctuations that are

characteristic of their small stream habitat including warming and periods of low oxygen. As benthic fishes that prefer shallow, fast-flowing habitats, rainbow darters likely rely on burst swimming to relocate within their narrow home ranges (a few metres) and to avoid predators. Using stop-flow respirometry, we tracked the aerobic oxygen consumption rate (MO_2) of rainbow darters as they recovered from loss of equilibrium (LOE, an ecologically relevant proxy for death) induced by hypoxia (PO_2 at LOE), warming (critical thermal maxima, CT_{max}), or exhaustive exercise. Regardless of the stressor used, rainbow darters recovered rapidly from LOE and returned to routine MO_2 within ~3 hours. Both hypoxia and warming had similar effects, with recovering fish having similar maximum MO_2, aerobic scope, total time to return to routine MO_2, and O_2 debt (oxygen consumed per gram throughout the recovery period). Exercise induced higher maximum MO_2, as well as a trend of greater recovery time and O_2 debt, suggesting that exhaustive exercise may be more physiologically disruptive than hypoxia or warming. Overall, these data suggest that even stressors that lead to the same outcome at the whole-animal level (inducing LOE) may vary in their overall recovery profiles and, presumably, their underlying physiological mechanisms.

Tooth ça Change, Tooth c'est la Même

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Abstract: When you think about it, most of how we identify other people and animals is by their faces including their teeth. Teeth tell us who an animal is, including what it eats, its age, health status, and life history. Teeth help tell the sprawling evolutionary history Earth's diverse vertebrate animals, living and extinct. This is in part because teeth display an amazing diversity – as well as precise species specificity – in terms of tooth number, form, and location in the mouth (and elsewhere!). No matter which species you choose, teeth develop in part from a dental epithelium. The evolutionarily ancient gene, p63, acts as a transcription factor in epithelium body-wide during organogenesis. Via high-throughput screening of transcripts and P63-bound proteins in toothless p63-/- mice vs. phenotypically normal p63+/- and p63+/+ littermates, we discovered differential expression and binding of a suite of genes. This p63-driven gene regulatory network (GRN) appears to be specific to teeth and exclusive of the jaw. It also appears to be active in all toothed animals. Our *in situ* hybridization and immunohistochemistry assays showed that many of these genes are expressed in wildtype dental epithelium (but not jaw mesenchyme) of mice and other toothed vertebrates including fishes, frog, and primates. We confirmed that this GRN includes primary as well as indirect targets of p63. Based on preliminary bioinformatics and organ culture analyses, we suggest that this GRN is important for epithelium integrity and movement (bending, folding, etc.), among other roles. Collectively our work thus far indicates that this p63-driven GRN is imperative for tooth development, peripheral for jaw formation, and deeply conserved across vertebrates.

Molecular and Pharmacological Characterization of the Hypothalamus-Pituitary-Interrenal Axis in Elasmobranchs

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Abstract: The hypothalamus-pituitary-interrenal (HPI) axis is an evolutionarily conserved feature of vertebrates that stimulates the synthesis of corticosteroids to respond to stressors. Phylogenetically ancient vertebrates, such as elasmobranchs (sharks and rays), exhibit some conserved features of an HPI axis. The initial step of the HPI axis is the release of corticotropin-releasing factor (CRF) by the hypothalamus to stimulate pro-opiomelanocortin (POMC) synthesis. Notably, elasmobranchs express CRF paralogs (e.g., urotensin I); yet, a CRF ortholog has not been described in any elasmobranch. Next, POMC is cleaved into adrenocorticotropic hormone (ACTH) that stimulates corticosteroid synthesis via the melanocortin-2 receptor (MC2R). In elasmobranchs, it appears that melanocyte-stimulating hormones – other post-translational products of POMC – can also activate MC2R; this phenomenon is unlike what is documented in bony vertebrates and has not been described in squaliform elasmobranchs (e.g., dogfishes). This study tested the hypothesis that elasmobranchs exhibit conserved features of an HPI axis at two key processes: 1) the production of CRF and 2) the activation of MC2R by ACTH. Therefore, we sought to determine whether a CRF-encoding gene exists among elasmobranchs, and test whether a representative squaliform MC2R can be activated by MSH-sized peptides. To address objective 1, CRF-like sequences were curated from available genomic resources. Phylogenetic analysis was conducted to distinguish CRF-like sequences from CRF paralogs. *crf*-like transcripts were detected in the hypothalamus of the Pacific spiny dogfish (*Squalus suckleyi*). To address objective 2, *S. suckleyi* MC2R was transfected into CHO cells and co-expressed with homologous accessory proteins (i.e., MRAP1 and MRAP2). Activation of dogfish MC2R was quantified using a luciferase reporter gene assay and demonstrated activation by both ACTH and MSH-sized peptides. Together, these findings demonstrate that elasmobranchs possess key elements of a functional HPI axis and suggests that functional characteristics of bony vertebrate HPI axes are derived traits.

The Importance of TLR Expression in Snail Haemocytes and Tissues

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Abstract: The snail, *Biomphalaria glabrata*, has become an important model organism since it is the obligate intermediate host for *Schistosoma mansoni*, a trematode parasite impacting millions of people annually. As mass drug campaigns targeting this parasite in the human host have proven ineffective for parasite population control, we aim to better understand the host-parasite interactions occurring within the snail. Although the field of gastropod immunology is relatively sparse, several immune factors have been described for their role in coordinating snail resistance to *S.*

mansoni. However, the source of the main effector cells, which have been postulated to be granulocytic haemocytes expressing a toll-like receptor (BgTLR), remains unknown in both spatial origin and differentiation history. Here, we present data collected by flow cytometry to show that the reactive oxygen species (ROS) used for parasite killing are predominantly generated by granulocytes sorted for BgTLR-positivity. Additionally, the hematopoietic boundaries of sorted haemocyte populations have been characterized using cell proliferation assays in the context of stimulation with a growth factor that is upregulated during infection. To address immune dynamics in non-haemocyte tissues, we have developed methods to create an "Atlas of Biomphalaria," in which entire snail sections are imaged to generate whole-snail datasets depicting histology, BgTLR expression, cell proliferation, and ROS production. These datasets will introduce an unparalleled reference for tissue morphology in snails. Thus, our findings provide fundamental knowledge useful to both researchers of snail-trematode interactions, as well as general learners and educators in malacology.

Are Sunscreens Better Together? A Comprehensive Comparison of Individual Ultraviolet Filters and off-the-shelf Sunscreens and their Effects on *Daphnia magna*.

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Abstract: Organic ultraviolet filters (UVFs) are an emerging contaminant of concern found in sunscreens and personal care products to protect against harmful ultraviolet radiation. Their use in sunscreens leads to widespread environmental contamination through leaching of these chemicals during recreational activities, posing a threat to aquatic ecosystems. Current research has focused on the toxicity of individual UVFs with few studies considering the effects of sunscreen mixtures. This study sought to compare the toxicity of five off-the-shelf sunscreen mixtures to the individual UVFs of avobenzone, homosalate, octisalate, octocrylene and oxybenzone in *Daphnia magna*. 48 h LC50 tests and 21 d exposures were determined for each sunscreen and UVF, revealing minimal relation between individual UVF concentration and the toxicity of each sunscreen product. Sunscreen mixtures exhibited lower toxicity, as <20% mortality was observed during exposures to mixtures containing individual UVFs at concentrations exceeding those which have been demonstrated to cause 100% daphnid mortality over 21 d. *Daphnia* exposed to sunscreens experienced several reproductive impairments, including a 70% reduction in overall reproduction and a 10-fold increase in non-viable offspring compared to controls. Interestingly, some sunscreen products reduced *Daphnia* growth, observed through a 65% reduction in body dry mass and a 35% shorter body length. While exposure to individual UVFs revealed similar reproductive impairments at lower tested concentrations, daphnid size was not impacted by individually tested UVFs, indicating the potential presence of a new mechanism of action for sunscreen mixtures. Overall, these results suggest that the mixture toxicity of sunscreens cannot easily be predicted by individual UVF exposures. This presents a worrying concern for risk assessment practices due to the popularity of testing single UVFs, excluding the potential effects of the emulsifiers, fragrances, and other sunscreen additives that alter the overall toxicity, and which may also be present in contaminated environments.

More than one way to Make an egg: Evolutionary Conservation and Novelty in an Early Syncytial Stage of Oogenesis

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Abstract: Germline cysts are syncytia formed by incomplete cytokinesis of mitotic germline precursors (cystoblasts) in which the component cells (cystocytes) are interconnected by cytoplasmic bridges, permitting the sharing of molecules and organelles. Among animals, such syncytia are a nearly universal feature of spermatogenesis, and are also often involved in oogenesis. Recent, elegant studies have demonstrated remarkable similarities between the female germline cysts of mice and flies, leading to conclusions that these syncytia are a conserved feature of metazoan oogenesis. Unfortunately, such claims ignore the well-described diversity of female germline cysts in animals, and their absence in significant taxonomic groups. Here, I explore the phylogenetic distribution and diversity of oogenic cysts in the animal kingdom, focusing on the hexapods: a clade in which such cysts have been lost, regained, and modified in several lineages – flies included. My aim is to build on the insights of recent comparative studies by calling for a more nuanced view of evolutionary conservation in this case. The underappreciated variability among metazoan oogenic cysts means that these structures are not intrinsically essential for oogenesis, nor do they necessarily serve the same functions in oocyte development.

Determination of the Stress Level Generated by Recreational Catch-and-Release of Striped Bass (*Morone saxatilis*)

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Abstract: Recreational fishing has been shown to impact the health and survival of fish individuals/populations. Catch-and-release is considered an effective practice promoting biological, economic, and social sustainability. This project aimed to evaluate stress levels caused by catch- and- release fishing on striped bass in the Baie-des-Chaleurs (Québec), considering the duration of the fight, the skills of the anglers, water temperature, and duration of air exposure. Following capture by volunteer anglers, fish were immediately sampled for blood plasma, gill, and liver tissues. Common primary and secondary stress indicators (cortisol, glucose, plasma ions) were used to quantify stress levels, but new "stress transcriptomic chips" designed and developed by the Gen-Fish consortium were also tested. Realized in collaboration with the Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs, this project will help assess the direct effects of catch-and-release practices on the health of striped bass, information that will be used to better regulate this fishing practice and ensure its sustainability.

Identification of Physiological Impacts and Biomarkers for Food Deprivation in Juvenile Chinook Salmon, with Application for non-lethal Assessment of Wild Populations

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Abstract: Risk assessments have identified prey limitation as one of the strongest risk factors for juvenile salmon migration survival under climate change. In British Columbia, Canada, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) may experience prolonged periods of food deprivation both upon first marine entry as well as during their first marine winter. Laboratory-based studies have demonstrated that Pacific salmon species can withstand food deprivation for approximately 4 weeks with minimal mortality. However, there is limited assessment of physiological impacts and development of tools that can be applied non-lethally to assess nutritional status of wild populations. In this study we will conduct lab-based food deprivation experiments to assess the physiological consequences of starvation and identify mRNA based biomarkers for food limitation in the gill of juvenile Chinook salmon. Trials will be conducted at both summer (16^{o}C; marine entry) and winter (10^{o}C; overwintering) temperatures, and will continue for up to 3 months, to identify biomarkers which may be conserved across seasons. Bi-weekly, physiological metrics will be taken and tissue samples (liver, gill, and muscle) will be collected for molecular analysis. At the end of food deprivation (when condition decreases 25% or mortality in any treatment reaches 20%), gill and liver samples will be collected for RNA-sequencing and subsequent bioinformatic analysis. Random-forest classification will be used to optimise the most predictive combination of 6-8 biomarkers to form a qPCR panel with the ability to detect food deprivation in non-lethally collected gill samples, and to build a specific classifier to be applied to wild fish. Ultimately, if successful, this suite of biomarkers will be integrated into existing Fit-Chip assay panels and deployed to assess the physiological state of wild Chinook salmon. This poster will present details of upcoming food deprivation trials, identification of biomarkers to be used in non-lethal sampling, and their application to wild populations.

Effects of Temperature and Live-well Water Conditioners on Plasma Cortisol and Lactate in Walleye (*Sander vitreus*) at Catch and Release Angling Tournaments

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Abstract: Walleye (*Sander vitreus*) are one of the main targets for anglers at catch and release angling tournaments in Canada and the Northern United States. Over the course of three years, experiments were carried out to assess the physiological condition of Walleye at seven different tournaments in Saskatchewan, Canada. Plasma cortisol and lactate concentrations were measured in Walleye angled at tournaments with cool (< 15°C) and warm (>18°C) lake water surface temperatures. Sampling occurred both immediately after weigh-in and following 4-hours in on shore live-wells with or

without water conditioner. Our data confirm those of previous studies which show fish from tournaments held at warmer water temperatures are in poorer condition and experience greater stress as indicated by higher plasma cortisol and lactate levels. Interestingly, control fish sampled at the tournament with the highest water temperatures had plasma cortisol and lactate concentrations more similar to cool water tournaments. We hypothesize that this was related to tournament regulations reducing the maximum number of fish that anglers could hold at any one time, thereby reducing the time spent in the boat live-well. We also provide data that suggest live-well water conditioners have properties that may be beneficial for fish angled and intended for release. Regardless of the temperature at the time of catch, those fish held in closed live-wells on shore without the addition of fresh lake water fared as well with respect to survival, and plasma cortisol and lactate, as those receiving a continuous supply of fresh lake water. In summary, tournament organizers should attempt to avoid scheduling tournaments in the warm summer months, but if they do it would be prudent to include regulations that reduce the time fish spend in the boat live-wells. Additionally, live-well water conditioners may offer some benefit to fish captured and held under adverse environmental conditions which are often associated with poor water quality and fish condition.

The Decomposition of Manure in Aquatic Ecosystems

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Abstract: Allowing livestock access to wetlands directly impacts their traffic and excretion to the surface water and riparian areas, and the use of deworming drugs results in manure containing a range of pesticides that may affect its decomposition by invertebrates. Hydrophobic anthelmintic drug residues are excreted in cattle manure and have been shown to exert non-target effects on invertebrate fauna that encounter dung as a part of their lifecycle, but the aquatic macroinvertebrate food web associated with manure decomposition and interactions with anthelmintics are not well understood. The goal of this study is to determine which macroinvertebrates colonize manure in an aquatic system and how it alters rates of decomposition of manure deposited into water. Secondly, to assess the implications of anthelmintic use in beef cattle on manure breakdown and colonization by macroinvertebrates in aquatic ecosystems. Invertebrate access to manure was shown to have a significant effect on the rate of decomposition by day one of the study. By day five of decomposition, manure that was open to invertebrate access had been completely consumed while manure was still retained in samples that restricted access to manure from invertebrates until the end of the experiment on day 35. Manure colonization was dominated by two taxa (>97.5%), nonbiting midge larvae (Chironomidae) and freshwater shrimp (*Hyalella azteca*). The preservation of wetlands is dependent on an understanding of all factors that impact the function and health of the ecosystem and its users. Moreover, the success of Best Management Practices for cattle grazing depends on a knowledge base of food web responses. An insight into the invertebrate species using manure deposited in waterways and the implications of deworming treatments on this process provided by this study will help best inform these practices.

Investigating the Inflammatory Response in Zebrafish Following Diurnal Temperature Fluctuations and Venlafaxine Exposure

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Abstract: Increased intensity and frequency of heat waves is predicted to have detrimental impacts on aquatic organisms. Thermal variability coupled with exposure to xenobiotic stress could place significant strain on the thermal acclimatory capacity and the inflammatory response of some fish species. The impacts of contaminant exposure have become an area of particular interest in addressing how waterborne contaminants and increased diurnal thermal extremes impact fish physiology. This study aims to address how known contaminants from wastewater treatment plant effluent coupled with diurnal temperature cycling impact laboratory zebrafish innate immune function. Here, we acclimated zebrafish to three respective temperatures: 25°C, 35°C and fluctuating diurnal temperatures between 25-35°C (12hrs:12hrs) for 2 weeks. Following acclimation, zebrafish were exposed to an acute heat stress of 35°C and an environmentally relevant concentration (1µg/L) of the antidepressant venlafaxine (VFX) for 24 hours. We compared basal mRNA expression at 25°C to exposed zebrafish to quantify differences in several gene transcripts in the brain and gills, specifically examining innate immune cytokines and heat shock proteins. Acute heat and VFX exposure had no impact on mRNA expression of *hsp70* and *hsp90*, suggesting that VFX does not significantly impact the heat shock response in the brain. Furthermore, the acute heat stress suppressed the expression of interleukin-1β (*IL-1β*) in the cycling temperature group, but these effects were not significant in the presence of VFX. Further investigation of the gill expression and enzymatic assays may provide a more comprehensive understanding of the metabolic and immune responses underlying multiple stressor impacts.

Elevated Temperatures Exacerbate Developmental and Latent Dilbit Toxicity in Coho Salmon (*Oncorhynchus kisutch*)

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Abstract: Diluted bitumen (dilbit), the major crude oil export product from Canada's oil sands, causes developmental and long-lasting effects in various species of fish, including Pacific salmon that are at an increased risk of exposure from pipeline spills. Water temperature is an abiotic factor that alters both dilbit chemistry and the physiology of fish in ways that can influence contaminant uptake and metabolism. Using a suite of whole-animal endpoints, we investigated the effects of a modest increase in water temperature (+3°C above ambient; consistent with present-day annual fluctuations in the Fraser River) on the toxicity of dilbit to early life stage coho salmon (*Oncorhynchus kisutch*). Embryos were exposed to uncontaminated water or to one of two environmentally relevant concentrations of dilbit (~0.3 µg/L and ~1 µg/L) from fertilization to swim-up, and then a subset of fish from each treatment was reared in uncontaminated water at

ambient temperature for an additional 6 weeks. Embryonic dilbit exposure increased mortality by 20% and reduced cardiorespiratory performance by 30%, and these effects were exacerbated at elevated temperature. Effects of dilbit exposure at elevated temperature on fitness metrics persisted despite return to clean water at ambient temperature; and a latent reduction in body mass and condition presented at fry stage. Combined, these results indicate that a small increase in water temperature can exacerbate the adverse effects of dilbit exposure on developing coho, suggesting that a spill occurring in a relatively warm year could have a greater effect on salmon populations than a spill in a cooler year. In addition, the increasing baseline water temperatures as a result of climate change may exacerbate the toxicity of a future dilbit spill. As the demand on Canada's crude oil grows, understanding the interactions between dilbit toxicity and environmental factors is critical for spill response, species impact, and risk assessments.

Dilbit Exposure Reduces Cardiorespiratory Performance in two Species of Pacific Salmon (*Oncorhynchus sp.*)

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Abstract: Diluted bitumen (dilbit) exposure is known to have developmental and latent toxic effects on teleost fish. A disruption to the cardiorespiratory system will have serious implications on the swimming performance of the adult salmonids during their freshwater migration, potentially affecting the population numbers of the next generation. Coho (*Oncorhynchus kisutch*) and sockeye salmon (*Oncorhynchus nerka*) embryos were exposed to two concentrations (low and high) of environmentally relevant water-soluble fraction of dilbit (WSFd). Hypoxia challenge tests, performed at swim-up stage of development, were used to assess the cardiac function. A subset of coho salmon was reared an additional 6 weeks in clean water and tested again and then the hypoxia challenge test was repeated. Both high and low exposure concentrations led to a significant reduction in the time to loss of equilibrium, indicating a reduced capacity of cardiac function. For coho salmon, the 6-week depuration period did not completely reverse this effect. These results suggest that sublethal exposure to dilbit leads to lasting impairment of the cardiorespiratory function in coho and sockeye salmon. A functional cardiorespiratory system is integral to the survival of the Pacific salmon during the challenging conditions of the marine and upstream migration phases of their life cycle. Long-term dysfunction of the cardiac system following exposure to a dilbit spill could have greater implications on the salmon populations of the British Columbia.

Removing the Brakes: Convergent Genomic Excision of the Regulatory N-Terminal Extension of Cardiac Troponin I in a Clade of Tachycardic Mammals

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Abstract: Mammalian cardiac troponin I (cTnI) is characterized by an N-terminal extension harbouring two protein kinase A (PKA) target residues ($Ser^{23/24}$). During adrenergic stimulation, $Ser^{23/24}$ phosphorylation reduces myofilament Ca^{2+} sensitivity, thereby increasing the rate of cardiomyocyte relaxation. Deletion of the N-terminal extension in transgenic mice mimics PKA phosphorylation and improves diastolic function, which may have therapeutic applications for chronic heart failure. Here, via gene annotation, transcriptome/mRNA analyses, and Western blots of PKA treated cardiac extracts, we show that exon 3 of *TNNI3*, which encodes the majority of the regulatory cTnI N-terminal extension including $Ser^{23/24}$, was independently pseudoexonized at least four times in shrews and moles. In contrast, we demonstrate that exon 3 of desmans, which are nested within the mole clade, is intact and evolving under strong purifying selection with maximum entropy modelling further suggesting this regulatory exon is likely capable of undergoing alternative splicing. Bat cardiac transcriptomes similarly reveal alternative splicing of *TNNI3* exon 3 in at least two distantly-related chiropteran families. Taken together, our findings suggest that alternative exon 3 splicing was ancestral for shrews and moles with the subsequent genetic assimilation of exon 3 skipping in these clades facilitating the evolution of persistently high heart rates without chronic adrenergic stimulation. Thus, by studying the hearts of mammals with exceptionally high heart rates, we have shown that nature realised the solution to habitually accelerate diastolic relaxation—by genetic truncation of cTnI—long before modern biomedicine. However, the convergent loss of this regulatory exon may represent a historical contingency that contributes to the inability of these small insectivorous mammals to exploit hibernation as a seasonal energy conservation strategy.

Learning about Risk and Safety in a Changing World

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Abstract: Predation is recognized as among the most important selective pressures faced by prey animals, influencing everything from morphology to life history and behaviour. Not surprisingly, the way in which animals learn to recognize risk has been at the cornerstone of antipredator decision making. However, with our preoccupation on understanding risk, a forgotten element of decision making involves recognition of non-predators and safety. We will discuss research on recognition of risk and safety in coral reef ecosystems, which are among the most threatened of all ecosystems.

Increases in global temperature combined with others stressors have led to huge tracks of bleached, dying and dead corals. When corals die, the remaining structures are often taken over by various algae, cyanobacteria and sessile invertebrates, which changes the physical and chemical landscape of the area. We addressed how this environmental degradation provides a key challenge for the ability of prey to catalogue unknown animals as safe or risky.

Interspecific Diversity in Propatagium Shape During Avian Wing Morphing

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Abstract: The ability of birds to change their wing shape allows them to perform complex aerial behaviours. This mechanism, known as "wing morphing", affords control over efficiency, stability, and maneuverability during flight. Previous comparative work has shown that dynamic, rather than static, metrics of wing shape are associated with flight behaviour, implying that diverse ecological pressures have caused interspecific variation in wing morphing capacity. However, one aspect of wing morphing has not yet been explored through a comparative morphological lens: the propatagium. This region of skin, muscles, and tendons between the shoulder and the wrist has been hypothesized to generate more lift than the skeletal and feather components of the wing, and adjustments to its shape and surface area could affect aerodynamic forces experienced by the bird. Although the propatagium has been informally observed to change shape during wing flexion and extension, its morphology has never been quantified, and its contribution to wing morphing is not well understood. Here we quantified how 3D propatagium morphology changes over the wing's range of motion, and explored how this differs across a range of avian species. Propatagial surface area was not substantially affected by wrist angle, but depended primarily on elbow angle, and was maximized in the same region of each species' range of motion. Relative changes in area and leading edge curvature, as well as the degree of curvature, were more pronounced in some species than others. Variation in these shape metrics suggests diversity in wing morphing functionality. Additionally, species that vary propatagium shape to a greater degree may have broader control over aerodynamic forces acting on the wing. Quantifying these metrics will allow us to determine whether dynamic wing shape differences are related to ecological factors, as well as providing a basis for investigation of the propatagium's aerodynamic properties.

Effects of Alternative Bisphenols – BPS and BPAF- on Zebrafish Spermatogenesis and its Consequences in Behavior

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Abstract: BPA is a plasticizer that has been extensively studied due to its role as an endocrine disruptor in the hypothalamic-pituitary-gonadal axis of several vertebrates. Alternative bisphenols have been developed to replace BPA.

Recent studies have shown that these compounds can also act as endocrine disruptors, and may be equal to, or even more harmful than BPA. In this work, zebrafish adult males were exposed to BPA alternatives – BPAF (0,15 µg/L) or BPS (0,5 µg/L) - for 14 days, and as controls, fish were kept in water with DMSO (0,00001%). After treatment, we evaluated zebrafish behavior parameters like stress/anxiety and antipredatory response, using Novel Tank and Alarm substance tests, respectively. In addition, we performed *in vitro* exposure using testis tissue culture treated with 0,5; 5 and 50 µgL of BPS or BPAF for 7 days. We then evaluated *in vitro* and *in vivo* (14 days) spermatogenesis by histomorphometric analysis of germ cells and testes gene expression (*pou5f3*, *nanog*, *dazl*, *scyp3l*, *cyp19a1a*, *star*, *cyp17a1a*, *esr1*, *esr2a*, *esr2b*, *dnmt5*, *tet1*, *ezh2*, *kdm6b, kat6a*, *hdac4*, *noxa*). Our results showed that BPAF exposure increased stress/anxiolytic behaviour and decreased antipredator response. Regarding the testes, in general, BPS and BPAF upregulated steroidogenic enzymes gene expression (*cyp19a1a*, *star* and *cyp7a1*) *in vitro* and *in vivo*. Moreover, *in vivo* exposure with BPS and BPAF stimulated epigenetic regulatory enzymes gene expression (*dnmt5*, *tet1*, *kat6a*, *hdac4*) and (*tet1*, *ezh2*, *hdm6b*, *kat6a*, *hdac4*), respectively. Concerning the histomorphometric data, BPS and BPAF *in vitro* and *in vivo* exposure reduced the frequency of primary spermatocyte in pachytene cysts and, *in vivo,* increased first meiotic division cysts, indicating stimulation toward finishing meiosis. Altogether, our data showed that BPAF interferes with fish behaviour and indicates that BPS and BPAF may have epigenetic effects and affect reproduction by stimulating steroidogenesis and modifying spermatogenesis towards meiosis.

Relaxing on the Wing: Myosin Conformation in Migrating birds

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Abstract: Migratory birds migrate under a tight energetic budget and benefit from energy-saving mechanisms. Myosin ATP turnover correlates directly with metabolic rate in resting skeletal muscle and varies with the physical conformation of myosin. Myosin conformations include super-relaxed (SRX) and disordered-relaxed (DRX), which are associated with low and high ATP turnover rates, respectively. We hypothesized that migratory birds modulate myosin conformation to reduce energy costs during migration and predicted a higher proportion of SRX myosin and lower ATP turnover in migrating birds. We tested this hypothesis by comparing myosin ATP turnover between a long-distance (Swainson's thrush) and short-distance (hermit thrush) migratory songbird. Next, we compared myosin ATP turnover between a migratory and non-migratory phenotype within a long-distance migratory songbird (blackpoll warbler). For each bird, we assessed myosin ATP turnover using mantATP chase, wherein we measured ATP turnover in resting flight muscle fibers using epifluorescence microscopy. We estimated the proportions of SRX and DRX myosin and measured ATP turnover rates for both myosin conformations.

We found similar myosin conformation proportions and DRX myosin ATP turnover between long- and short-distance migrants, but SRX myosin ATP turnover times were shorter in the long-distance migrant. These data suggest limited interspecific variation in myosin conformation during migration. In contrast, we found a trend suggesting higher proportion of SRX myosin in the migratory phenotype in the blackpoll warblers, similar ATP turnover times in DRX

myosin, but significantly lower ATP turnover times in SRX myosin. These data suggest that flight muscle myosin is seasonally flexible and may contribute to lower resting metabolic costs during migration. Our data support our hypothesis and indicate that migratory birds modify their flight muscle myosin conformation as a potential energy-saving mechanism and that much of the variation in myosin conformation may be the result of seasonal flexibility, rather than variation in migratory strategy.

Flight Muscle Mitochondria are Seasonally Plastic in a Migratory Songbird

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Abstract: Birds remodel their flight muscle metabolism prior to migration to meet the high energetic demands of migratory flight. This remodeling includes increases in both oxidative capacity and defence against reactive oxygen species. The degree of plasticity mediated by changes in these mitochondrial properties is poorly understood but could be explained by two non-mutually exclusive hypotheses: variation in mitochondrial quantity or individual mitochondrial function. We tested these hypotheses using yellow-rumped warblers (*Setophaga coronata*), a songbird which biannually migrates two to five thousand kilometres. We predicted higher flight muscle mitochondrial abundance and substrate oxidative capacity and reduced reactive oxygen species emission in migratory warblers captured during autumn at Long Point, ON. Non-migratory warblers were sampled following acclimation to short-day photoperiod. We assessed mitochondrial abundance via citrate synthase activity and assessed mitochondrial function using high-resolution fluororespirometry on isolated mitochondria to infer capacities for substrate oxidation and reactive oxygen species emission. We measured rates of respiration and H_2O_2 emission in oxidative phosphorylation and leak conditions in addition to flux through electron transport system protein complexes. We found higher citrate synthase activity in the migratory phenotype, indicating higher mitochondrial abundance during migration. We also found higher oxidative phosphorylation respiration fuelled by fatty acids in the migratory phenotype, yet H_2O_2 emission rates were similar between phenotypes. This higher oxidative phosphorylation capacity was supported by higher flux through electron transport system complexes II-III and IV, while flux through complex I was similar between phenotypes. We also found higher leak respiration and lower H_2O_2 emission rates in the migratory phenotype for both substrates. These data suggest that flight muscle mitochondria are remodeled to increase oxidative capacity during migration without increasing reactive oxygen species formation. Our findings support both hypotheses and indicate that mitochondrial function mediates seasonal metabolic plasticity in the pectoralis flight muscle of migratory songbirds.

Uncertainty About Novel Predation Risk in a Changing World

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Abstract: The Anthropocene is defined by human-induced rapid environmental changes including habitat loss/fragmentation, the spread of exotic species, harvesting, various pollutants, and climate change. In many cases, these environmental changes force animals into novel situations where they face uncertainty in decision making, potentially leading to decision errors and thus negative fitness consequences. In this talk, I will discuss research on uncertainty about novel predators for wood frog tadpoles, *Lithobates sylvaticus*, focusing on their learning and memory of novel predators following unpredictable variance in either the timing, intensity, frequency, or diversity of risk. I will also highlight the potential for such cognitive studies to serve as an innovative conservation tool, where the early detection of impairments can assist environmental planning and management in our rapidly changing world.

Regulation of the Gill Corticotropin-Releasing Factor System During Smolting and Seawater Acclimation of Atlantic Salmon

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Abstract: Anadromous fishes—such as Atlantic salmon (*Salmo salar*)—undergo major osmoregulatory changes to tolerate transitions between freshwater and seawater. Several of these responses have been directly linked to changes in circulating hormone levels, but the potential contributions of many local endocrine regulators are unknown. Corticotropin-releasing factor (CRF) is a peptide hormone which serves several key osmoregulatory functions in mammals and insects, but whether the peripheral CRF system serves similar functions in fishes is unclear. Here, we performed experiments using Atlantic salmon which aimed to evaluate the potential osmoregulatory function(s) of the CRF system in the gills. We initially assessed transcriptional changes in the branchial CRF system of parr and smolts during the spring smolting period, as well as following transfer to seawater during peak smolting in May. Few seasonal changes were observed, but widespread changes in transcript abundances of peptides, receptors, and binding proteins within the CRF system were observed during seawater acclimation. To determine whether the observed transcriptional changes might be driven by elevated cortisol levels associated with seawater acclimation, we used *in vitro* gill filament cultures to assess the direct influence of cortisol on the branchial CRF system. In general, transcriptional changes in filaments that were incubated with cortisol were distinct of those observed during our *in vivo* study suggesting that changes in the branchial CRF system are not mediated by cortisol. We are currently performing RNA-Seq on gill filaments that have been cultured with different CRF peptides to determine the major transcriptional targets of this hormone system in the gills. Collectively, our data implicate the CRF system in responses to osmotic stress in the gills and provides novel insights into the osmoregulatory functions of the peripheral CRF system in fishes.

Buddies and Bullies in Sable Island Feral Horses: Connecting to Individual Fitness and Population Ecology

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Abstract: Sociality is a common feature of many mammalian species, yet individual social relationships between animals in groups are rarely scaled up to their influence for fitness and population dynamics. Feral horses (*Equus ferus caballus*), the model species for my research, present complex social relationships within their groupings, called bands. The purpose of my research is to determine what causes social affiliations between horses, how they are influenced by ecological constraints, and in turn influence individual fitness and population dynamics. In summer 2022 I collected field data on spatial associations between horses in bands (n = 10) in Sable Island National Park Reserve. Proximity measures between band members were obtained by 10 min scan samples during 20 h of observations per band. Associations indices per each dyad within the bands were then computed as a proportion of time individuals spent in close proximity (≤ 3 m) out of all scan samples. Results show that in every band preferred and avoided companionships are being present (standard deviation of association indices is significantly higher than expected by chance). Consequent analysis indicates that almost every individual within a band has at least one preferred social partner that they associate with significantly more than with anybody else. Horses exhibit slightly disassortative associations with regards to sex and age, yet the results are not statistically significant. I aim to further explore the ways preferred associations may influence individual fitness, in relation to stress, body condition and parasite load, and reproductive success. On the population level I will investigate differences in sociality across bands in light of several limiting ecological and demographical factors, such as population density, operational sex ratio, habitat quality and freshwater availability. My research will provide important insights into links between sociality, individual fitness and population ecology.

Coping with the Heat in the Endothermic Poikilothermic Eusocial Bumblebee *Bombus Impatiens*

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Abstract: Habitat temperature variation is a central driving force that shaped many behavioural, morphological, and physiological adaptations of animals. Some species have complex thermal biology such as endothermic poikilothermic insects. Certain groups of insects also evolved eusociality that leads to group behaviours that help regulate nest temperature, which is central to many species of bees and their success. Bumblebees are well known for their adaptations that improve their function in a cold environment, but how well they can cope with warm conditions is of concern for this group of bees. In this series of studies, we investigated 1) the ability of various cast members to function in warm conditions, 2) the energy expenditure and effectiveness of the colony to control nest temperature in variable environmental temperatures, 3) the longer-term consequences of high temperature challenges on the colony activity

budget and growth. At the individual level, the critical thermal maximum was determined and showed little variation among workers, drones and queens, but developing larvae are much more sensitive to warm temperature. We also found no impact of the presence or absence of the insulating pile, typical of bumblebees, on the metabolic rate of workers, drones or queens flying in warm conditions. Regulating nest temperature for developing larvae is of utmost importance and bumblebee colonies were effective at it through changes in colony metabolic rate. Beyond 30°C, however, nest temperature increased, and such thermal challenge was unsustainable. Individuals worked collectively to maintain nest thermal conditions by increasing their fanning behaviour in warm conditions. When exposed long term, workers abandoned their nest, and the number of larvae produced was impaired. Overall, this work shows the multifaceted thermal biology of bumblebees and the impact of acute and chronic warm temperature on the function of the individuals and colonies.

Immune Responses Elicited by Mosquitoes, *Culex Territans*, Infected with *Hepatozoon Sipedon* from the Blood of Common Gartersnakes

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Abstract: Little is known about immune responses elicited by mosquitoes that are infected with *Hepatozoon* species acquired when these insects take blood from garter snakes. Production of reactive oxygen species (ROS) is a typical immune response of mosquitoes against other apicomplexan parasites, including *Plasmodium* species, the causative agents of malaria, and against *Hepatozoon* species that are acquired from the blood of frogs. Previous work in our laboratory revealed that oxidative activity increased significantly in the haemocoel of *C. territans* mosquitoes that had taken blood meals from snakes with heavy infections of *Hepatozoon sipedon* (i.e., parasitaemia of 5%) when compared to a control group of uninfected individuals. The objectives of this study, therefore, were to analyze the ROS response elicited by *Culex territans* when infected with lighter infections of *Hepatozoon sipedon* (i.e., parasitaemia of 0.1%) from the blood of Common Gartersnakes (*Thamnophis sirtalis*), and to identify if there is a generalized immune response generated by mosquito vectors against infections with *Hepatozoon* species from different vertebrates. Mosquitoes that fed on the blood of lightly infected snakes were dissected to separate the midgut and haemocoel, and the concentration of peroxide from each of these two organs was determined with a peroxide assay as a surrogate for oxidative activity. Production of peroxide was observed both in the haemocoel and in the midgut of infected mosquitoes, and we are currently analyzing data to determine if there are differences in oxidative activity among organs and among infection cohorts. Comparison of these data with those of peroxide responses of mosquitoes infected with *Hepatozoon* species of frogs reveal that production of reactive oxygen species appears to be generalized immune response in mosquitoes infected with *Hepatozoon* species, but is elevated in different regions of the body depending on which tissues are infected by different species of these parasites.

Retention of Sea Water Ionoregulatory Machinery in an Exclusively Freshwater, Non-Parasitic Lamprey

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Abstract: Fishes maintain ion balance in freshwater (FW) and sea water (SW) environments using ionocytes which utilize various ion transport proteins working in concert to either take-up or excrete ions. A key transporter in both FW and SW ionocytes is the sodium potassium ATPase (NKA), a transmembrane protein located on the basolateral membrane which uses ATP hydrolysis to drive active transport to take-up K^+ and to extrude Na^+. In anadromous sea lamprey (*Petromyzon marinus*), NKA is weakly expressed in the ionocytes of FW-dwelling ammocoetes, but following metamorphosis into parasitic juveniles, there is a massive upregulation of NKA which coincides with the appearance of large SW ionocytes used for ionoregulation after their migration to SW. When adult sea lamprey returns to FW to spawn, upstream SW ionocytes are subsequently lost which corresponds with decreases in NKA abundance. Less is known regarding NKA abundance changes in landlocked populations of sea lamprey, which invaded the Great Lakes, or in non-parasitic American brook lamprey (*Lethenteron appendix*) which live exclusively in FW. Using immunohistochemistry and western blot analysis, we demonstrated that the patterns of NKA expression in the gills of landlocked sea lamprey were virtually identical to their anadromous counterparts, characterized by low NKA expression in the larval and adult life stages, but higher NKA expression during the juvenile parasitic phase. Remarkably, a near identical pattern of NKA expression was observed following metamorphosis of American brook lamprey, in which a parasitic, seawater phase is absent. We conclude that this brief transient period of greater NKA expression in the American brook lamprey is consistent with the presence of SW ionocytes in FW, and that it may represent an evolutionarily conserved trait related to their descent from a parasitic, anadromous lamprey ancestor. Indeed, their nearest relative is the anadromous, parasitic Arctic lamprey (*Lethenteron camtschaticum*).

Anatomical Investigation of Regular Dorsal Dimples on *Varroa destructor*, a Devastating Parasitic Mite of Honey Bees

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Abstract: *Varroa destructor* (Anderson and Trueman) parasitizes immature and adult stages of the European honey bee (*Apis mellifera* L.), posing a serious threat worldwide. Mistakenly interpreted for decades as biting damage inflicted to mites by adult bees and hive associates, in earlier studies we showed that regular dorsal dimples (RDD) instead are developmental faults in 10% of adult female mites. RDD exist as indentations in a maximum of two predictable, symmetrical locations per mite. RDD are situated directly above a set of obliquely-oriented muscles which run dorso-ventrally within each side of the mite's body. These muscles repeatedly expand and contract in a critical manner, allowing *Varroa* mites to ultimately achieve their widened, crab-like shape at the final molt of the adult female. Previous

work indicated that RDD themselves may decrease mite reproductive success, a potential benefit to the honey industry largely dependent on acaricides for *Varroa* control. Accordingly, this study focused on anatomical characterization of the musculature below RDD in resin-embedded mites. An increased cross-sectional area compared to regions where dimples were absent, could indicate sustained muscle contraction that becomes permanently fixed beneath a hardened, permanent dimple during the process of the adult mite's final, rapid sclerotization. Non-dimpled mites (controls) were examined for comparison to mites having one or two RDD. The attachment points, paths, plus lengths and widths of the dorsal-ventral muscles were tracked and measured from sections. Six such muscles exist per lateral side per mite, but only the outer few of those showed a significant increase in cross-sectional area, reflecting the muscles' relative position beneath the deepest portion of an RDD indentation. These results are consistent with the hypothesis that mite sclerotization can fix contraction of the dorso-ventral muscles in place, thus manifesting formation of up to two RDD on an adult mite's surface, and potentially reducing the female mite's internal body cavity available for egg development. As such, this study holds promise as an applicable strategy for implementation towards non-chemical control of *Varroa*.

Damage by Ants to the Honeybee Parasitic Mite, *Varroa destructor*

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Abstract: Ants (Formicidae) are social hymenopterans commonly associated with nests of honeybee colonies. Associations of ants with bees generally are antagonistic, ranging from individual ants that stealthily enter hives to forage on combs or at hive middens, to extreme situations wherein an entire ant colony may nest in the dry cavity between a hive's inner and outer covers whereby the warmth generated by a honey bee colony can be trapped and favourably utilized by ants. Moreover, the hive interior may present occasions wherein ants encounter free-roaming mites of *Varroa destructor* Anderson and Trueman, a major parasitic species of honey bees. Furthermore, many ant species are a source of formic acid and other defensive compounds. Formic acid is a registered compound utilized by beekeepers for *Varroa* control in Canada. This motivation led to determining the impact of three ant species in Saskatchewan, namely *Formica fusca*, *Lasius neoniger*, and *Tapinoma sessile*, on groups of ten adult female mites of *V. destructor*, in the laboratory. Five worker ants of one of these three species were sealed within petri-dish arenas containing ten mites and held in darkness at 24°C; control arenas contained mites without ants. Half of the arenas were modified with screen mesh that allowed ant vapours to pass, but prevented direct contact (e.g., biting) of mites by the captive ants. Other arenas contained formic acid itself and mites, but no ants. Formic acid killed mites rapidly, whereas vapours from ants (separated from mites) did not increase mite mortality. When in direct contact, greatest damage to *Varroa* was caused by ants of *F. fusca* and most commonly occurred as amputation or dislocation of mite legs. Blistering of the mite idiosoma, evidently caused by chemical discharge from the ants, was also noted. The latter form of damage presented the clearest distinction between mite damage caused by adult ants versus adult honey bees.

Changes in Gene Expression During Cold Acclimation Allow *Drosophila Melanogaster* to Offset the Effects of Chill Injury

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Abstract: Insects, including *Drosophila melanogaster,* suffer from chilling injury in the cold, in part because of a loss of ion and water balance when subjected to cold stress. Loss of ion and water balance has been linked to temperature induced changes in membrane-bound transporter activity (e.g. Na^+/K^+ ATPase, H^+-ATPase). Cold acclimation can improve low temperature tolerance and is associated with an increase in water and ion transport rates in cold acclimated flies, but a paradoxical reduction in the activity of these ATPases in the same tissues. These findings raise questions about whether the current models of ion transport apply to cold-acclimated insects, or if modulation of other transporters allow for improved renal function in the cold. We propose the use of transcriptomic sequencing of the renal and gut tissues to investigate the regulation of genes that may be responsible for such changes, in the hopes of narrowing down the broader mechanisms responsible for the mitigation of chill injury in cold acclimated insects.

Exercise Recovery in High Altitude Deer Mice (*Peromyscus maniculatus*)

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Abstract: Animals living at high-altitude are faced with harsh conditions such as low levels of oxygen. This makes it difficult for individuals to perform daily tasks that require aerobic metabolism. However, deer mice have evolved physiological adaptions that allow populations at high altitude to survive in these conditions. One activity important for survival is aerobic locomotion and the rapid recovery of muscle metabolism after a bout of exercise. Past work has shown that hypoxia acclimated high altitude mice have a greater reliance on carbohydrates to power exercise than low altitude mice and show a significant depletion of muscle glycogen. However, it is unclear how quickly these mice can replenish these glycogen stores to the resting condition. The gastrocnemius muscle of high-altitude deer mice has a more aerobic phenotype and a greater capacity to oxidize lipids than in low altitude mice. This suggests that high altitude mice may recover more quickly from exercise than their lowland counterparts due to a greater capacity to power glycogen replenishment using lipids stored in muscle as intramuscular triglycerides (IMTG). To explore this possibility, I used low and high-altitude deer mice born and raised in common lab conditions and acclimated to chronic hypoxia. I determined changes in oxygen consumption following aerobic exercise and sampled muscle at various time points during recovery to examine changes in key metabolites, including glycogen and IMTG. I found there was depletion in glycogen stores during exercise in both populations, but [glycogen] did not return to resting levels following 90 minutes of recovery. Similarly, IMTG's did not significantly change with exercise or during the recovery period in either populations These data suggest that muscle recovery from aerobic exercise is not influenced by altitude ancestry in deer mice.

The Molecular Physiology of the Poeciliid Gill Epithelium TJ Complex: Sex-Specific Differences

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Abstract: Poeciliids are a sexually dimorphic family of livebearing fishes, many members of which exhibit a high degree of physiological plasticity. This study considered the sex-specific molecular physiology of the gill epithelium tight junction (TJ) complex of a Poeciliid, Endler's livebeaer (*Poecilia wingei*), in response to alterations in water salt content. Experimental fish were laboratory-reared but originated from Lagunos de los Patos, Venezuela (10°25'45.8" N; 64°12'18.2" W). Claudin (Cldn) TJ proteins known to exhibit abundance in the gill epithelium, and some degree of sensitivity to salinity change were targeted. Fish were acclimated to either freshwater (FW) or salt water (SW, 30-32‰) for two weeks. Male and female fish were housed in the same holding tanks, separated by a plastic mesh. Gill was collected for analysis of *cldn* mRNA abundance by qPCR analysis. Data was examined either (1) as a collapsed data set (i.e. combined results for male and female fish) or (2) separately (i.e. male and female fish independently). Collapsed data indicated that many *cldns* altered in response to salinity (i.e. elevated *cldn-8d, -10d, -10e, -10f, -28a, -28b, -28c* or reduced *cldn-3b, -3c, -3d* mRNA levels). However, it was observed that some of these changes were driven by one sex only. For example, a salinity-induced elevation in *cldn-8d* and *cldn-28a* mRNA abundance was driven by changes in the gill of female fish only, while male fish did not exhibit a significance alteration in mRNA abundance. In addition, the mRNA abundance of *cldn-3d* and *-8c* were found to differ between the gill of male and female fish irrespective of salinity. This study suggests that sex-specific differences in the molecular physiology of the gill TJ complex occurs in fishes and that these may be present independent of salinity change or may only occur when fish are exposed to altered environmental conditions.

Lessons from a Long-Lived Fish: Phenotypic Flexibility in Response to Chronic Thermal Stress

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Abstract: White sturgeon (*Acipenser transmontanus*) are one of the largest and longest-lived freshwater fish in North America but through historical overfishing and current range restrictions, many populations are at risk. With the rise in global temperatures, characterizing thermal sensitivities and identifying the associated mechanisms may be beneficial for species conservation. We investigated thermal stress responses of three distinct populations of white sturgeon from the Fraser and Nechako Rivers in British Columbia and the Sacramento River in California (USA). Fish were acclimated to either control temperature (14°C) or warm temperature (20°C, recommended river temperature limit for salmonids in BC), and were subjected to a series of phenotypic assessments to determine their thermal acclimation capacity. Critical

thermal maxima (CT_{max}) were measured for each population at their respective acclimation temperatures to quantify their acclimation response ratio (ARR), which is high in earlier life stages of white sturgeon. Furthermore, we quantified the effect of these temperatures on aerobic performance by using intermittent-flow respirometry to quantify standard metabolic rate (SMR) and routine metabolic rate (RMR) for each population, and additionally measured maximum metabolic rate (MMR), following exhaustive exercise for the Nechako River population. Finally, we sought to understand if thermal stress impacted their ability to cope with an additional stressor (low oxygen) and measured hypoxia tolerance in each population at their respective acclimation temperatures. We find that warm-acclimated fish demonstrate high ARR and pronounced compensatory responses to temperature (e.g., maintained SMR and hypoxia tolerance). These data indicate this may be associated with increased aerobic capacity (e.g., increased hematocrit, reduced splenic somatic index) and increased energy store utilization (e.g., reduced hepatosomatic index). When paired with biochemical and tissue-level metrices of aerobic and anaerobic capacity, these data will allow us to holistically assess how white sturgeon can cope with thermal stress, and if these plastic responses are population-specific.

Using Environmental DNA to Inform Conservation Decisions for Freshwater Fishes in Canada

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Abstract: Environmental DNA (eDNA) offers a promising and economical supplement to capture-based sampling because it is sensitive, non-invasive, and requires relatively little field effort. This is particularly important for freshwater fishes that are overlooked during traditional surveys because of their small size, rarity, and/or reputation as “coarse fish” or “non-charismatic” species. Thus, our lab uses eDNA to help map the distribution of underappreciated freshwater fishes to help inform Committee on the Status of Endangered Wildlife in Canada (COSEWIC) status assessments and Fisheries and Oceans Canada’s Species at Risk recovery efforts. For example, the three native lamprey species found in Manitoba (Northern Brook, Silver, and Chestnut lampreys) had all been assessed by COSEWIC as Data Deficient. It was unknown whether the relatively few records to date reflected the species’ actual distribution or were due to a lack of targeted sampling, especially for the burrowed larval stage. Our eDNA surveys helped show that Northern Brook Lamprey has a very limited distribution in southeastern Manitoba and Silver Lamprey is found in disjunct areas in the Nelson and Winnipeg River basins, contributing to updated assessments of Endangered and Special Concern, respectively, for the Saskatchewan-Nelson populations of these species. We are finding Chestnut Lamprey eDNA in several river systems in southwestern Manitoba, important information when this species is next assessed by COSEWIC. Similarly, we developed eDNA assays for the Endangered Carmine Shiner, a small minnow whose known Canadian distribution is limited to southeastern Manitoba. Carmine Shiner has been the focus of targeted sampling by Fisheries and Oceans Canada, but continued monitoring will be important to test for potential range contractions due to habitat loss or a northward shift due to climate warming. Other species of conservation concern that we are surveying with eDNA include Bigmouth Buffalo, Golden Redhorse, Hornyhead Chub, Deepwater Sculpin, and Pacific Lamprey.

Characterizing the Antimicrobial Activity of Skin-Secreted Peptides from the North American Wood Frog (*Rana sylvatica*)

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Abstract: Antimicrobial peptides (AMPs) are short, amphipathic peptides which exhibit broad-spectrum antimicrobial activity. AMPs appear to be a key innate immune barrier in amphibian skin, as many diverse AMPs have been isolated from amphibian skin secretions. The North American wood frog (*Rana sylvatica*) has previously been reported to produce only two AMPs (brevinin-1SY in adults and temporin-1SY in metamorphs), fewer than is typical for this genus. While a basic assessment of brevinin-1SY antibacterial activity has been made, the antimicrobial activity of temporin-1SY has not been previously characterized. The aims of this study were to characterize the antimicrobial activity of these AMPs and assess their ability to inactivate the viral pathogen frog virus 3 (FV3). We performed minimum inhibitory concentration (MIC) assays on a panel of model microorganisms (including Gram-negative bacteria, Gram-positive bacteria and fungi). Additionally, we performed viral inactivation assays with FV3 to determine whether *R. sylvatica* AMPs might serve to prevent ranavirus infection. Our findings suggest that brevinin-1SY and temporin-1SY differ in their inhibitory activity against various classes of microorganisms and likely play complementary roles in defending the skin surface against colonization by harmful microbes. Incubation with *R. sylvatica* AMPs was observed to reduce the number of infectious FV3 virions *in vitro*, suggesting that they may provide direct ranaviral defense at the skin surface. As *R. sylvatica* are susceptible to ranavirus infection and skin is a potential route of entry for these pathogens, our research provides insight into how the *R. sylvatica* AMP arsenal contributes to protection against population-threatening disease outbreaks.

The Role of Conservation Physiology in the Science and Management of SARA-listed Fishes

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**Presenting Author*

Abstract: Effective conservation of fishes listed under Canada's *Species at Risk Act* (SARA) requires scientific knowledge spanning life history, species distribution and abundance, genetic structure, habitat, and threat topics. A review of research progress for SARA-listed species in the Great Lakes St. Lawrence-River basin indicated that greatest research progress has been achieved for population and habitat topics, with lower progress on threats and approaches for species recovery. Despite research progress on habitat topics, the identification of physiological thresholds for SARA-listed species remains a notable gap. The lack of temperature and oxygen thresholds for most species has influenced the identification of critical habitat, the assessment of the severity of threats, and the role of threat mitigation in species recovery. I will identify reasons for the lack of progress in identifying physiology thresholds for SARA-listed fishes, review

promising non-lethal approaches to obtain species responses to thermal stress and hypoxia, and identify scenarios where knowledge of physiological responses to common stressors would improve the science and management of SARA-listed fishes in Canada.

Comparing Glucose Acquisition Strategies Between two Ancient Fish Species: Lake Sturgeon (*Acipenser fulvscens*) and Pacific Spiny Dogfish (*Squalus suckleyi*)

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Abstract: The uptake of glucose within the gastrointestinal tract (GIT) is largely unexplored in ancient fish such as lake sturgeon (*Acipenser fulvescens*) and Pacific spiny dogfish (*Squalus suckleyi*). Interestingly, within the GIT of Acipenserids (e.g. *A. fulvescens)* and Chondrichthyes (e.g. *S. suckleyi*) evolved an organ with scroll-like folds termed the spiral valve. Due to its structure, the spiral valve presumably slows down the passage of chyme and increases the gastrointestinal epithelial surface area to maximize nutrient absorption, suggesting it to be primarily responsible for nutrient acquisition. However, there is currently minimal functional data to support this claim and no data looking at feeding state. Here we aim to determine the functional role of the spiral valve in carbohydrate digestion and glucose acquisition during different feeding states and to determine if this role changes in organisms with different life histories. To test this, we assessed glucose digestibility and transport capacity along the GIT of *A. fulvescens* and *S. suckleyi* in fed and fasted fish. Digestibility was assessed through biochemical assays for the carbohydrate digesting enzyme maltase, while transport capacity was determined via i) mRNA abundance of the primary dietary glucose transporter (SGLT1), and ii) through *in vitro* intestinal fluxes. The spiral valve had an elevated role in glucose digestion and transport in *S. suckleyi* in comparison to *A. fulvescens.* Within the spiral valve, there were no significant postprandial changes in SGLT1 mRNA abundance nor maltase activity in *A. fulvescens* in comparison to *S. suckleyi*, supporting their feeding strategies with *A. fulvescens* being continuous feeders and *S. suckleyi* being opportunistic feeders. This study provides further insight about the functional role of the spiral valve in ancient fish along with increasing our understanding of the evolutionary history of glucose acquisition in vertebrates.

3D Digitization as a Means Of Engaging Undergraduate Students In Zoology Research

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Abstract : Three-dimensional (3D) imagery and digitization have become cornerstones of comparative morphology and developmental studies. Despite 3D visualization being commonplace in research, and 3D technologies becoming more ubiquitous, their use is seldom implemented in zoology courses. Across biological sciences, observational skills are crucial, but can also pose an obstacle to student learning and future participation in research. This is especially true when working with small, fragile, or rare specimens, issues commonly encountered in fields such as entomology and paleontology. These issues are further exacerbated for undergraduates in the context of distance-learning.

Here, we propose to use digitization and 3D modelling as a way of introducing students to digitization research, virtual morphology, and current methods in the field of comparative morphology and development. We have adapted a 3D photogrammetry workstation to scan small specimens and developed a workflow for low-cost photogrammetry of larger specimens. Our work has produced 3D surface models representing 10 invertebrate orders as well as 3 vertebrate orders for virtual anatomy teaching. Furthermore, we have developed learning activities that engage student learning through interaction and annotation of these digital models and collaborative discussions in online and blended learning environments.

Finally, our work offers a pipeline through which undergraduate students can become more familiar with modern research methodology in comparative morphology and engage in research in a classroom setting or via supervised research experiences.

Head, Girdles, Knees and Toes: Morphological And Topological Variation In The Pectoral Girdle Across the *C. eos-neogaeus* Hybridization Complex

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Abstract: Across Teleosts, variation in the skeletal structure and morphology of the pectoral girdle is tied to ecological differences between taxa. However, little attention has been given to how variation in the topology and shape of the pectoral girdle resulting from hybridization affects its disparity. Indeed, because of its ecological role, the pectoral girdle is a system of interest to understand ecological differentiation between parental species and hybrid lineages.

Here we analysed the structure and morphology of the pectoral girdle of members of the *Chrosomus eos-neogaeus* hybridization complex using anatomical networks and geometric morphometrics to assess topological and morphological disparity and establish patterns of modularity and integration in both parental species and across five hybrid lineages found across Québec. Our results show that, while the overall structure of the pectoral girdle does not vary much within the complex, bones within the pectoral girdle show significant diversity in morphology and presence of sutures. Indeed, *C. neogaeus* displays a pectoral girdle characterized by enlarged coracoids and radials, and unexpected bone fusion between the cleithra, coracoids and radials while *C. eos* displays a gracile and unsutured pectoral girdle. *Chrosomus eos-neogaeus* hybrids display morphologies that are separate from both parental species but show similarity with *Chrosomus neogaeus* by displaying more densely connected bones within their pectoral girdles than *C. eos.* Finally, our results support similar patterns of modularity across the complex but with different directions of

morphological variation, suggesting that modularity plays a role in the phenotypic differentiation of *C. eos-neogaeus* hybrids and parents.

Arctic Char Dealing with Diel Oxygen Fluctuations: A Fish that is not Short of Breath?

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Abstract: Due to the increased eutrophication of aquatic environments, the daily oscillation between normoxic and hypoxic conditions could be amplified. At the metabolic level, lack of oxygen and reoxygenation can have serious consequences on fish as a result of altered ATP balance and an elevated risk of oxidative damages. Fish must adjust their oxygen management to thwart the deleterious effects of fluctuating oxygen concentration. This adjustment can be achieved by modulating their metabolic rate to equilibrate their energetic budget and by regulating their mitochondrial metabolism to improve oxygen use and limit ROS generation. Although hypoxia is a major concern for ecosystems around the world, our knowledge about the response of fish to cyclic hypoxia is limited, especially in hypoxia-sensitive species which are the most threatened by such environmental change. Our objective was to characterise the effects of cyclic hypoxia on metabolism and oxidative stress in Arctic char (*Salvelinus alpinus*). We hypothesised that, when exposed to multiple days of cyclic hypoxia, Arctic char alters its metabolic activities and its oxygen management due to the pressure on the aerobic metabolism. We characterized the hepatic proteome, monitored the metabolic rate, and measured mitochondrial respiration and *in vivo* ROS generation in several tissues, of fish exposed to up to 15 days of cyclic hypoxia. The first days were distinguished by the near absence of hepatic proteome modulation in fish exposed to cyclic hypoxia, but it strongly differed from control fish after fifteen days. The standard metabolic rate remained stable throughout the experiment, but we observed a decrease in spontaneous activities during the hypoxic phases. Regarding mitochondrial metabolism, we characterized a tissue-specific response. These results revealed how the hypoxia-sensitive Arctic char is able to modulate its metabolism to cope with episodes of heavy oxygen fluctuations.

Torpid 13-lined Ground Squirrel Liver Mitochondria Resist Anoxia-Reoxygenation Despite High Levels of Protein Damage

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**Presenting Authour*

Abstract: Hibernation confers resistance to ischemia-reperfusion injury in tissue, but the underlying mechanisms are still unclear. Suppression of mitochondrial respiration in torpor may contribute to this tolerance. To explore this idea, we exposed isolated liver mitochondria from torpid, interbout euthermic (IBE) and summer 13-lined ground squirrels

(Ictidomys tridecemlineatus) to five minutes of anoxia, followed by reoxygenation (A/R). We also compare these responses to mitochondria from a non-hibernator, the rat. Maximum respiration rates of mitochondria from torpid ground squirrels were not affected A/R, but in IBE and summer these rates decreased by approximately 25%. Rats showed the greatest decrease in maximum respiration rate, by more than 50% following A/R. We also compared net ROS production before and after A/R exposure among groups, revealing seasonal differences; mitochondria from summer squirrels produced more ROS than IBE. Net ROS production did not differ before and after A/R in any group. Measurements of oxidative damage to these mitochondria before and after anoxia-reoxygenation demonstrated elevated damage to protein but not lipids in all groups. Despite the elevated levels of protein damage, torpid mitochondria maintained maximal respiration rates after anoxia-reoxygenation, suggesting ischemia-reperfusion tolerance at the mitochondrial level when metabolically suppressed.

From the Epigenome to Performance: Effects of Heatwave Acclimation in an Ancient Endangered Fish

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Abstract: Climate change is causing an increase in the frequency and severity of heatwaves, posing a significant threat to organisms globally. In aquatic environments, heatwaves are often associated with low environmental oxygen, which is a deadly combination for organisms such as fish. However, surprisingly little is known about the capacity of fishes to induce plastic responses to these interacting stressors. This issue is particularly critical for species of conservation concern such as sturgeon – one of the most endangered groups of fishes on earth. Here we investigated whether heatwave acclimation increases thermal and hypoxia tolerance in white sturgeon (*Acipenser transmontanus*) and characterized underlying plasticity in mRNA levels and global DNA methylation. Heatwave acclimation increased thermal tolerance (measured as critical thermal maximum, CTMax) and induced complete compensation of hypoxia tolerance. Complete compensation of this type is almost unknown in fishes and highlights the impressive plasticity of white sturgeon. This was associated with a transient increase in baseline levels of mRNAs involved in acute high temperature and hypoxic responses (*HSP90a, HSP90b, HSP70* and *HIF1a*) and increased plasticity in response to acute stressor exposure. Global DNA methylation was sensitive to heatwave acclimation and rapidly responded to acute thermal and hypoxia exposures, and this plasticity increased in heatwave acclimated fish. For the first time in fishes, we demonstrate a rapid change in DNA methylation in response to both acute high temperature and hypoxia exposure, highlighting a previously unappreciated mechanism of thermal compensation. Thus, we demonstrate that white sturgeon have tremendous capacity to improve tolerance and increase plasticity in response to heatwave acclimation.

Exploring The Roles of Reactive Oxygen Species Generators and Scavengers in Free Radical Homeostasis During Acute Hypoxia In naked Mole-rat Cortex

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Abstract: Neuronal death during acute hypoxia and following reoxygenation is partially attributable to oxidative damage caused by deleterious bursts of reactive oxygen species (ROS). This oxidative damage is thought to be attenuated to some degree in the brains of hypoxia-tolerant species, either by comparatively lower ROS generation or elevated ROS scavenging. Naked mole-rats (NMRs, Heterocephalus glaber) are hypoxia-tolerant mammals and we hypothesized that ROS homeostasis would be better maintained in NMR cortical neurons during acute hypoxic exposure and following reoxygenation. We predicted that adaptations in both ROS generation and scavenging contribute to this tolerance. To test this, we measured ROS flux in cortical neurons using corrected total cell florescence from live brain slices during a normoxic to hypoxic (1% O_2) transition, and following reoxygenation (20 mins each). We found that, relative to hypoxia-intolerant mouse brain, ROS flux in NMR cortical neurons is stable during normoxia-hypoxia-normoxia transitions. Next, we pharmacologically inhibited specific ROS generators and scavengers to evaluate their impact on NMR neuronal ROS homeostasis. These experiments revealed a primary role for mitochondrial ROS generators and scavengers, with minimal input from xanthine oxidase or NADPH oxidase. These results suggest that NMRs may have adapted a strategy to attenuate deleterious ROS overproduction in hypoxia and following reoxygenation, which would support life in an intermittently hypoxic environment.

Effects of Amino Acids on Olfactory Development and Plasticity in age – 0 Lake Sturgeon *Acipenser fulvescens*

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Abstract: The rearing environment plays an important role in the phenotypic development of fishes. However, little research has examined the influences of the environment on the development and plasticity of olfaction. The olfactory epithelium in fishes is composed of three well-described olfactory sensory neurons (OSNs) the microvillous, ciliated, and crypt cells. With populations of OSNs present as early as 5.5 days post-fertilization (DPF) in Acipenserids, the goal of this study was to determine the developmental plasticity of olfactory epithelia in lake sturgeon (*Acipenser fulvescens*). We manipulated the developmental environment with the inclusion of L-alanine, L-lysine or L-glutamic acid to the recirculating holding tanks twice daily during water changes to maintain a relatively consistent concentration (~5 x10^{-5}M) from 10-20 DPF prior to exogenous feeding. We chose L-alanine, L-lysine, and L-glutamic acid as they represent neutral, basic and acidic amino acids that correspond to the amino acid receptors in the olfactory epithelium. We measured mRNA transcript abundance of the microvillous OSN marker (TRPC2) and receptor genes (V2R 26, V2R 1) and

ciliated OSN marker (OMP) and receptor genes (OR 1, TAAR 1) throughout development. Olfactory epithelia were sampled from individuals at 10, 21, 26, 31, 50, 65 and 80 DPF. At approximately 1-year post-fertilization imprinting effects were measured using electro-olfactogram (EOG) to the corresponding amino acids lake sturgeon were treated with during the larval stage. Previous studies observed changes in receptor mRNA transcript abundance but not populations of OSNs. This leads to the question do changes in the environment act only at the receptor level or also at the level of the neuron? Such observation may further add to the activity-dependent survival of OSNs and the importance of creating wild-type environments in hatchery settings.

Diet Impacts Thermal Performance and Tolerance in Fish

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Abstract: The Anthropocene has caused dramatic changes in the environment, including the nutritional landscape that many ectothermic animals experience. Diet provides the energy and nutrients that ectotherms require to thrive, grow, and respond to environmental stressors. Changes in food availability or quality could profoundly impact performance and environmental tolerance. However, ectotherms often have the capacity to choose their diet in the wild and thus ectotherms could potentially actively regulate their performance through diet selection. We have been investigating how diet impacts performance (whole animal, organ, and cellular-level) and thermal tolerance in marine fish. Two different studies examined how diet options (broad vs simple diets) impacted performance in opaleye (*Girella nigricans*) and California killifish (*Fundulus parvipinnis).* Both studies found the surprising trend that cardiovascular performance was reduced in the broad vs simple diet. Another study examined how diet impacts thermal acclimation rate and capacity in opaleye. Opaleye thermal limits and acclimation rates were not affected by diet; however, acclimation capacity for maximum heart rate was diet-sensitive. A final study examined how diet quality (DHA and EPA fatty acid supplementation or not) and diet quantity impact performance and thermal tolerance in opaleye. Both diet quality and quantity impacted metabolic and cardiac performance. All these studies demonstrated that diet can impact performance, in trait-specific ways. Our work suggests that diet is an important determinant of thermal performance on environmentally relevant timescales.

The Effects of Diet Quality on Developmental Plasticity of Size and Flight Energetics in the Hawk Moth, *Manduca sexta*

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Abstract: Animals vary tremendously in body size which has profound impacts on most of their function. Not only does size vary across species, but environmental conditions experienced during development induce substantial variation in adult size. In holometabolous insects, the larval stage of development is crucial in determining the final size of adults

but also various body proportions. Here we assess how developmental plasticity impacts the effect of size on animal function through manipulation of nutrient content in the larval diet of the hawk moth, *Manduca sexta*. By manipulating the nutrient quality of the diet, we can induce size variation to investigate the effects of developmental plasticity through body size on flight energetics and flight muscle metabolic capacity by measuring wingbeat frequency, wing loading, metabolic rate, and enzyme metabolic capacity. The experiment underway shows sexual dimorphism of body to wing proportions with males having smaller wings in proportion to their body compared to females in our treatment group containing half the normal nutrient content. It is hypothesized that changes in body size due to developmental plasticity will impact flight energetics and flight muscle metabolic phenotype, ultimately predicting that small size impose high wingbeat frequency, high mass-specific metabolic rate, and higher metabolic capacity of the flight muscle. The next steps in our process are to explore growth rate among treatment groups with predictions leading to lesser nutrient quality rearing decreased growth rate resulting in smaller final body size. Furthermore, metabolic measurements will provide insight on how the influence of size effects metabolic capacity, allowing assessments on the effects of developmental plasticity through nutrient manipulation on flight energetics of the hawk moth.

Temperature Induced Developmental Phenotypic Plasticity Impacts Morphological and Metabolic Traits Associated with Flight in the Hawk Moth, *Manduca sexta*

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Abstract: Animals vary substantially in size and shape within and among species, which broadly impacts functional properties such as locomotion and metabolism. In flying insects, the impact of body and wing size variation on locomotion and metabolism can be easily quantified through analysis of two linked traits: wingbeat frequency and metabolic rate. Smaller individuals tend to have a higher wingbeat frequency and consequently higher mass specific flight metabolic rates due to higher energy demands associated with faster muscle contraction rates. Ambient temperature during insect species development is an overarching cause of developmental phenotypic plasticity and increases the range of intraspecific diversity in size and shape. We experimentally induced phenotypic plasticity in *Manduca sexta* larvae using various developmental temperatures to explore plasticity's association with traits that are currently known to co-vary. The developmental experiment underway shows that as expected, warmer reared individuals matured as smaller adults. Current trends also show sexual dimorphism of body to wing proportions with males having smaller wings in proportion to their body compared to females. During this talk, I will present evidence that morphological variation as a result of developmental temperature and plasticity has functional consequences on: wingbeat frequency and metabolic rate. The next step includes exploring the thoracic muscle metabolic phenotype through quantification of the activity of key enzymes involved in *Manduca sexta's* metabolism. Experiments at the cellular level will complement current whole animal measurements to further shed light on whether there is a benefit or trade off associated with plasticity in response to temperature during insect development.

Potentiation of the PFOA Uptake in Daphnia (*Daphnia magna*) by TiO_2 Nanoparticles

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Abstract: Hydrophobic persistent organic pollutants (POPs) such as polyaromatic hydrocarbons (PAHs) and perfluorinated organic acids (PFOAs) are near-ubiquitous in the environment. They are known to negatively affect living organisms even at very low concentrations. Recently, our lab has demonstrated that certain hydrophobic plastics can adsorb PAHs and potentiate uptake of these PAHs in the marine pacific oysters and zebrafish. We also hypothesized an exponential increase in organics uptake when adsorbed by other hydrophobic nanoparticles. We hypothesized that the similarly processes may be occurring in other species and that this potentiation would increase as particle size decreases. In this research we used ^{14}C- PFOA to explore the potential for binding of PFOA to titanium dioxide (TiO_2) nanoparticles and whether that association could potentiate the uptake of PFOA into Daphnia (Daphnia Magna). We developed a novel radiotracer-based method to track the uptake of radioactively labeled PFOA in the presence and absence of 5, 25 and 100 nm TiO_2 nanoparticles. Our study demonstrates that the presence of TiO_2 nanoparticles significantly increases the rate of uptake of PFOA and that 5 nm TiO_2 nanoparticles have higher rates of potentiation when compared to 25 and 100 nm TiO_2 nanoparticles. It is also demonstrated that presence of TiO_2 nanoparticles results in a significant increase in PFOA sub chronic effects such as lipid peroxidization (LPO) using the standard test for thio-barbituric acid-reactive substances (TBARS). We also show that the presence of 5 nm TiO_2 nanoparticles increases Lipid peroxidation PO compared to the 25 and 100 nm TiO_2 nanoparticles.

Potentiation of the PFOA Uptake in Pacific Oyster (*Magallana gigas*) by PS-NP Nanoparticles

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Abstract: Hydrophobic persistent organic pollutants (POPs) such as polyaromatic hydrocarbons (PAHs) and perfluorinated organic acids (PFOAs) are near-ubiquitous in the environment. They are known to negatively affect living organisms even at very low concentrations. Recently, our lab has demonstrated that certain hydrophobic plastics can adsorb some PAHs and potentiate uptake of these PAHs into the freshwater zebrafish embryo. We also hypothesized that the exponential increases in specific surface area of nanoplastics when compared to microplastics could exacerbate this potentiation of uptake. We hypothesized that similar processes may be occurring in seawater species and that PFOA uptake would be increased in the presence of smaller sized plastics. We used radiolabelled 14C-PFOA to explore the potential for adherence of PFOA to polystyrene nanoplastics. Secondly, we assessed whether the presence of nano plastics would potentiate the uptake of PFOA into a marine (Pacific Oyster (Magallana gigas)) species. We developed a novel radiotracer-based method to track the uptake of PFOA in the presence or absence of either 500 or 20 nm nano

plastics. Our study demonstrates that the presence of nanoplastics can significantly increase the rate of uptake of PFOA and that smaller 20 nm nanoplastics have significantly higher rate of uptake when compared to 500 nm nano plastics. We also demonstrate that presence of nano plastics significantly increases thio-barbituric acid-reactive substances (TBARS) as a measure of lipid peroxidization (LPO) and that 20 nm nano plastic invokes greater increases in LPO compared to 500 nm nanoplastics.

The Role of Octopamine in Crustacean Acid-base Regulation

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Abstract: Crustacean endocrinology is a vastly understudied area of research. While many recent studies have been conducted on the acclimation and adaptation capacity of crustaceans to a changing environment, only few have investigated internal hormonal balance and how it might be affected. The current study hence aimed to identify and characterize endocrine components in acid-base regulation in the green crab, Carcinus maenas. We show here that the biogenic amine octopamine suppressed branchial proton excretion and promoted branchial ammonia excretion, likely due to affecting metabolic and mitochondrial performance in the gill(s). Furthermore, mRNA abundance for branchial octopamine receptor (OCT-R) was affected by environmental change that challenges acid-base homeostasis, including hypercapnia (elevated pCO2) and high environmental ammonia. Our findings strongly support a role for octopamine as an endocrine component in basic crustacean acid-base homeostasis, as well as being a regulatory component in response to environmental challenges that green crabs encounter on a regular basis and/or with future global change.

Immune Performance in Mosquitoes Reflects Species- and Life-stage Specific Investment Across Temperature

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Abstract: The thermal environment can have a major impact on the outcome of disease in insects and other ectotherms. Because the immune system mediates the interaction between pathogens and their hosts, its response to temperature is crucial for host survival. High temperatures can increase the rate of immune activity; however, the thermal performance of the immune system is also shaped by physiological trade-offs and adaptations to pathogen thermal performance. Thus, we can expect variation in how the immune system responds to temperature among populations, species, and even life stages. To determine if variation occurs among species and life stages, we compared the thermal performance of the melanization and encapsulation responses in four species of mosquitoes: *Aedes aegypti, Aedes japonicus, Culex pipiens* and *Culex territans*. We injected both larval and adult, female stages of mosquitoes with a mock parasite (a Sephadex bead) for 1 h or 6 h, respectively, at 10 C, 18 C, 26 C or 32 C. We then quantified the strength of

the melanization and encapsulation responses by measuring the darkness of the beads as well as the width of the layer of cells surrounding the beads. Increasing temperature improved the melanization response in adults but had little impact on melanization in larvae. Larvae in all species invested heavily in melanization relative to adults, suggesting that larvae and adults differ in the trade-offs that shape immune performance. The strength of melanization was sometimes bimodal, depending on the temperature and species, suggesting that there may be different immune strategies maintained within a population. These immune strategies may support the success of mosquitoes under a wide range of thermal environments.

Sounds Like Trouble: The Effects of Anthropogenic Noise on the Ecology and Cognition of Prey Fishes

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Abstract: Noise produced by anthropogenic activities is increasing in many aquatic ecosystems, and so is our need to understand the numerous sublethal effects of such exposure on aquatic wildlife. We first focused on the effect of noise from small motorboats, since its occurrence can dominate soundscapes in coastal communities, the number of noise-producing vessels is increasing rapidly and their proximity to aquatic life has the potential to cause deleterious effects. We present data on the effect of boat noise exposure on fish survival and cognition, highlighting both clear immediate and more hidden/latent effects of noise on wild coral reef fishes. Using a well-established fish model, we will also discuss the role of noise intensity and predictability on the cognitive performance of zebrafish in several learning tests under laboratory conditions.

Investigating the Role of 11β-hydroxysteroid Dehydrogenase Type 2 in Mediating Stress-specific Effects of Cortisol on Neurogenesis in Adult Zebrafish (*Danio rerio*)

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Abstract: Stress-induced increases in cortisol during acute or mild stress can have stimulatory effects on cell proliferation in the brain. In contrast, sustained increases in cortisol during chronic stress can suppress brain cell proliferation. The cortisol-inactivating enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-hsd2), may modulate this differential response to cortisol. 11β-hsd2 is localized to known neurogenic regions of the adult zebrafish brain, but its role in regulating adult neurogenesis during stress is unknown. We are testing the hypothesis that 11β-hsd2 mediates cortisol-induced changes to brain cell proliferation using standardized acute (1-min air exposure) and chronic (social subordination) stressors. Zebrafish were acutely stressed using a 1-min air exposure stressor, and then temporally

sampled throughout a 48 h recovery period. The acute stressor was repeated after 24 h recovery in a subset of fish. Plasma cortisol was significantly elevated 15 min after a single stressor and recovered within 24 h, and this same pattern was seen in fish exposed to a second stressor. Under chronic stress, subordinate fish exhibited significant elevations in plasma cortisol levels relative to dominant and group-housed fish. During acute stress, *11β-hsd2* transcript abundance significantly decreased in the zebrafish brain 24 h after a single and repeat stressor. In contrast, 11β-hsd2 protein expression in the brain was elevated at these timepoints. The observed changes in 11β-hsd2 protein expression are in line with previous studies showing an increase in 11β-hsd2 enzymatic activity in the brains of zebrafish 24 h after an acute stressor. On the other hand, subordinate fish exhibited no changes in 11β-hsd2 protein expression relative to dominant or group-housed fish. These results demonstrate stressor-specific regulation of 11β-hsd2 in the brain, which may contribute to the differential effects of cortisol on brain cell proliferation. Ongoing histological analysis will determine how the observed changes in 11β-hsd2 protein abundance relate to stress-induced changes in brain cell proliferation.

Are Measures of Hypoxia Tolerance Repeatable in Zebrafish *(Danio rerio)?*

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Abstract: Individual variation in tolerance of low O_2 (hypoxia) is well documented within many fish species. However, fewer studies have examined whether hypoxia tolerance is a repeatable trait based on commonly used measures of hypoxia resilience. Hypoxia tolerance is often determined via loss of equilibrium (LOE) experiments, with a longer time until LOE corresponding to greater hypoxia tolerance. Adult wildtype zebrafish were exposed to three repeated LOE trials held at two-week intervals. Time to LOE was measured at PO_2 = 10 ± 0.5 mmHg. Time to LOE exhibited a bimodal distribution, with the majority of individuals having time to LOE values less than 140 minutes but a subset with time to LOE values between 230-280 minutes. Time to LOE across trials was repeatable; using a linear mixed model repeatability (R) of time to LOE was estimated as 0.549 (95% CI: 0.294, 0.745) (N= 31-14)). These data suggest that individuals within the population vary in hypoxia tolerance in a bimodal fashion, and that an individual's hypoxia tolerance is a repeatable measure.

Does Psilocybin Affect Behaviour of an Isogenic Mangrove Fish (*Kryptolebias marmaoratus*)?

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**Presenting Author*

Abstract: Hallucinogenic "magic" mushrooms are becoming more prominent as a resource offering potential therapeutic properties. Psilocybin is the primary compound in these hallucinogenic mushrooms and has been known to produce anti-anxiety and stress-reducing effects in humans. It interacts with serotonin receptors in the brain that mediate anxiety and stress, to produce a calming effect which can result in changes in mood and personality. The use of non-human

models is compelling as an approach to better understand the effects of psilocybin with more robust conclusions. My research investigates the behavioural effects of psilocybin in the emerging model fish species, the mangrove rivulus (*Kryptolebias marmoratus*). Rivulus have socially dynamic behaviour and are known to be aggressive towards conspecifics and their mirror reflection. We are testing the anti-anxiety/stress-reducing properties of psilocybin by examining aggressive behaviour at low (500 mg/L), medium (1000 mg/L) and high (3000 mg/L) concentrations. If psilocybin reduces social aggression in rivulus, then psilocybin-treated fish will engage in fewer aggressive behaviours compared to control fish. To test this, we socially stimulated a focal fish with its mirror reflection and 24 h later, treated the same fish with a psilocybin dose (20 min) prior to the addition of a mirror. We measured a range of behaviours including frequency and time spent in aggressive behaviours. Our early results demonstrate that at a low psilocybin dose, there is considerable variation in the behavioural response of these homozygous fish. Specifically, we observed a bimodal response to psilocybin suggesting that there may be responders and non-responders to this hallucinogenic compound.

Assessment of the Effects of Simulated Microgravity Using a 3D Clinostat on Adult and Larval Zebrafish Bone

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Abstract: Vertebrate models like zebrafish have become a main interest to study the effects of simulated microgravity (SMG) on ground. Those effects have resulted in important findings between the responded of living organism and simulated microgravity in comparison with the experienced by astronauts during space flights. For this study we exposed both zebrafish larval and adult bone to simulated microgravity using a 3D clinostat during one to three days. We analyzed the effects on bone tissue and bone cells using transgenic zebrafish (bone markers) and whole larval staining, enzymatic staining, gene expression by RT-qPCR which involved several bone genes and apoptosis. The ossification was reduced in several skeletal elements in larval zebrafish. We also found that the effects differed regarding the function of the bone resorbing (osteoclast) and the bone depositing (osteoblast) cells. These findings elucidate our understanding of the response of developing bones, bone maintenance and the effects of simulated microgravity. Further studies are needed to understand more aspects of cellular communication and the description of molecular pathways involved in these response patterns. This study was funded by the Canadian Space Agency and NSERC.

Torpor as an Energy Saving Strategy to Cope With Metabolic Challenges in Deer Mice (*Peromyscus maniculatus*)

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Abstract: High-altitude environments are cold and hypoxic (low O_2). Endotherms at high altitude face the significant metabolic challenge of sustaining high rates of heat generation (thermogenesis) to maintain body temperature (T_b) despite potential constraints on tissue O_2 supply. This challenge may be exacerbated during periods of low food availability. A potential strategy to overcome these challenges is to enter a state of torpor, which involves dramatic reductions in T_b and metabolism. We are investigating this issue in deer mice. Mice from populations native to high altitude and low altitude were born and raised in captivity. Adults were acclimated to warm (25°C) normoxia or cold (5°C) hypoxia (~12 kPa O_2 for 6 weeks) and then subjected to measurements of resting metabolism (O_2 consumption rate) and T_b during food deprivation. During food deprivation in warm normoxia, low-altitude mice readily enter torpor with the onset of the daytime inactive phase, reducing metabolic rate and T_b for approximately 6 hours, following by the resumption of normothermia later in the inactive phase. During food deprivation in cold hypoxia, entry into torpor starts earlier in the middle of the active phase and appears to be more pronounced. Therefore, low-altitude deer mice will readily use torpor to cope with food limitation, but its greater use in cold hypoxia may limit night-time activity. Future research will expand upon these findings in high-altitude deer mice, to examine whether torpor is a viable energy conservation strategy in high-altitude environments.

Influence of Hemoglobin-O_2 Affinity on Aerobic Capacity in Deer Mice (*Peromyscus maniculatus*)

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Abstract: High-altitude environments present a significant metabolic challenge to endotherms, as cold temperatures increase the metabolic demands of thermogenesis while low O_2 availability (hypoxia) can constrain tissue O_2 supply. Evolved increases in hemoglobin-O_2 (Hb-O_2) affinity are pervasive across high-altitude taxa, but the influence of such increases on aerobic capacity (maximal O_2 consumption) in hypoxia remains contentious. Recent findings from mathematical modelling suggests that the influence of Hb-O_2 on aerobic capacity is dependent on other traits in the O_2 transport pathway, namely the capacity of tissues to extract O_2 from the blood, but this possibility has yet to be tested in empirical studies. We are examining this issue in deer mice. Mice from populations native to high altitude and low altitude were born and raised to adulthood in captivity. Low-altitude mice were acclimated to warm (25°C) normoxia and high-altitude mice were acclimated to cold (5°C) hypoxia (~12 kPa O_2 for 6 weeks), creating two groups with very different capacities for O_2 transport in hypoxia. We are now using pharmacological treatments to manipulate Hb-O_2 affinity and to then measure aerobic capacity for thermogenesis using respirometry. This is being complemented by measurements of the underlying determinants of aerobic capacity across the O_2 transport pathway (e.g., arterial O_2 saturation, heart rate, phenotypes of thermogenic tissues). This research will shed insight into the mechanistic basis for variation in aerobic capacity and will improve our general understanding of phenotypic integration and the evolution of complex and coordinated performance traits.

Comparing Diet Composition of Aerial Insectivorous Birds in Ontario and Saskatchewan

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Abstract: In North America, large population declines have been recorded in aerial insectivorous songbirds over recent decades. Prey quality can influence the health and growth of nestling birds. For example, aquatic-emergent insects supply more beneficial omega-3 polyunsaturated fatty acids than terrestrial insects leading to better growth. In addition to dietary sources, some birds can endogenously synthesize long-chain polyunsaturated fatty acids by conversion of shorter-chain precursors. By combining fatty acid compositions of blood with naturally occurring stable H isotope ratios (δ^2H) in feathers, diet quality and dietary sources of acquired and synthesized fatty acids can be inferred. In southern Ontario, purple martin (*Progne subis*) and tree swallow (*Tachycineta bicolor*) nestlings had lower feather δ^2H values compared to barn swallows (*Hirundo rustica*), indicating a more aquatic-emergent diet. Lakeshore purple martins and tree swallows also had higher omega-3 fatty acids levels in their blood compared to inland populations. Yet, barn swallows having higher δ^2H, or the most terrestrial diet, still had high levels of long-chain omega-3 fatty acids, suggesting the ability to synthesize long-chain omega-3 fatty acids. Southern Ontario birds are expected to have lower δ^2H feather values and higher levels of omega-3 fatty acids associated with the influence of the Lake Erie compared to Saskatchewan that has agriculturally intense landscapes with small wetlands. If birds are unable to consume or make these key fatty acids, they will incur direct and indirect health consequences. Together these tools allow us to assess species and landscape differences in their use and dependency on aquatic-emergent insects, and to better understand which species can be vulnerable to changes in the nutritional landscape.

The Emergence of the French Heartworm *Angiostrongylus vasorum*

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Abstract: *Angiostrongylus vasorum* is a blood-dwelling cardiopulmonary nematode of canids. It mainly infects dogs and foxes and can lead to severe and sometimes fatal disease in dogs. The parasite is present in Europe and Newfoundland and has been increasingly reported in the past two decades in both dogs and foxes from known endemic foci and from new areas where *A. vasorum* had not been described before. In Switzerland necropsy studies revealed an increasing *A. vasorum* prevalence from 22% to 82% over 6 years in the examined fox population. Serological data supported the drastic increase in Switzerland: 3955 tested fox blood samples collected between 1986 and 2017 led to the conclusion that *A. vasorum* had been present in Switzerland since the 1980s at low levels (2%), and that around the turn of the millennium the prevalence started to increase (33%) in the fox population. The observed prevalence increase in

Switzerland is representative of other endemic areas, supports the important role of foxes as reservoir hosts, and is in line with increasing reports of canine angiostrongylosis described from several European countries. The spread of *A. vasorum* however is not limited to Europe. The parasite was recently detected in wild canids in Nova Scotia and Prince Edward Island and is an emerging parasite in Canada. The reasons for the dramatic expansion of *A. vasorum* remain largely unknown. Contributing factors are hypothesized to be the increasing number of foxes in endemic regions, movement of dogs from endemic to non-endemic areas, and increased dispersion and/or survival of intermediate hosts due to climate change.

Atlantic Salmon (*Salmo salar*) Gill Filaments Regenerate Following Physical Damage

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Abstract: The gills of marine-farmed Atlantic salmon are vulnerable to physical damage from a diverse range of stressors which can contribute to "complex gill disease" (CGD), and greatly compromise gas exchange and osmoregulation. Regeneration of damaged gill has been reported for some fish species, however it is not known for Atlantic salmon, which is an economically important aquaculture species in Canada. We used a gill filament resection model to investigate regeneration and the cytological and functional capacity of regenerated tissue in Atlantic salmon in fresh water. Two levels of filament resection were investigated: 30% and 50% of filament distal length removed from 16 filaments on first branchial arch. Filament length was measured in the same 8-12 individuals at 1, 2, 4, 8, 12,16, and 20 weeks post-resection (wpr). Additional resected and control fish were terminally sampled at these times for immunohistochemistry and Na^+/K^+-ATPase (NKA) activity measurement. At 20-wpr, 38% of the resected filament length was regenerated in both resection groups. However, 50% resected filaments showed significantly higher absolute regenerative growth (mm) than 30% resected filaments. The regeneration rate varied significantly between individuals, but was not correlated with initial body size, condition factor, or overall fish growth rate. Immunohistochemistry showed an increase in cell proliferation at the filament tip shortly after resection. Neuroepithelial cells (NECs), nerve fibres, and ionocytes were identified at the tip of regenerating filaments as early as 4-wpr and their numbers increased during the experiment. In both resected groups more than 50% and 90% of NECs were innervated by 12- and 20-wpr, respectively. Gill NKA activity in regenerated tissue was not significantly different from that of internal control and non-resected control group. Our results demonstrate that the gill of Atlantic salmon is capable of significant regeneration and functional recovery, which has important implications for recovery from CGD in aquaculture.

Genomic and Transcriptomic Analysis of the Whirling Disease-resistant Gunnison River Rainbow Trout

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Abstract: Whirling disease is an ecologically and economically debilitating disease of rainbow trout *Oncorhynchus mykiss* caused by the parasite *Myxobolus cerebralis*. In this study, we are focusing on a promising Whirling disease resistant rainbow trout strain developed in the Gunnison River, Colorado, which can play an instrumental role in helping rainbows recover from the scourge of whirling disease. We analyzed the genomes and transcriptomes of this resistant strain after challenging it with *M. cerebralis* at different time points; T0, T1hour, T1day, T2days, T4days, and T24days. A total of 60 genomes and 140 RNA transcriptomes were used in this study. Trimmed reads of each sample were mapped separately to the reference genome of *O. mykiss.* After running the variant calling function at different time points and filtering shared variants, 27,123,539 SNVs, 791,905 MNVs, 1,451,724 Insertions, 1,394,336 Deletions, and 195,446 Replacements were identified. After removing variants in control reads, the number was reduced to 1,059,700, representing variants possibly associated with whirling disease resistance. Among these, we found that only 8,402 SNVs, 297 MNVs, 119 Insertion, 159 Deletions and 15 Replacement could cause a change in the amino acid composition of a protein. Transcriptomic profiling identified ~50 differentially expressed genes following *M. cerebralis* challenge. Many of these genes displayed a pattern of increased transcript abundance at either day 1 or day 2 post-challenge compared to control fish, and many were identified as possessing immunological function. Interferon-induced protein 44 was the highest differentially expressed immune gene with 7.8-fold change and was expressed in time points 1d, 2d, 4d, and 24d. These results can help to understand the resistance mechanism of the Gunnison River rainbow trout strain, which in turn can help in controlling this disease in North American salmonids.

The Diversity of Carbonic Anhydrases: Insight Into Regulation of the Internal Environment in Fishes

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Abstract: The carbonic anhydrases (CA) form a family of zinc metalloenzymes that catalyze the reversible reactions of CO_2 and water: $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$. Although blood and gill were identified as tissues of high CA abundance in the years soon after the enzyme was discovered in 1933, it has taken modern molecular techniques to fully appreciate the diversity of CA isoforms. Our work has focused on identifying the physiological roles of CA isoforms in fish tissues, particularly with respect to CO_2 excretion, ion transport and acid-base balance. The tissue and cellular location of CA play critical roles in determining its function. For example, CA in the cytoplasm of gill epithelial cells catalyzes the hydration of CO_2, providing protons for acid secretion and sodium uptake. By contrast, membrane-associated CA in contact with the blood flowing through the gill catalyzes the opposite reaction (dehydration), contributing to CO_2

excretion in elasmobranch fishes. The regulation of CA within a tissue offers additional mechanisms for fine-tuning its actions. The diversity of CA isoforms coupled with the availability of new tools for probing their function continues to offer intriguing insight into the physiology of fishes.

Capitalizing on Automated Quantification of Spontaneous Novelty Preference to Infer Working Memory Capacity Following Acute *Cannabis* Smoke Exposure in Rats

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Abstract: Working memory (WM) allows for the temporary storage and use of information over short periods of time and is essential for cognitive functions like learning and memory. The amount of information held in WM is called working memory capacity (WMC). Traditionally, WM and WMC tests require a long training period, learned rules, and may evaluate qualities like recency instead of WM-based recognition. In the present study, we use novel spontaneous behavioural tests to assess WMC in rats after low- and high-THC *Cannabis* smoke exposure in two modalities using objects and odours. This test capitalizes on rodents' innate preference for novelty and uses sets of either 6 identical or 6 different stimuli to evaluate WMC under low- and high- cognitive loads, respectively. When rats display novelty preference, they interact with novel objects or odours by sniffing, chewing, or looking at the item for a longer duration. This interaction is normally scored using stopwatches, but this scoring approach can introduce subjectivity and human error. To reduce this effect, supervised machine learning was used to predict and quantify the interaction behaviour. Sub-optimal machine-generated predictions were supplemented with traditional stopwatch scoring, which constitutes a novel human-machine hybrid approach. This approach effectively demonstrated rats' novelty preference under control conditions. We also show that high-THC, but not low-THC, *Cannabis* smoke induced WMC deficits in a load-dependent, and modality-specific manner. These results have implications for the effects of *Cannabis*, and *Cannabis* smoke in particular, on cognition. In addition, the human-machine hybrid approach could be optimized for use in a variety of tasks and species.

City Living: The Influence of Urban Stress on Early Rates of Neurogenesis in Eastern Grey Squirrels (*Sciurus carolinensis*)

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Abstract: As the fastest growing habitat type on the planet, urban environments encroach upon existing ecosystems at a pace never seen before, yet the severity and implications of these alterations are greatly unexplored. One means through which we may gain insight on the impact of urbanization on wildlife is through 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 urban and habitats, making them a fitting representative population, for both their success in these environments and their reliance on neurogenesis, an integral part of their caching behaviour. Using urban and natural populations, our study applies a comparative approach to investigate potential trade-offs of an urban lifestyle in wildlife. With physiological methods, we examine the stress of mothers and offspring, and its effects on the rates of neurogenesis in juveniles. By testing novel theories related to the ecological and physiological outcomes of environmental stress, this research helps illuminate the specific mechanisms that are influenced by rapid, anthropogenic, environmental changes and moreover, contribute to the development of conservation strategies associated with modified landscapes.

Implications of a Fluctuating Estuarine Environment on the Upper Thermal Tolerance in the Invasive Green Crab (*C. maenas*)

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Abstract: The introduction of invasive species can have a devastating impact on an ecosystem, often through predation and competition with native organisms. The European green crab (*Carcinus maenas*) is an extremely successful invasive species, thriving in marine environments around the world, and known for their tolerance to environmental stress. The success of an invasive species often depends on their ability to thrive in new environments. Here, using a multi-stressor approach, we evaluated the upper thermal tolerance of the green crab in combination with various environmental challenges present within estuarine environments. In particular, we investigated how changes in environmental salinity, dissolved oxygen, and waterborne copper influence the critical thermal maximum (CT_{max})and fitness of the green crab. First, six male crabs were assigned to each of the following treatment groups, control, 50% oxygen saturation, 150% oxygen saturation, 50% salinity, 20% salinity, 0% salinity, 200 µg/L, or 600 µg/L copper, and CT_{max} was assessed. No significant differences in CT_{max} were found in response to changes in environmental salinity or dissolved oxygen. However, control green crab exhibited a CT_{max} of approximately 37.6 ± 0.3 °C, while copper exposed green crab exhibited significantly reduced CT_{max} values in both the 200 µg/L (36.7 ± 0.1 °C) and 600 µg/L (36.6 ± 0.3 °C) treatments. Heart rate, maximum metabolic rate, resting metabolic rate, and copper accumulation in the heart, hemolymph, and gill are also assessed. This research offers valuable information regarding *C. maenas*' tolerance to environmental stress, which may play a vital role in their invasive success.

Developing an OpenArray Chip to Analyze the Expression of Genes Important in the Thermal Stress Response of Acipenser Sturgeons

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Abstract: In a natural aquatic ecosystem temperature fluctuation happen on a daily and seasonal basis. Organisms living in these environments have developed certain adaptations that enable them to survive and reproduce successfully in the natural range of thermal fluctuations. However, human activities have had detrimental effects on the temperature of these ecosystems. Reasons such as global warming and warm water discharge from industrial and residential areas acutely increase the temperature of some aquatic ecosystems, which makes it difficult for animals to respond physiologically. This increase in temperature can affect aquatic animals' life from different aspects, one of which is triggering physiological and cellular responses to maintain homeostasis. Thus, studying the effects of thermal stress on the expression of genes involved in different physiological responses is a tool to investigate the thermal tolerance and physiological plasticity of aquatic animals. In this study, we developed assays for an OpenArray chip to analyze the expression of important genes involved in detoxification, apoptosis, hypoxia, immune system, and endocrine disruption, in Acipenser sturgeons, exposed to acute thermal stress. This work is associated with the Genome Canada funded project GEN_FISH (Genomic Network for Fish Identification, Stress, and Health). The primers and probes used in this OpenArray chip are designed based on the conserved region of genes belonging to different species of Acipenser taxa and their efficiency has been successfully tested in four Acipenser species *A. brevirostrum, A. fulvescens, A. oxyrhynchus, and A. transmontanus*. By using this high-throughput OpenArray chip, we can analyze the expression of 56 genes in 96 samples (duplicate reactions) in most of the Acipenser species.

Energy Dynamic in Mountain Pine Beetle Larvae During Overwintering

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Abstract: Mountain pine beetles (MPB, *Dendroctonus ponderosae*) is a notorious species of bark beetle that causes major economic and ecological damage to pine forests in Canada. Native to western Canada, these beetles have spread eastward across the Rocky Mountains into Alberta, despite the harsh winter climate. The 4th instar larvae of MPB can withstand the winter season by utilizing overwintering strategies like cryoprotectant accumulation, basal maintenance suppression, dormancy, and food cessation. Most physiological processes are suppressed during overwintering; however, basal maintenance, post-dormant recovery, and repair rely on bioenergetics. As feeding ceases over winter, prudent use of stored macromolecules, such as protein, carbohydrate, and lipid as well as lowering the basal

maintenance cost might help beetles survive through winter and by extension disperse successfully in summer. The shift in the macromolecule depletion can indicate the different survival pathways during overwintering. Therefore, we determined the whole-animal concentration of each macromolecule during overwintering, by exposing overwintering MPB larvae to stepwise temperature decreases (0, -5, -10°C) and collecting subsets of beetle larvae at each temperature. We hypothesize that there will be a preferential shift in fuel use from carbohydrate to lipid with decreasing temperature, accumulating sugar polyols for cryoprotection and that there will be a lower energy demand for basal maintenance with decreasing temperature. This data will help us to understand the underlying mechanisms of the invasive capacity of MPB and manage it as well as improve models of beetle survival in their native and invasive ranges.

Urbanization and Biodiversity: Citizen Science Confirms Species-Area Relationship for Birds in Urban Parks

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Abstract: Habitat loss is a major contributor to the biodiversity crisis, and urbanization is a major factor by reducing the amount of habitat available for wildlife. In this study, we tested the hypothesis that anthropogenic land use affects avian species richness in urban parks. We used data collected by citizen scientists that were deposited to eBird to determine avian species richness at ten parks in London, Ontario for two different periods: the breeding season, and the winter. For each park, we calculated the extent of forest, grassland, and anthropogenic land use, which was measured as either a percentage of the total park size or as an absolute area. We found no correlation between anthropogenic land use and species richness. However, the total forest area and total grassland area – but not the percentage of park that was forest or grassland – were both positively correlated with avian species richness at the parks. Indeed, bird species richness correlated with the total park area. This result confirms the well-established species-area relationship, that species richness is higher in large habitats than in small habitats. This confirmation indicates that citizen scientists can be relied on to collect valuable data on species presence. We suggest that large tracts of viable habitat be protected to maintain biodiversity in urban areas.

A Cercarial Invadolysin Interferes with the Host Immune Response and Facilitates Infection Establishment of *Schistosoma mansoni*

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Abstract: *Schistosoma mansoni* overcomes immune responses in it's human host by utilizing a vast array of immunomodulatory factors. Among these factors, proteases are heavily featured throughout the entirety of the intra-mammalian life cycle stages. We demonstrate that one protease, an invadolysin termed SmCI-1, is contained within the acetabular glands of *S. mansoni* cercariae, and released early on during infection and transformation into

schistosomulae. This invadolysin is involved in creating an immunological milieu favorable for survival of the parasite. SmCI-1 functions as a metalloprotease with the capacity to cleave collagen type IV, gelatin and fibrinogen. We then show that SmCI-1 treatment modulates cytokine release from human peripheral blood mononuclear cells, providing compelling evidence that SmCI-1 promotes an anti-inflammatory microenvironment by enhancing production of IL-10 and suppressing the production of inflammatory cytokines like IL-1B and IL-12p70 and those involved in eosinophil recruitment and activation, like Eotaxin-1 and IL-5. Additionally, complement component C3b is cleaved by this protease, resulting in inhibition of the classical and alternative complement pathways. We proceeded to generate SmCI-1 knockdown in cercariae using RNA interference, and successfully perform the first knockdown of a cercarial factor prior to emergence from the snail host. Using these SmCI-1 knockdown cercariae, we demonstrate the key role of SmCI-1 in protecting schistosomula from lysis mediated by the human complement system. Finally, we utilize the SmCI-1 knockdown cercariae in a mouse model of infection, and demonstrate a reduced survival of SmCI-1 KD cercariae as compared to control worms, thereby confirming a role for parasite survival in mammalian hosts. This work significantly expands both our understanding of schistosome invadolysins, while laying the foundations for using RNAi as a tool for knocking down and studying cercarial factors involved in mammalian penetration.

Embryonic Thermal Stress and its Effect on the Stress Response During Embryogenesis and Early Life Stages in Yellow Perch (*Perca flavescens*)

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Abstract: The global effects of climate change and industrial thermal effluents are causing a rise in fresh water temperatures. As ectotherms, temperature can influence all aspects of fish biology, particularly during key developmental stages. Yellow perch (*Perca flavescens)* is a cool water fish with ecological, cultural, and economic importance in North America. As a fish that spawns in the near shore environment, they may experience warmer than normal embryonic incubation temperatures. We set out to assess if and how chronic non-optimal embryo incubation temperatures might alter the characteristics of the heat shock response to an acute stressor in embryos and post-hatch fish. Embryos were reared at 12℃, 15℃ (optimal), and 18℃ throughout embryogenesis and then transferred to common garden conditions (18℃). Embryonic and larval Yellow perch were subjected to one of three acute heat shock treatments at 3, 6, or 9℃ above their incubation temperature at various points throughout development prior to sampling and quantifying transcript levels of key heat shock proteins. Preliminary data suggest that fish reared at 18℃ exhibit greater increases in transcript abundance of heat shock protein 90A and 70-2 following acute heat shock than those reared at 15℃. Data on all heat shock protein transcript levels in fish at the three incubation temperatures in response to the three acute heat shocks throughout development will be presented and discussed.

Did Trans-Regulatory Changes to Sox9 and Runx2 Decrease Cartilage Gene Expression During Osteoblast Evolution?

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Abstract: Changes in transcription factors (trans-regulatory elements) and/or their binding sites (cis-regulatory elements) can cause evolutionary changes in gene expression. In the vertebrate skeleton, limited data suggest that bone-forming osteoblasts of earlier-diverged vertebrates express more genes typical of cartilage-forming chondrocytes than osteoblasts of later-diverged vertebrates. We hypothesized that changes to the coding sequences of the critical skeletal cell transcription factors Sox9 and Runx2 caused osteoblasts to decrease chondrocyte gene expression during vertebrate evolution. To test this hypothesis, we first used laser-capture microdissection to compare *in vivo* transcriptomes of three main skeletal cell types in mouse, chick, and gar: 1) immature chondrocytes (IMM), which typically express *Col2a1* and *Acan*; 2) mature chondrocytes (MAT), which typically express *Col10a1* and *Ihh*; and 3) osteoblasts (OST). Pairwise differential gene expression analyses revealed that gar OST expressed significantly higher levels of some chondrogenic markers, including *Col2a1*, *Acan*, *Sox6*, and *Col10a1*, compared to mouse or chick OST. Gene co-expression network (GCN) analyses in gar showed increased positive correlations between genes in IMM and OST, suggesting that these two cell types are more similar in earlier diverged vertebrates compared to later diverged vertebrates. To evaluate whether such changes were due to Sox9- and/or Runx2-related changes, we are transducing mouse pre-osteoblastic cells (MC3T3-E1.4) with recombinant FLAG-tagged *Runx2* and *Sox9* from four vertebrate clades: gar, frog, chick, and mouse. To figure out whether changes in Sox9 or Runx2 activity led to our observed evolutionary changes in OST gene expression and whether Sox9 or Runx2 from different species bind to the same genomic loci, we will perform RNA-seq and ChIP-seq analyses, respectively, on transduced cells. In addition to helping define changes to the gene regulatory network (GRN) underlying osteoblast evolution, this study will evaluate the relative contributions of trans-regulatory changes in cell type evolution.

Nesfatin-1-Like Peptide Enhances Reproductive Hormones in Hypothalamic Neurons and Pituitary Gonadotrophs and Stimulates Testosterone Secretion in Mice

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Abstract: Nesfatin-1-Like peptide (NLP), processed from nucleobindin-1 (NUCB1) shares very high similarity with nesfatin-1, a reproductive and metabolic peptide. NUCB2 (nesfatin-1 precursor) mRNA and protein are significantly increased in the hypothalamus of rats during puberty-to-adult transition. Nesfatin-1 stimulates GnRH and gonadotropin

synthesis and secretion. We hypothesized that NLP, like nesfatin-1, stimulates reproductive hormones in mice. NUCB1/NLP presence in mouse brain and gonads *in situ*, and its possible effects on hypothalamic neurons and gonadotrophs *in vitro* was studied. Further, whether synthetic NLP affects testosterone secretion *in vivo* in mice was studied. NUCB1/NLP-like immunoreactivity was detected in murine hypothalamic neurons (GT1-7 cells) and gonadotrophs (LβT2 cells), as well as in mouse brain and gonads. NLP (100 nM) significantly upregulated GnRH mRNA and protein abundance in GT1-7 cells, and LHβ (1 and 24 h) and FSHβ (1 h) mRNA abundance in LβT2 cells. GnRHR protein expression was upregulated by 1 and 10 nM NLP in LβT2 cells. Subcutaneous NLP infusion using osmotic mini-pumps increased plasma testosterone levels in male mice. Based on these results, we conclude that NLP is a reproductive hormone stimulatory peptide in mice.

Developmental Rate, Mitochondrial Function and Mitonuclear Interactions in Advanced-Generation Hybrids After Selection for Fast Development

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Abstract: Genetic incompatibilities in hybrid organisms can produce physiological dysfunction and loss of fitness. Interactions between genes encoded by the mitochondrial and nuclear genomes may play disproportionate roles in these effects, as poor mitochondrial performance can impair metabolic rate and other biological rates with likely links to fitness. The extent of these mitonuclear incompatibilities varies among hybrid individuals resulting in selection to maintain compatible genotypes, and providing the opportunity for performance and fitness to recover over successive generations of hybridization. However, the physiological and genetic mechanisms underlying these incompatibilities, and the recovery of performance in advanced-generation hybrids, are generally unknown. To investigate these mechanisms, we generated reciprocal hybrids of the intertidal copepod *Tigriopus californicus* between populations from San Diego and Santa Cruz, California. After fifteen generations, we assessed nuclear allele frequencies using Pool-seq, created six independent lines of each reciprocal cross, and scored developmental time to metamorphosis. Overall, we found patterns consistent with fitness recovery: developmental rates were relatively high compared to previous work in early-generation *T. californicus* hybrids, and allelic variation tended to be biased such that, between the crosses, proportionally more of the nuclear genome came from the same population as the mitochondrial DNA. We conducted four additional generations of hybridization, and selected for fast development in half of the lines. Developmental rate did not respond to this selection, and changes in allele frequencies were complex, suggesting impacts of both mitonuclear interactions and nuclear-only mechanisms. However, in both reciprocal crosses, the ATP synthesis rates of mitochondria isolated from selected lines were higher than those of mitochondria from unselected lines. Taken together, our study suggests that improved compatibility of mitonuclear interactions is a key aspect of performance and fitness recovery in advanced-generation hybrids, and that selection for phenotypes consistent with high fitness may result in improved mitochondrial performance and efficiency.

Pathogen Evolution in a Warming World

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Abstract: Recent years have highlighted that many species – including our own – sit perilously between the threats of infectious disease outbreaks and warming temperatures. Our understanding of the ecology of hosts and pathogens under warming is advancing rapidly. However, the potential of pathogens to adapt to shifts in temperature, and how this will impact on host health and the severity of disease outbreaks, remains unknown. Here, we experimentally evolved a natural bacterial pathogen (genus: *Leucobacter*) in nematode worms, to test how changes in average and extreme temperature may drive pathogen virulence and fitness. We found that pathogen evolution – at the phenotypic and genetic scale – can be profoundly shaped by the temperature experienced during infection. Cool and optimal temperatures selected for the pathogen to become substantially more virulent. In contrast, the pathogen's ability to evolve increased virulence was eliminated under warm temperatures and under regular heatwave conditions. Within-host pathogen proliferation also varied across evolved populations, suggesting that temperature can shape the fundamental relationship between virulence and transmission. At the genetic level, a potential trade-off has emerged between the molecular changes that underpin adaptation to warming and to infectivity. Our results demonstrate that global change will have great repercussions for the evolution of pathogens. These insights allow us to predict on the ability of climate change to shape the evolution of pathogens. Understanding the potential for pathogens to evolve is essential in mitigating future pandemics and the extinction of species in the face of global change.

The Effects of Light and Heat on the Behaviour of Potential Second Intermediate Host Snails (*Stagnicola elodes*)

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Abstract: Host manipulation is often documented for parasites that use trophic transmission. However, many parasite life cycles also require non-trophic transmission, where larval parasites must encounter and penetrate the next host in the life cycle. The role of host manipulation in non-trophic transmission is unclear despite its potential benefit to larval parasites. In echinostome trematodes, non-trophic transmission commonly occurs between gastropod first hosts and a wide variety of invertebrate second hosts (including snails). In lab-controlled conditions, these parasites manipulate snail behaviour by altering the navigational behaviour of uninfected second hosts to infected first hosts. However, the generality of host manipulation is unknown because attraction has only been demonstrated in one pair of heterospecific snails. Further, the effect of natural conditions (e.g. light and heat) on host manipulation are unknown. We hypothesized that light and heat would increase attraction between conspecifics, but only when one individual was parasitized. This result would be evidence for parasite-modified behaviour because the parasites could be more easily transmitted from

the infected to the uninfected snail. Using a Y-maze, we assessed the behaviour of uninfected *Stagnicola elodes* in response to no stimuli (negative control), vegetation (positive control), uninfected conspecifics, and echinostome-infected conspecifics (n = 30 trials per treatment). All treatments were conducted at ambient room light and temperature (21.1˚C and 165 Lux) and with increased light and heat (25.3˚C and 5500 Lux). We video recorded the behaviour of each responder for 30 minutes to measure the average distance from the stimulus, the time spent in direct contact, the time spent in each arm, and the arm first entered. We will discuss whether parasitism and light and heat have an additive effect on snail attraction and discuss the influence of host specificity. This research will broaden our understanding of the contexts in which host manipulation occurs.

Lessons Learned from Xenografting of Testis Tissue from Diverse Mammalian Donor Species into Host Mice

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Abstract: Testis function is a complex process of spermatogenesis and steroidogenesis which is difficult to be fully replicated *ex situ*, prompting the introduction of various *in vivo* and *in vitro* models. We have developed, optimized, and used testis tissue xenografting (TTX) as a model to study and manipulate testis function of diverse mammalian species using recipient mice. TTX is a procedure in which small fragments of testis tissue from practically any immature mammalian donor can be ectopically grafted under the back skin of immunodeficient mice. The grafts survive and respond to the host mouse gonadotrophins by initiating testicular development and potentially completing all stages of spermatogenesis. As a result, this TTX system efficiently uses the recipient mice as a bio-incubator for grafting experimentations from phylogenetically distant species. Thus far, we and other groups have observed complete spermatogenesis after TTX from immature donor testis tissues of a wide range of species including lab animals (*e.g.*, mouse, rat, hamster, rabbit), companion animals (*e.g.*, dog, cat), farm animals (*e.g.*, pig, goat, sheep, horse, cattle, alpaca), and even wildlife species (*e.g.*, ferret, monkey, deer, bison). Moreover, viable progenies have been produced using the sperm extracted from such testis grafts (*i.e.*, mice, rabbits, pigs). Importantly, with every new species, we have learned new lessons about the comparative physiology of the testis. For instance, grafts from some farm animals (*e.g.*, pig, goat, sheep) grow faster and show a higher percentage of fully formed sperm than from some others (*e.g.*, horse, bull). The level of compatibility and interactions between the grafts and the hypothalamic-pituitary axis of the host may explain some of the observed differences in spermatogenic efficiency after TTX. Additionally, we have learned that grafts from neonatal, immature, or younger age donors have significantly higher potential for survival and development than pubertal or mature donors. Owing to this unique animal model, our ability to study and manipulate testis function has also dramatically increased, which complements the availability of other *in vitro* and *in vivo* models in understanding the development, germ-somatic cell interactions, and mechanisms that control spermatogenesis. Moreover, these models allow experimentations that are otherwise difficult or impossible to be performed directly in the target species. Equally important, these animal models can have a wide range of experimental and potential clinical applications in fertility preservation and genetic conservation of endangered species. Therefore, the TTX model is becoming a standard method of evaluating the developmental potential of immature testis tissue of practically any mammalian donor species.

Ion Regulation Under Acidic Conditions in Zebrafish (*Danio rerio*) Lacking Functional Corticosteroid Receptors

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**Presenting authour*

Abstract: Exposure to acidic water increases diffusive ion loss in freshwater fishes. Zebrafish exposed to highly acidic (pH 4) water restore ion balance by elevating ion uptake through increases in the abundance of ion-transporting cells (ionocytes) and proteins. In larval zebrafish, cortisol acting through glucocorticoid (GR) but not mineralocorticoid receptors (MR) were implicated in initiating these compensatory responses. Specifically, pharmacological blockade or knockdown of GR in larval zebrafish prevented the compensatory increase in sodium (Na^+) uptake. The present study used CRISPR/Cas9 technology to generate GR and MR knockout (KO) lines to test the hypothesis that cortisol plays a role in the compensatory responses to pH 4 water in adult zebrafish. Whole-body Na^+ and calcium (Ca^{2+}) levels, cortisol concentrations, and the abundances of three ionocyte subtypes [H+-ATPase- rich (HR) cells, Na+-K+-ATPase-rich (NaR) cells, and Na+-Cl--cotransporter (NCC) cells] were measured in wildtype (WT), GR-KO, and MR-KO zebrafish exposed for seven days to control or pH 4 water for seven days. Despite the absence of cortisol signaling via GR, GR-KO fish tolerated pH 4 water without mortality. By capitalizing on gene editing technologies, the present study deepened our understanding of how cortisol and its receptors influence ion regulation in adult zebrafish.

Contrast-Enhanced Ultrasound Imaging of Rainbow Trout Intestinal Inflammation

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Abstract: Intestinal health is important for both pathogen protection and nutritional uptake. Chemical contaminants, dietary irritants or disease may inflame the intestine and lead to serious health consequences. Post-mortem histological analysis is the traditional method of detecting intestinal inflammation. More recently, contrast-enhanced ultrasound (CEUS) imaging has been established for use in human clinical settings as a non-invasive alternative method of assessing intestinal inflammation. By providing real-time visualization of vascular perfusion, CEUS allows quantification of changes in blood flow, which are commonly observed in inflamed and diseased tissues. Measurement of these changes can be used to assess the degree of inflammation. We induced acute inflammation of the distal intestine of pre-adult rainbow trout (~50 g) through rectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) and ethanol. After 24 h, we injected an ultrasound contrast agent (microbubbles with a lipid shell) through the caudal vein of anaesthetized rainbow trout and imaged vascular perfusion with a VEVO3100 ultrasound system. We detected a difference in vascular perfusion, calculated as peak enhancement, between control and TNBS-inflamed trout intestines. A decrease in

perfusion was observed in the TNBS-inflamed intestines, consistent with mammalian models of bowel inflammation. Ex vivo histological examination of the intestines showed significant inflammation of TNBS-treated intestines as compared to controls, including thickening of the lamina propria of the intestinal folds and infiltration by immune cells. The ability to evaluate intestinal health using minimally-invasive CEUS imaging techniques presents novel opportunities for longitudinal study and without mortality, and this technique should be applicable in a variety other internal organs. Newer, highly portable CEUS systems will allow application of this tool in diverse settings from field research to industry.

The Physiology of Torpor in Ruby-Throated Hummingbirds

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Abstract: Ruby-throated hummingbirds (*Archilochus colubris*) are seasonal long-distant migrants found in eastern North America. Hummingbirds have a small body size (2.5-5 g) and high surface-area-to-volume ratio, so maintaining their typical day-time body temperature (Tb) of ~40℃ is energetically costly. To survive cold nights, hummingbirds use torpor, a state of inactivity characterized by low T_b and metabolic rate (MR) that can be spontaneously reversed using endogenously produced heat. In mammalian models of hibernation and daily torpor, mitochondria have been implicated as a major site of metabolic suppression. In mitochondria isolated from torpid mice and Siberian hamsters, phosphorylating respiration is suppressed by as much as 30% compared to normothermic, active animals. Much less is known about mitochondrial function in avian daily torpor. The goal of my PhD is to determine how cellular and mitochondrial physiology change to facilitate metabolic suppression in hummingbirds experiencing daily torpor. I used flow-through respirometry to measure the rate of O_2 consumed and CO_2 produced by birds when ambient temperature is lowered to 10℃. Preliminary data show that the hummingbirds' MR dropped by ~90%, Tb dropped to ~10℃ and the RQ dropped from ~1 (carbohydrate metabolism) to ~0.7 (lipid metabolism). Next. I used high-resolution respirometry to measure respiration rate in isolated pectoralis (flight muscle) mitochondria. The fuel-use shift I observed on the whole-animal level was mirrored at the level of the mitochondria: oxidative phosphorylation was suppressed in torpid birds (by ~50%) when the mitochondria were fuelled with a carbohydrate-based substrate, but not when fuelled with a lipid-based substrate. These findings highlight interesting contrasts between birds and mammals and provide insight into their evolutionary paths to heterothermy.

Growth Factors Used in a Novel Testicular Tissue Culture System Improve Outcomes of *In Vitro* Spermatogenesis

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Abstract: *In vitro* spermatogenesis (IVS) has important applications including fertility preservation of prepubertal cancer patients; however, thus far, IVS has only been achieved using mouse models. To study the effects of growth factors on

the maintenance of testicular tissue integrity, germ cell numbers, and potential induction of IVS using a porcine model, we cultured small testicular fragments (~2 mg) from 1-wk-old piglets under 6 different media conditions (DMEM+10%KSR alone or supplemented with either GDNF, bFGF, SCF, EGF, or a combination of all) for 8 weeks. Overall, tissues supplemented with GDNF and bFGF had the greatest tissue (seminiferous tubule) integrity and least number of apoptotic cells. GDNF-supplemented testicular tissue had the greatest number of ggerm cells (gonocytes) per tubule, followed by bFGF-supplemented tissues. There was evidence of gradual maturation of somatic (Sertoli) cells, especially in the mixed group. Moreover, histological examination, and expression analysis of c-KIT (a marker of differentiating spermatogonia and spermatocytes) and STRA8 (a marker of the pre/meiotic stage germ cells) in all growth factor-supplemented groups confirmed the induction of IVS. Moreover, GDNF- and bFGF-supplemented tissues had greater numbers of seminiferous tubules with spermatocytes compared to others. In conclusion, overall, GDNF and bFGF supplementation better maintained the tissue integrity, gonocyte numbers, and induction of IVS over the length of culture. These findings have implications in taking us one step closer to optimizing culture conditions for achieving complete IVS using this important animal model.

Quantitative Analysis of the Morphology-function Relation in the Armour of the Basal Ray-finned Fish, *Polypterus senegalus*

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Abstract: Fish armour is composed of skeletal elements originating from the dermis of skin, such as scales, scutes, or bony plates. Over the last 460Ma fish armour has evolved into a varied arrangement of scales, bony plates and scutes across Chondrichthyes and Osteichthyes. The scales of the basal ray-finned fish, *Polypterus senegalus*, are hypothesized to possess features of both ancient (rhombic) and modern (mostly elasmoid) scales which makes this fish an interesting model to study the functional changes of fish armour over evolution. The laminate microstructure of individual fish scales has been shown to gradually dissipate energy from external to internal layers, reducing damage. How individual scales overlap and engage with each other also strengthens the overall protective capacity of the integument system, resisting deformation and even storing and releasing strain energy to accelerate swimming strokes. This study uses histological techniques to quantify the structural changes in *Polypterus* armour over ontogeny and assess how scale shape, tissue structure and overall integument morphology may alter armour function. We predicted that mature fish armour would consist of thicker scales with more compact laminate structures, greater overlap between scales and result in greater body stiffness. Preliminary data suggest that fish scales increase in chondrification and calcification as they grow, eventually becoming completely calcified. Preliminary mechanical testing of whole bodies showed that mature fish tend to have greater body stiffness compared with small fish, and that body armour contributes more to bending stiffness than to torsional stiffness. Future work to model scale overlap and resultant mechanical function will help determine how changes in scale morphology relate to swimming speed and performance across ontogeny.

Investigating the Potential Toxicity of Copper Nanoparticles on *Daphnia magna* From Agricultural Run-off

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Abstract: Many pesticides for agricultural pest management use have the potential to leach into the environment causing detrimental effects on surrounding aquatic biota. Nanoformulations or nanopesticides (e.g., copper nanoparticles (CuNPs)) are becoming increasingly popular as they are cost-effective, have improved solubility, and provide a greater surface area for application ensuring a targeted mode of pesticide delivery. While useful for reducing pests, nanoformulations exhibit novel properties with unknown effects compared to their conventional counterparts due to their reduced size. However, NPs are relatively unstable, aggregate, and fall out of solution, raising concerns about the long-term effects on aquatic organisms and their environmental deposition. We aimed to compare and contrast the already established impacts of both acute and chronic toxicity of dissolved Cu to those of CuNPs found in agricultural pesticides, as the CuNPs toxicity is currently unclear. The 48-hour lethal median concentration (LC50) of CuNPs will be tested using the freshwater crustacean *Daphnia magna*, as they represent a critical species in many food webs. The acute LC50 concentration for ionic Cu was determined to be 38.4 µg/L (95% CI 28.85-47.95). The acute CuNP LC50 was not obtained due to a lack of mortality observed in the highest CuNP concentration of 2560 µg/L, suggesting that CuNPs are less toxic than ionic Cu. Short-term acute pulsed exposures will be assessed to mimic environmental contexts, during which *D. magna* will be monitored for reproductive success. Finally, a chronic 21-day exposure (with Cu and CuNPs) will be conducted to understand the sub-lethal mechanisms of toxicity of these formulations. Understanding the toxicity of nanopesticides used in agriculture will allow us to understand the potential risk associated with NPs and allow for better regulatory limits on pesticides to protect sensitive species. This project is funded by the Alberta Conservation Association.

Using Transcriptomics to Understand the Effects of Environmental Stress on Aquatic Ectotherms

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Abstract: Environmental and anthropogenic stressors can disrupt homeostasis and negatively affect aquatic organisms, potentially leading to impacts on growth, reproduction and survival over time. The focus of the research in the Jeffries Lab is to investigate how various environmental and contaminant stressors can affect an individual's fitness through examination of responses at multiple levels of biological organization, and how these responses can potentially scale up to population-level consequences. We measure responses at the level of the transcriptome using various approaches (e.g., High-throughput qPCR, RNA-sequencing) to characterize the cellular responses of ectotherms to stressors relevant to aquatic ecosystems. The cellular-level responses are integrated with tissue or whole-organism level performance indices to gain a more comprehensive understanding of the effects of exposure to environmental stressors and to

address whether populations and species can persist in changing or disturbed aquatic environments. Advancements in the field of transcriptomics have facilitated the use of these tools on wild animals and species of conservation concern. The work in the Jeffries Lab has primarily been focused on fishes, however we have begun studying the effects of environmental stressors on bivalves in the Arctic. In recent years, we have also incorporated DNA sequencing approaches into our research to understand population structure in wild fishes. The work in the Jeffries Lab typically focuses on species that are economically important, invasive or of conservation concern and combines approaches used in the fields of physiology, ecological genomics and ecotoxicology.

A One Health Approach to Ticks and Tick-borne Diseases in People, Animals, and the Environment at an Interface Zone in Canada

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Abstract: The province of Saskatchewan represents 2 interface zones in Canada: the historical eastern limit of the Rocky Mountain Wood Tick (*Dermacentor andersoni*) and the western limit of the American dog tick (*D. variabilis*), and the western distributional limit of established populations of *Ixodes scapularis* (black-legged tick), the main vector of Lyme disease in North America. Our primary motivation for surveillance is to detect establishment of this tick in Saskatchewan. In 2018 and 2019, we conducted passive surveillance for ticks recovered from pets and people as part of a free identification program based on physical submissions. In 2020, we added a citizen science-based initiative called eTick, where people can submit digital photos of ticks for identification, which led to an increase in the number of *Ixodes* spp. detected. We also identify physical specimens of ticks submitted to the provincial human health laboratory. Following detection of *I. scapularis* via passive surveillance, we conduct reactive surveillance by dragging in the environment where the tick was acquired. We also conduct active surveillance involving standardized transects in likely habitat for *Ixodes* spp. All *I. scapularis* submitted are tested via PCR for DNA of*Borrelia* spp., *Babesia microti,* and *Anaplasma phagocytophilum*. Despite regular annual detection of *I. scapularis*through passive surveillance, active surveillance indicates that endemic populations do not yet occur in Saskatchewan. However, tick PCR and serology in coyotes and dogs suggests local transmission of *Borrelia burgdorferi*. We conclude that animals make excellent sentinels for invasive ticks and tick-borne diseases, frequent use of environments by dogs may alter the abundance and diversity of ticks, and adventitial ticks regularly introduce*Borrelia* spp. (and other pathogens) into Saskatchewan. This One Health approach can be used to develop models, necessary to predict and mitigate risks of emerging ticks and tick-borne diseases in a rapidly changing environment.

2D to 3D: Exploring Variation of Niche Metrics Across Consumers in a Coastal Arctic Ecosystem and Implications on Interpretation

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Abstract: Stable isotopes have been used extensively to study ecological niches of consumers across ecosystems, but have typically been analyzed using only two dimensions (2D; $\delta^{13}C$ and $\delta^{15}N$) despite the ecological niche being an n-dimensional hypervolume. By adding more dimensions to a niche model we can better quantify the realized niche of a consumer, which is the actual set of conditions an individual utilizes when competition and predation are taken into account. This will help better differentiate the ecological roles of each species within the system. We compared 2D ($\delta^{13}C$ and $\delta^{15}N$) versus three dimension (3D; $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) niche characteristics among invertebrates, fish, seabirds and marine mammals in an Arctic coastal system across a wide range of trophic positions and functional groups. All species inhabit the coastal waters of Southampton Island, Nunavut in summer and were collected from 2016-2019. We calculated 2D and 3D niche size along with overlap at the species level where the two dimensional niche sizes ranged from 1.30‰ – 54.76‰ for nudibranchs and sea stars respectively. All niche sizes increased when $\delta^{34}S$ was added and ranged from 3.09‰ – 249.12‰ for murres and sea stars respectively. The increase in niche size was highly variable between species ranging from in 0.23‰ – 194.36‰. Niche overlap between species mostly decreased (74%) when $\delta^{34}S$ was added to the model, while the niche overlap increased in a few species (12%) or had no change (14%). The magnitude of change in niche overlap varied greatly, with the largest increase in niche overlap being 12.6% and the largest decrease in niche overlap being 93.87%. Overall using a three dimensional niche model provides a better estimate of species' realized niche and ecological role within that system. This is key to understand how a species may be impacted by environmental shifts occurring in the Arctic Ocean.

Naked Mole-Rats and Mice Exhibit Varying Sickness Behaviour and Microglia Activation Patterns Following Acute Hypoxia and LPS Exposure

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Abstract: Mounting an immune response is energetically expensive but hypoxia compromises aerobic energy production. Hypoxia-tolerant organisms, which typically minimize energy demand in hypoxia, must therefore make

trade-offs between hypometabolism and immune competency in hypoxia. We hypothesized that the immune response of hypoxia-tolerant naked mole-rats (NMRs) and hypoxia-intolerant mice to bacterial challenge (e.g., lipopolysaccharide, LPS) is differently impacted by hypoxia. To test this, we treated male NMRs and CD-1 mice with phosphate buffered saline (sham) or LPS (1.5 mg/kg) injections in either hypoxic (11% O_2) or normoxic (21% O_2) conditions. We scored sickness behaviours as a non-invasive measure of immune system activation and then sacrificed animals to evaluate microglia activation using immunohistochemistry. We found that NMRs and mice both exhibited sickness behaviours following LPS and hypoxia exposure, but NMRs had a longer latency in hypoxia. In NMR brain, LPS and hypoxia treatments both induced microglia proliferation after 24h, but without an additive effect, whereas these changes were reversed at 48h, such that NMR microglia were more activated in the double treatment at this timepoint. Interestingly, no differences were observed in breeding males. Mice generally had higher cell counts at all time points, however, the number of microglia in the LPS+hypoxia group decreased drastically from 2 to 48h. These results suggest that hypoxia activates an immune response in NMRs, like mice, and does not impair immune responsiveness to a bacterial challenge.

Chitosan Nanoparticles Containing Spirulina Protein Hydrolysate Ameliorate Hepatic Lipid Metabolism and Altered Redox Homeostasis in Non-alcoholic Fatty Liver Disease in Rats

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Abstract: Marine bioactive peptides have attracted much attention due to their role in metabolic pathways and potential application to treat metabolic diseases such as obesity, hypertension and hyperlipidemia. Spirulina platensis protein hydrolysate (SPH) has been found to exhibit anti-obesity action. This research aimed to develop a novel oral formula of spirulina platensis protein hydrolysate-loaded chitosan nanoparticles (SPH-CSNPs), and determine whether SPH-CSNPs can inhibit nonalcoholic fatty liver disease (NAFLD). The nanoparticles with high stability, bioavailability, and favorable efficiency were synthesized, and the cytotoxicity study confirmed the safety of the formula at low and high doses. The results demonstrate that the diet supplementation of SPH-CSNPs efficiently suppressed the increased weights in body and livers of high-fat diet-fed rats. The hepatic lipid accumulation was significantly decreased in treatment groups, which were attributed to decreased expressions of lipogenic genes (SREBP1c, ACC1, AMPK and FAS), and increased expressions of fatty oxidation-related genes (PPAR α and CPT1). Additionally, SPH-CSNPs downregulated the proinflammatory cytokines (TNF-α, IL-6) genes. Administration of SPH-CSNPs upregulated the expression of antioxidant enzymes in liver, and reduced biomarkers of oxidative damage (AOPP, AGE, MDA and PCO) in hyperlipidaemic rats. In conclusion, SPH nanoformulations might exert attenuation effects through activation of PPARα pathway and provides protection against redox imbalance in NAFLD in rats.

Detection of Southern Ontario Amphibians Using Environmental DNA Barcoding and Comparison to Conventional Methodologies

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Abstract: Amphibians are a globally declining taxa, warranting the need for effective species monitoring and conservation. Environmental DNA (eDNA) barcoding is an emerging, non-invasive, method for species monitoring by detecting unique sequences of nucleic acid, specific to a target species, from environmental samples and removes the need for direct observations of taxa. Our research aims to determine if eDNA barcoding is a sensitive method of detection for six amphibian species (wood frog, spotted salamander, green frog, northern leopard frog, American toad, spring peeper) in vernal pools in Southern Ontario compared to conventional detection methods (auditory and visual surveys). We hypothesize that eDNA barcoding will provide equal or greater accuracy for detection of the target amphibians, compared to conventional survey methods. From April to July 2019, conventional surveys and eDNA collections were conducted in collaboration with *rare* Charitable Research Reserve, Cambridge, ON. Conventional detection methods included collection of daily auditory data using acoustic song meters and weekly to biweekly auditory and visual surveys according to the amphibian marsh monitoring protocol. Audio data was analyzed using advanced cluster analysis in Kaleidoscope Pro and remaining conventional data was tallied. Alongside conventional surveys, duplicate water samples containing eDNA were collected at multiple sampling locations around the periphery of three vernal pools. After water collection, eDNA was concentrated by filtration, extracted, and quality controlled. Species-specific eDNA barcoding assays were optimized for species detection using quantitative PCR and detections of target species in eDNA samples is currently underway. Each survey method (eDNA/auditory/visual) will be assessed for overall ability to detect species presence over a spatiotemporal scale. Results will provide evidence needed to evaluate the ability of eDNA detection methods to enhance ongoing amphibian monitoring by end users in academia, government, and private industry. [Funded: CFREF-GWF]

Batrachochytrium dendrobatidis Cell-free Supernatants Affect the Survival and Cellular Immune Responses of Amphibian Skin Epithelial-like Cell Lines

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Abstract: *Batrachochytrium dendrobatidis* (Bd) is a pathogenic fungus that infects amphibian skin tissue, causing the often-fatal disease chytridiomycosis. Previous studies have revealed that cell-free supernatants (CFSs) of Bd, or specific

metabolites present in Bd CFSs, may contribute to its ability to evade host immune responses though lymphocyte inhibition. Our preliminary data suggests similar effects of Bd CFSs on a frog renal epithelial cell line. Since amphibian skin is the main tissue targeted by Bd, we sought to uncover whether Bd CFSs alter the viability and immune gene regulatory responses of frog skin epithelial cells using two *Xenopus laevis* skin epithelial-like cell lines, Xela DS2 and Xela VS2. We first demonstrate that Bd CFSs inhibit survival of these skin cell lines, as do a subset of the metabolites present in Bd CFSs. To determine whether Bd CFSs may alter immune gene regulation, we co-treated Xela DS2 or Xela VS2 cells with Bd CFSs and the immune stimulant zymosan and examined the levels of immune gene transcripts. Co-treatment of these cells with Bd CFSs and zymosan resulted in reduced proinflammatory immune gene transcript levels relative to cells treated with zymosan alone. This trend persisted when cells were pre-treated with Bd CFSs followed by zymosan treatment, or pre-treated with zymosan followed by Bd CFS treatment, suggesting that Bd CFSs can prevent the establishment of, or turn off, proinflammatory gene signatures, respectively. However, treatment with specific metabolites found in Bd CFSs did not mimic the effects of Bd CFSs on immune gene transcript levels, suggesting that molecules other than the metabolites examined may be involved in the immunomodulating effects of Bd CFSs. These findings suggest that Bd CFSs can alter immune gene expression in amphibian skin cell lines, which may underly its pathogenic mechanisms *in vivo*. [Funding: NSERC RPGIN-2017-04218 to BAK; NSF 2147467 to LAR-S]

Small GTPases Regulate Pituitary Cell Functions in Goldfish *(Carassius auratus)*

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Abstract: The vertebrate pituitary gland is an important tissue where hormonal regulators of multiple physiological functions are produced and secreted. For example, luteinizing hormone (LH) regulates processes of sexual maturation and growth hormone (GH) regulates somatic growth and metabolism. Pituitary cell types, such as LH-secreting gonadotrophs and GH-secreting somatotrophs are often regulated by multiple hypothalamic factors, which, while generally activating unique receptor systems, ultimately propagate signals through conserved intracellular regulatory elements to influence hormone secretion. One important family of intracellular regulators is the monomeric small GTPases, a subset of which (Arf1/6, Rac, RhoA, and Ras) has highly conserved cellular functions across vertebrates including the control of secretory vesicle exocytosis in many cell types across biological systems. However, the roles of these effectors in neuroendocrine systems, especially in basal vertebrate models, have been under-studied. Here, we utilize the well-characterized goldfish (*Carassius auratus*) neuroendocrine model to investigate the roles of these small GTPases in basal and agonist-dependent hormone release from dispersed goldfish pituitary cells in column perifusion experiments. Inhibition of these small GTPases rapidly elevated basal LH and GH secretion, except for Ras inhibition which selectively increased basal LH release. However, variable effects were observed with regard to acute LH and GH secretion responses to the two goldfish native gonadotropin-releasing hormones (GnRH2 and GnRH3). GnRH-dependent LH release, but not GH secretion, was mediated in part by Arf1/6 GTPases. In contrast, inhibition of Rac and RhoA GTPases selectively enhanced GnRH3- and GnRH2-dependent GH release, respectively, while Ras inhibition only enhanced GnRH3-evoked LH secretion. Together, our results reveal novel divergent cell-type- and ligand-specific roles

for small GTPases in the control of goldfish pituitary hormone exocytosis in both unstimulated and GnRH-evoked release. (Supported by grants from NSERC and Faculty of Science, University of Alberta.)

Effect of Simulated Ocean Acidification on Olfaction in the Coastal Yellow Shore Crab

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Abstract: Coastal upwelling events bring up deeper high carbon dioxide (CO_2) waters to the surface, making coast lines particularly variable in CO_2. Additionally, atmospheric CO_2 is absorbed by our oceans, leading to ocean acidification with end-of-century levels predicted to increase to three times current day levels. Therefore, coastal areas are already more variable environment, and will become even more so in the future. Ocean acidification has been shown to alter olfactory behaviours such as foraging and predator sensing in crustaceans. However, data on coastal species that already experience fluctuating and higher CO_2 levels, is scarce. Yellow shore crabs, *Hemigrapsus oregonensis*, a species found on the Pacific coast, were exposed for 14-days to control (~700µatm, pH = 8.0; current CO_2 levels), medium (~1500µatm, pH = 7.7, current upwelling events), and high (~3000µatm, pH = 7.1, future upwelling events) CO_2 levels. After their behaviour was analyzed including baseline locomotion, odorant preference, and time to locate odorant source with an attractive food cue, putrescine (butane-1,4-diamine). Crabs exposed to medium and high CO_2 levels took three times longer to locate the odorant source when compared to control crabs. Similarly, crabs exposed to medium and high CO_2 levels took longer to indicate preference to the odorant side of the behaviour tank. There were no significant differences in baseline locomotion, thereby indicating this is an impairment in olfaction rather than a result of changes in metabolism. Changes in ability to locate odorant sources as a result of ocean acidification may potentially impact the ability of coastal crab species to find food and sense their environment as a whole. Current upwelling events demonstrated olfactory impairment indicating lack of adaptation to current levels and with upwelling events predicted to increase in length and severity, this impairment will likely worsen.

Chasing the Red Queen

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Abstract: Hosts and parasites can continuously and reciprocally evolve, locked in a process of constant change. These coevolutionary interactions are named after the Red Queen, who pointed out to Lewis Carroll's Alice the need to 'run to stay in the same place'. I will talk about the development of my career-long fascination with the Red Queen, and how my group has chased these interactions in the lab using experimental evolution.

Mechanism of Selenite Uptake in the Water Flea, *Daphnia magna*

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Abstract: Coal mining activity is one of the major sources of the toxicant selenium to aquatic systems. In the province of Alberta, the potential toxicological impacts of aquatic selenium is of renewed interest with the proposed development of 14 new mining permits being issued along the North Saskatchewan River. While the major route of selenium uptake to macroinvertebrates is via the diet, the uptake of selenite ($HSeO_3^-$), the most prominent form in waters at circumneutral pH, can be an important contributor to selenium body burden, and in turn is relevant to trophic transfer of selenium and thus toxicity to fish. In the current study, radiolabeled selenite (Se^{75}) was used to characterize selenium uptake in the water flea, *Daphnia magna*. By analogy with selenite in plants and algae, we hypothesized that selenite uptake would occur via an anion transporter. The concentration-dependence (1 to 32 µM) of selenite uptake was determined in one-hour uptake assays in artificials waters that independently varied in bicarbonate, sulfate, selenate, chloride, and phosphate concentrations. A finding of phosphate-dependence coupled with inhibition of uptake with a phosphate transporter inhibitor (foscarnet) indicated that selenite uptake is likely mediated by a phosphate transporter at low concentrations. At higher concentrations, selenite uptake is bicarbonate-dependent although this effect may be indirect as it was not inhibited by a bicarbonate transporter inhibitor (4,4'-Diisothiocyano-2,2'-stilbenedisulfonic acid; DIDS). These findings suggest that concentrations of phosphate and bicarbonate in coal mining affected waters could potentially alter selenite toxicity to aquatic organisms.

Wide Thermal Tolerance of Invasive Sea Lamprey: Past and Future Implications for The Great Lakes

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Abstract: Parasitism by invasive sea lamprey (*Petromyzon marinus*) contributed to declines in Great Lakes fisheries during the 1900s. Their ability to osmoregulate in freshwater for their entire life cycle and their generalist diet are thought to be key factors that allowed them to successfully occupy the Great Lakes. However, less is known about the thermal physiology of sea lamprey, which spend their first 3-7 years burrowed in the substrate of streams as filter-feeding larvae before metamorphosing into free-swimming, parasitic juveniles (that spend 12-20-months in the lakes). To test the hypothesis that larvae have greater thermal tolerance than juvenile sea lamprey, thermal performance tests were completed to determine how metabolic rate varies with temperature. Absolute metabolic scope (AMS), the difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR), was determined by measuring oxygen consumption ($\dot{M}O_2$) at 6-8 different temperatures for larval and juvenile sea lamprey (6-31°C, and 5-25°C respectively) using intermittent-flow respirometry. SMR was generally lower in larvae, but in both cases increased with temperature. SMR increased at higher rates in juveniles, suggesting juveniles were more sensitive to increasing temperatures.

Between 10-20°C, Q10 averaged 1.75 for larvae compared to 2.79 in juveniles. Between 21-25°C and 25-31°C, larval Q10 increased to 2.03 and 3.81, whereas for juveniles, Q10 dropped to 1.74 between 20-25°C. AMS of larvae increased from 6.6 to 10.9 μmol O2 $g^{-1}h^{-1}$ between 10-25°C and peaked at 15.8 μmol O2 $g^{-1}h^{-1}$, with a corresponding T_{opt} of 26.6°C. In juveniles, AMS was stable from 10-20°C, averaging 10-11 μmol O2 $g^{-1}h^{-1}$, before increasing to 14.3 μmol O2 $g^{-1}h^{-1}$ at 25°C. Based on their lower Q10 and more robust thermal performance curve, we conclude that larvae are more tolerant to increasing temperatures than juveniles, which could have implications on juvenile survivability as juveniles live in colder lakes during this life stage.

Transcriptomic Response and Gill Microbial Community Changes of Dominant Fish Species Under Steep Abiotic Gradients in one of Europe's Largest Estuarine Habitats

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**Presenting Autor*

Abstract: Holistic transcriptome studies provide a mechanistic understanding of an organism's response to changing environmental conditions. The combination with physiological and ecological research on wild fish has the potential to elucidate factors determining resilience and is increasingly used in the framework of conservation biology. This study focusses on the linkage of biotic and abiotic factors with gene expression patterns in dominant fish species (smelt, ruffe, zander) along the spatiotemporal gradients of tidal Elbe estuary. To account for tissue specificity of transcriptomic adjustments, we consider liver as a central metabolic organ and gills with respiratory, excretory, and immunological functions. Gills require a high connectivity between the surrounding water and the animal's bloodstream, making them an important potential entry point for pathogens. We study the interaction of the host and its gill microbiome and link this information to various physiological measurements. The aim is to show possible local adaptations of fish of different trophic levels and life history guilds and draw conclusions about factors influencing fish health in the heavily anthropogenically influenced Elbe estuary. For this purpose, fish were caught with a stow-net vessel along the tidal Elbe between 2021 and 2022, tissue samples and swabs were immediately collected on board. We use RNAseq along with 16S-rRNA metabarcoding, shotgun metagenomics, LC-MS3 for long-term scale cortisol level determination together with further physiological measurements. We found spatial and temporal clustering in tissue transcriptomes accompanied by clustering in bacterial composition with pronounced differences between fish species. Stress response pathways are identified from differentially expressed genes and compared between species and seasons. Distribution, function and

possible pathogenicity of bacterial taxa are inferred by means of metagenome analyses. Multiomics techniques are used to show the interplay of bacterial mucus community and the host in response to changing abiotic factors, local adaptations as well as impacts on fish health.

Shining a Light on Bat Activity: Artificial Light Increases Foraging Activity in Red Bats (*Lasiurus borealis*) in Saskatchewan

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**Presenting Author*

Abstract: Artificial Light at Night (ALAN) poses a global threat to biodiversity, particularly for nocturnal and migratory species. Artificial Light at Night (ALAN) can create problems with migration, hibernation, feeding activities, and lead to increased predation risks for many species. The effects of ALAN on bat species has been studied worldwide and is often seen to have detrimental consequences on these nocturnal animals. It has previously been shown that bats emergence time relates to their prey availability and predation risk. However, with the development of ALAN, this creates a new and easier way to access phototactic prey species at night for nocturnal insectivores. For this study I hypothesized that, while ALAN has many negative effects on nocturnal species, bats will use ALAN sources to their advantage due to the higher abundance of insects around them. My results showed a significant difference in activity with detectors placed near light sources compared to detectors away from light sources. Species composition indicates that the red bat (*Lasiurus borealis*) was the main contributor to this exploitation of ALAN for energetic gain. These results show that while red bats are willing to exploit artificial light, other species may be experiencing negative side effects from ALAN being introduced to their habitats. The adverse impacts of ALAN on nocturnal species needs to be continually studied and solutions must be proposed to save insectivorous bats and other species affected by artificial light.

Impacts of Pathogen Infection on the Winter Behaviour and Physiology of the Black-legged Tick, *Ixodes scapularis*

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*Presenting Author

Abstract: Since the early 1990s, the range of the black-legged tick (*Ixodes scapularis*) has expanded in Canada, increasing the risk of tick-borne pathogen infection in humans (e.g. Lyme disease). One factor that may contribute to the ability of *I. scapularis* to invade Canada is physiological and behavioural changes caused by pathogens that help the ticks survive the Canadian winter. Recent research indicates that *I. scapularis* infected with pathogens (e.g. the causative agent of Lyme disease, *Borrelia burgdorferi*) can survive the winter season better than uninfected *I. scapularis*. The mechanism through which this occurs is largely unknown but may be linked to changes in activity and cold tolerance. To determine how pathogen infection alters *I. scapularis* behaviour and physiology in the winter, we are comparing patterns of winter

activity and gene expression between infected and uninfected *I. scapularis.* We maintained wild-caught, male and female adult *I. scapularis* collected in Nova Scotia in an outdoor enclosure for 5.5 weeks in November-December 2022, and monitored their activity using TriKinetics Locomotor Activity Monitors (LAMs). After this period, we recorded tick survival and preserved the ticks at -80°C for subsequent DNA and RNA extraction. We will test the ticks for four of the most prevalent pathogens in the Atlantic provinces, including *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Borrelia miyamotoi*, and *Babesia microti*. We will also examine the expression of three target genes related to cold-tolerance and host-seeking behaviour in the ticks including: (1) a putative antifreeze glycoprotein (*iafgp*); (2) glycerol-3-phosphate dehydrogenase (*gpdh*), and; (3) heat-shock protein 70 (*hsp70*). Our research aims to discover why pathogen infection improves the ability of infected *I. scapularis* to survive winter conditions, which has critical implications for tick-borne disease transmission. Overall, this work will help to understand the drivers of northern range expansion and the future of tick-borne diseases in Canada.

Coho Salmon (*Oncorhynchus kisutch*) Females With High Circulating Cortisol Levels Have Reduced Fitness

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**Presenting Author*

Abstract: High baseline corticosteroid levels during life history transitions is common in vertebrates and viewed as an adaptive response to mobilize energy stores to fuel enhanced metabolic demands. Semelparous Pacific salmon have high circulating cortisol during their upstream spawning migration and encounter additional stressors along the way. Cortisol plays an integral role during oogenesis and early development. In excess, cortisol has the capacity to impair oocyte development in maturing adults and alter the developmental trajectory of their offspring. However, it is unknown whether this occurs in wild Pacific salmon where there is natural variation in baseline cortisol levels among females. Here we collected wild female coho salmon (*Oncorhynchus kisutch*) from the Interior Fraser Watershed, BC Canada, to test whether the reproductive success of female coho salmon decreased when plasma cortisol increased. Furthermore, we determined whether high maternally derived cortisol in the eggs would negatively impact the growth and stress performance of the offspring. We found that females maintained their investment in egg size, but egg number decreased with increasing plasma cortisol in larger females. Females with high plasma cortisol deposited more cortisol in their eggs and there was a shift in egg nutrient composition. The eggs of each female were fertilized and reared separately in vertical flow stacks until the onset of exogenous feeding. Survival to the eyed stage decreased with increasing maternally derived egg cortisol. At the fry stage, condition factor decreased, and the magnitude of the stress response increased as their exposure to maternally derived cortisol in the egg increased. Our results demonstrate that there are fitness consequences for female coho salmon with high plasma cortisol. Moreover, the offspring exposed to high maternally derived cortisol in the egg had reduced survival, a greater capacity for energy substrate breakdown coupled with lower condition factor, and a hyperresponsiveness to stressors.

Mechanisms of diapause and cold tolerance in the Colorado potato beetle

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Many temperate insects enter diapause (a state of dormancy) and enhance their cold tolerance to survive the winter. During diapause, Colorado potato beetles (CPB) stop developing, lower their metabolism, and change their physiology to avoid freezing. The extent to which diapause confers cold tolerance in CPB, and other overwintering insects, is currently unknown. In my PhD, I used CPB to improve our understanding of the mechanisms underlying metabolic suppression during diapause and cellular protection at sub-zero temperatures in insects. First, I used RNA-seq to compare gene expression in fat body and flight muscle of diapausing and non-diapausing CPB to explore how insect diapause is modulated by tissue-specific processes. During diapause, fat body played a larger role in driving hypoxia- and immune-related processes, whereas processes mediating proteostasis and mitochondrial metabolism were more important in the flight muscle. Given the importance of mitochondrial metabolism-related processes in the flight muscle, I next tested the hypothesis that flight muscle mitochondria modulate metabolic suppression during diapause in CPB. Indeed, metabolic suppression during diapause in CPB is driven, in part, by Parkin-mediated mitophagy and thus degradation of flight muscle mitochondria. Further, CPB reverse this mitophagy and activate mitochondrial biogenesis in anticipation of their emergence from diapause, which leads to the re-establishment of their metabolic rate. Finally, I used RNA-seq to explore the mechanisms underlying cold tolerance in diapausing CPB and found that cold-tolerant beetles activate the chaperone response to a greater extent than less cold-tolerant diapausing counterparts, and that this enhanced chaperone response leads to a greater capacity for chaperone-mediated protein repair. Together, these studies improve our understanding of the physiological and cellular mechanisms insects use to survive the winter.

Investigating Thermogenic Mechanisms in Honeybees

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Abstract: In recent years, an important decline of the honeybee's populations is observed, due in part to climate change. The colony's transition between summer and winter is crucial to its survival but is not well understood. We have recently demonstrated that honeybees' mitochondria undergo drastic changes during winter: winter bees have lower CI-linked respiration but increased mtG3PDH- and CII-linked respiration. These results suggest that ATP production might be lower during winter and therefore costly shivering thermogenesis might not be the only mechanism used to produce heat in honeybees. We hypothesize that the increased use of G3P could activate uncoupling proteins to produce heat, as G3P has been shown to uncouple bumble bee mitochondria. In this study we investigate thermogenesis mechanisms and mitochondrial reprogramming in honeybees (*Apis mellifera*) exposed to low temperatures and fed with reserpine (to inhibit thermogenesis). Specifically, UCP activation will be determined by measuring oxygen consumption, with G3P as a substrate, and adding UCPs inhibitor (GDP). We will also measure ATP production by spectrophotometry to determine G3P contribution to ATP production in isolated mitochondria. After optimisation of isolation method and respiration

protocol, we found an interesting effect of cytochrome C on respiration that is specific to honeybees. Preliminary results show that cytochrome C increases respiration, normally indicating a loss of outer mitochondrial membrane integrity. However, detection of mitochondria incubated with TMRM (a marker of membrane potential) by flow cytometry showed that mitochondria were polarized. We also optimized a new assay to determine ATP production rates in fresh isolated mitochondria to evaluate the contribution of each substrate to ATP production. Thus, this study will provide new knowledge about thermogenic mechanisms in honeybees, which is vital in addressing the decline of honeybee population due to climate change.

Lactate Sensing by Neuroepithelial Cells Isolated From the Gills of Killifish (*Fundulus heteroclitus*)

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Abstract: Lactate, a by-product of anaerobic metabolism, is gaining more attention as a signalling molecule in respiratory control in both the mammalian carotid bodies and fish gills. Indeed, circulating lactate can stimulate ventilation in air- and water-breathing vertebrates. Recent evidence suggests lactate acts on O_2- and CO_2/H^+-sensitive chemoreceptors located in the mammalian carotid body. While analogous receptors (neuroepithelial cells or NECs) in fish gills are presumed to also function as lactate sensors, direct evidence is lacking. Previous research in the field has shown that circulatory lactate stimulates cardiorespiratory reflexes in water-breathers, e.g. teleost fish (catfish and rainbow trout) and denervation or removal of the first gill arch inhibited lactate sensing. This suggests a branchial location for the sensors, however, both the cellular and sub-cellular mechanisms are unknown. Here, using ratiometric Fura-2 Ca^{2+} imaging, we show that chemosensitive NECs isolated from killifish gills respond to lactate (5–10 mmol/L; pHe ~7.8) with intracellular Ca^{2+} elevations. These responses were inhibited by an L-type Ca^{2+} channel blocker (nifedipine; 0.5 µmol/L), a monocarboxylic acid transporter (MCT) blocker (α-cyano-4-hydroxycinnamate; 300 µmol/L) or a competitive MCT substrate (pyruvate; 5 mmol/L). These data provide the first direct evidence that gill NECs act as lactate sensors.

A Transcriptomic- and Metabolomic-level Investigation of Ant Brains Infected with the Larvae of the Iconic Manipulator *Dicrocoelium dendriticum*

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Abstract: Wood ants infected with larvae of the lancet liver fluke, *Dicrocoelium dendriticum*, leave their nests in the cool hours of the afternoon, ascend a herbaceous plant, and attach themselves to a flower petal with their mandibles. They remain attached to the plant overnight, then detach the following morning as temperatures rise. They repeat this bizarre

attach/detach sequence throughout the summer. Uninfected ants from the same nest do not engage in these odd behaviors. During the manipulation, ants do not defend themselves from predators, and do not perform typical worker duties for their queens. We seek to understand the mechanisms that underlie this complex host manipulation. Using transcriptomic and metabolomic approaches, we compared gene expression patterns and biogenic monoamine concentrations in the brains of infected and uninfected *Formica aserva* collected in southern Alberta, Canada. With a lab set-up, we recreated the manipulation cycle to mimic pre-attached, attached, and post-attached stages of the manipulation, and sampled ant brains from each stage. We found a total of 4062 transcripts that were differentially expressed between infected and uninfected ants, including those involved in environmental sensing (odorant, vision, gustatory), circadian rhythm, immune response, muscle contraction, the production of biogenic monoamines, and hormones. Genes involved in odorant and vision were downregulated in attached infected ants. Vision genes were upregulated in post-attached infected ants. Genes involved in serotonin synthesis were also downregulated during the post-attached stage in infected ants. These results paralleled results of our neurotransmitter concentrations: mean brain concentrations of tyramine and serotonin were 22% and 17%, lower respectively, in infected ants compared to uninfected ants during the detachment phase. These reductions paralleled a 40% reduction in overall host activity in infected versus uninfected ants during the detachment phase. Overall, this study helps us better understand the complex parasitic manipulation in the *D. dendriticum*–ant system.

Migration in the Spotlight: A Comparative Investigation of Behavioural Responses to Artificial Light at Night in Nocturnal Migrant and Nonmigratory Songbirds

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Abstract: Artificial light at night (ALAN) is a rapidly growing pollutant as it currently outpaces the growth of its leading cause, urbanization. Varying levels of ALAN generate a wide range of responses, physiological and behavioural, reported in a daily growing number of species. Nocturnal migratory songbirds may be particularly vulnerable given their long-distance migration which increases their exposure risk to ALAN during a potentially sensitive window. We hypothesized that increasing levels of ALAN may alter migratory behaviour and energetics of foraging and flight activity in both nocturnal migrant and to a lesser extent in nonmigrant birds. Here, we used a captive experimental study design across three seasons to compare the activity and behaviour of two nocturnal migrant species, Gambel's white-crowned (*Zonotrichia leucophrys gambelli*) and White-throated sparrows (*Z. albicolis*), and a nonmigrant species, the House sparrow (*Passer domesticus*). Birds were video recorded in captive trials conducted in spring, summer, and autumn seasons, testing a range of ALAN intensities (0.15, 0.5, 1.5 and 10 lux) relative to a dark control treatment (< 0.01 lux). ALAN had little impact on House sparrow activity, which remained largely dormant at night, and did not change their behaviour. In migratory sparrows, we found nocturnal activity increased in response to increasing ALAN, along with changes in observed patterns of migratory restless behaviours (beak-up and beak-up-flight). Migratory behaviour had a threshold response, where increasing ALAN stimulated beak-up and beak-up-flight, but the highest intensity diminished it, suggesting 10 lux may be a threshold for behavioural compensation. This suggests migratory songbird behaviour

shifts in response to changes in perceived photoperiod, an established consequence of ALAN. We speculate changes to perceived photoperiod interfere with the circadian mechanisms that regulate migratory activity and behaviour. With effects apparent, ALAN may have unknown consequences on migration success, individual survival, and fitness in the wild.

Physiology and Transcriptomics Revealed Putative Local Adaptation to Fresh Water in Prickly Sculpin (*Cottus asper*)

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Abstract: Our study aims to identify the molecular and physiological adaptations that are associated with freshwater colonization by fish with marine ancestors. To do so, we integrated transcriptomics and functional enzyme analyses to study intraspecific variation in osmoregulation among populations of prickly sculpin (*Cottus asper*) from different habitats that had been acclimated to fresh water or sea water. Prickly sculpin are euryhaline freshwater fish with marine ancestors and they are broadly distributed across British Columbia (BC). We collected prickly sculpin from three distinct habitat types that differ in their degree and relative time of isolation from the marine environment, including coastal rivers with access to estuaries, isolated coastal lakes, and lakes from the interior of BC which contain populations that have likely resided in freshwater the longest. Analysis of gill transcriptomics revealed that salinity and habitat had effects on gill gene expression profiles. In addition, there was a significant interaction between salinity and habitat, whereby genes associated with gill ion regulation (e.g. carbonic anhydrase XVb, solute carrier family 12 member 2, and Na^+/K^+-transporter) responded to salinity differently across fish from the different habitats. Our physiological analysis further suggests that prickly sculpin from the interior lakes had the greatest freshwater osmoregulatory ability as evidenced by higher gill Na^+/K^+-ATPase and H^+-ATPase activities in ion-poor water compared with fish from the other habitats. In contrast, coastal river populations showed the greatest seawater osmoregulatory ability as indicated by the high gill and anterior intestine Na^+/K^+-ATPase activity as well as greater intestinal precipitate formation, whereas fish from the interior lakes had the lowest seawater osmoregulatory ability. Together, our study provides new insights into mechanisms underlying the post-glacial freshwater radiation in prickly sculpin and other fishes that have colonized fresh water.

Elucidating the Impact of Thyroid Hormones on Heart Regeneration

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Abstract: Thyroid hormones are commonly known to help regulate metabolism in mammals. However, recent studies have shown that thyroid hormones are involved in many processes and their roles differ depending on the species and

system of interest. Previous studies have suggested that greater levels of thyroid hormones in mammals compared to heart regenerating species may play a role in preventing successful heart regeneration. However, exactly how thyroid hormones influence the capacity of the heart to regenerate remains unclear. In this study, it was predicted that thyroid hormones alter the production of collagen increasing heart stiffness and ultimately preventing the proliferation of cardiomyocytes following damage. To determine how thyroid hormones influence collagen production in the heart, rainbow trout (*Oncorhynchus mykiss*) cardiac fibroblasts were exposed to one of three thyroid hormone triiodothyronine (T_3) *in vitro* for x weeks and then the expression levels of collagen related genes including *col1a1, mmp2, mmp9 and timp2* were quantified. Results show no change in expression. To determine whether T_3 influences heart stiffness, zebrafish (*Danio rerio*) were exposed to T_3 at a concentration of 5nM for two weeks. The hearts of these animals were then damaged using cryoinjury and collected at 3-, 7- and 14-days post injury. Heart stiffness, measured using atomic force microscopy, was higher in the T_3 exposed zebrafish following damage and remained elevated. In addition, a spike in heart stiffness, seen in control zebrafish following damage, appeared to trend back towards basal stiffness levels by day 7. Overall, these results suggest that T_3 may play a role in impacting heart regeneration through acting on the production of extracellular matrix components.

Analyzing the Pathways Involved in Heart Regeneration Using a Comparative Approach

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Abstract: In humans, damage to the heart occurs following a myocardial infarction (heart attack), which results in the death of a large region of contractile tissue composed of cardiomyocytes. The human heart is incapable of reforming this damaged tissue, which is instead replaced by collagen and a fibrotic scar, creating a non-functional region of the heart. In contrast, a few species have been shown to display regeneration of the heart following injury, including zebrafish, neonatal mammals and various amphibians such as newts. Recently, preliminary work has suggested that the leopard gecko (*Eublepharis macularius*) is capable of heart regeneration and has numerous characteristics in common with other heart regenerating species. Therefore, in this study, the heart regeneration process of leopard geckos was explored through time using a proteomic approach. To do this, the hearts of adult leopard geckos were damaged via cryoinjury and allowed to regenerate prior to being harvested at 3-, 14-, 30- and 100-days post-injury. The proteins composing the wound site were extracted and sent for mass spectrometry analysis. Significantly upregulated and downregulated proteins between the controls and various timepoints were identified and grouped into gene ontology (GO) terms. Preliminary results show changes in biological processes such as extracellular matrix organization and metabolic processes, suggesting modifications in collagen production and cellular proliferation, as would be expected following heart damage. Overall, these results may help elucidate the essential components required for successful heart regeneration, and provide further understanding as to why this does not occur in humans.

Pulmonary Ionocytes Play a Role in pH Regulation in Primary Human Bronchial Epithelial Cell Culture

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Abstract: Recent single-cell RNA sequencing analysis of airway epithelia revealed a previously undescribed cell type, pulmonary ionocyte. Pulmonary ionocytes express very high level of Cystic Fibrosis Transmembrane conductance Regulator (CFTR) mRNA and, thus, were proposed to be the site of development of Cystic Fibrosis (CF), an autosomal recessive disease caused by mutations in the gene encoding for CFTR channel. However, pulmonary ionocytes are rare, and account for only about ~1% of all epithelial cells. More recent publications confirmed the existence of ionocytes but also described a population of secretory cells, including club cells, also express CFTR mRNA. The functions of the various cell types and their potential roles in CF pathophysiology are not fully understood and there is still controversy about which cell types are responsible for the development of CF lung disease. We hypothesize that different cell types specialize in performing distinct tasks for maintaining the integrity of the airway surface liquid layer. Using high-resolution self-referencing ion-selective microelectrode, we measured single-cell ion transport properties of ionocytes and club cells in healthy and CF primary Human Bronchial Epithelial (pHBE) cell cultures. We showed that activating CFTR with forskolin+IBMX elicited different responses in ionocyte and club cells in Non-CF pHBE cell cultures. After forskolin+IBMX stimulation, H^+ was transported across ionocytes from the apical (lumen) to the basolateral side, making the lumen pH basic. This H^+ transport was reduced by 50% with the carbonic anhydrase inhibitor, acetazolamide. Forskolin+IBMX triggered Na^+ and Cl^- transport into the lumen via club cells. In CF pHBE cell cultures, ionocytes and club cells did not respond to forskolin+IBMX stimulation. Our results indicate that ionocytes contribute pH regulation in the airway. Defective ionocyte-related pH changes may contribute to CF pathogenesis. Club cells contribute to ion and liquid transport. Abnormal club cell function would cause reduced airway hydration in CF.

Environmental Gradients and Swimming Morphology as Driving Factors of Inland Lake Fish Abundances

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Abstract: Water's physical properties, such as its high density, create demands that fishes must overcome to access resources. Accordingly, it has long been hypothesized that fish morphology reflects swimming ability and that a fish's swimming ability limits the habitats it can occupy. Yet, in real environments, factors like temperature, water quality, and

geological history are often the drivers of species distributions over regional scales. Here, we unify biomechanical and ecological perspectives to understand the relationships between swimming morphology and habitat occupancy and how important these biomechanical relationships are for fish abundances in inland lakes relative to other well-known drivers. We collected linear measures of body and fin morphology from museum specimens of the most common Michigan inland lake species and performed a principal components analysis to characterize the morphospace they occupy. Our initial analysis on 8 of the sampled species indicates that strongest sources of morphological variation among these species are traits related to feeding, streamlining, stabilization, and pectoral and caudal fin use. Alongside, we performed regressions to examine how these species' abundances vary along environmental gradients using data from standardized lake surveys conducted between 2003-2019 by the Michigan Department of Natural Resources. We find that environmental factors like temperature are the most important predictors of fish abundances, but factors related to flow levels and presence of refuges correlate to a lesser extent with fish species abundances. Taken together, our two lines of analysis lead us to suggest that, after regional filters set which species are found in a water body, swimming ability may be influencing relative abundance or microhabitat use. Along with future swimming studies using live fishes, this work ultimately will aid in identifying selection pressures that may have influenced fish diversity and clarifying swimming specializations that may constrain habitat use and ecological role.

The Effect of Strain Rate on Adhesive Performance in the Purple Sea Urchin *(Strongylocentrotus purpuratus)*

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Abstract: The intertidal zone is one of the most dynamic and challenging marine habitats. For example, benthic invertebrates in the intertidal often lay down permanent glue to hold position in the face of crashing waves and predation, and physiologically adapt to heat stress, changes in salinity, and exposure to air during low tides. Rather than holding tight in these conditions, some benthic invertebrates, like the purple sea urchin, manage to move from place to place. Sea urchins use a duo-glad adhesive system with every step their adhesive tube foot discs take. While the tube foot disc is the site of adhesion, tube foot attachment failure may occur in either the tube foot-substrate adhesive interface, or in the tube foot stem, which attaches the distal tube foot disk to the water vascular system of the sea urchin. Tube foot stem strength is strain rate dependent, where faster applied forces increase the strength of the tube foot stem. It is unknown if interfacial adhesive performance is similarly strain rate dependent. The purpose of our study is to test for rate-dependent adhesive performance in sea urchins at the whole animal and the individual tube foot level. We measured tube foot adhesive performance at both levels (whole animal and tube foot) at strain rates that varied from 10%-250% of the tube foot length. Contrary to our prediction, adhesive performance was not strain rate dependent at either level. We did find evidence for a relationship between number of tube feet broken following whole animal dislodgement and the force required for dislodgement, suggesting that the primary point of failure in the tube foot system is the tube foot stem, rather than the adhesive glue.

Developing Larger Atlantic Salmon (*Salmo Salar*) Smolts for Aquaculture: Can it be Done?

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Abstract: Atlantic salmon aquaculture combines the use of land-based, freshwater recirculating aquaculture systems (RAS) and marine net pen operations. In freshwater (FW) RAS, a photoperiod manipulation mimicking a winter signal followed by an abrupt switch to constant light is used to induce smoltification, which prepares salmon for seawater (SW) entry. Developing larger, more physiologically robust smolts is an industry goal to reduce time spent in net pens, while yielding higher growth rates and survival. Using five independent RAS at the University of British Columbia's InSEAS research lab, we developed 200g, 500g, and 1250g smolts by exposing fish reared under constant light (24:0, hours light:dark) at 14℃ to a 12:12 winter photoperiod for 8-weeks followed by a 24:0 photoperiod for 4-weeks, prior to SW transfer. Blood, gill, and muscle samples were collected at the end of 4-weeks in 24:0 FW, 24h SW, and 1mo SW for plasma ions, whole blood parameters, gill Na^+/K^+ ATPase (NKA) activity, and muscle water content (MWC). Results from the plasma ions and osmolality data showed differences with no apparent patterns. Hemoglobin data indicated significant cellular swelling at 24h and 1mo SW for photoperiod manipulated (PM) and non-photoperiod manipulated (non-PM) fish for the 200 and 500g smolts. MWC in SW illustrated significant cellular shrinkage and swelling for 500 and 1250g non-PM fish, respectively, compared to the FW timepoint. The magnitude of cellular shrinkage was significant for 500g non-PM smolts when compared to PM smolts at 24h SW. Gill NKA activity increased significantly upon 24h and 1mo SW for 200 and 500g smolts and at 1mo SW for 1250g smolts. The 500 and 1250g PM fish demonstrated significantly higher NKA activity at 24h SW than non-PM fish. These parameters, along with NKA isoform expression data, will provide insight into whether developing larger smolts is possible for aquaculture purposes.

The Fruit fly, *Drosophila nigrospiracula*, Exhibits Threat-Specific Defensive Behaviour (Non-Consumptive Effects) in Response to Infection Versus Predation Risk.

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Abstract: Non-consumptive effects (NCEs) collectively refer to changes in an organism's behaviour, physiology or morphology that are caused by the perceived threat of predation or infection. Behaviours associated with NCEs have an associated fitness cost as they are either energetically costly in and of themselves, they can decrease the time available for reproduction or reduce access to high quality foraging grounds. NCE-related behaviours have frequently been investigated in predator-prey systems, less often in parasite-host associations, and few studies have used the same species as both prey and host. We investigated the behaviour of the fruit fly *Drosophila nigrospiracula* when exposed to visual and odor cues of a parasitic mite (*Macrocheles subbadius*) or a predatory spider (*Salticus scenicus*). Using video

analysis of fly behaviour, we first established that *D. nigrospiracula* could differentiate between parasitic and non-parasitic mites, and between predatory spiders and non-predator insects of a similar size. We then demonstrated that flies showed diametrically opposed responses to parasite vs. predator in certain behaviours; relative to controls, they moved faster and spent longer grooming in the presence of mites but slower and less time grooming in the presence of spiders. These behaviours reflect a threat-specific response, as grooming and evasion are defence mechanisms against the attachment of slow moving ectoparasites such as mites, while slower movements and reduced extraneous activity such as grooming are appropriate defences against the spider, an ambush predator. These responses show that *D. nigrospiracula* possesses a degree of behavioural plasticity and a sophisticated ability to tailor their NCE-related behaviours to predator or parasite threats. Our results also suggest that there may not be a predictable, linear relationship between the magnitude of an NCE behaviour and the fitness cost of the threat-type, and so may provide new perspectives on how parasitism should be incorporated into NCE research.

A Widespread Host-Parasite Association in the Strait of Georgia Provides a Unique Opportunity for Studying the Effects of Climate Change on Parasitism in the Marine Environment

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Abstract: Decades of research have clearly shown that the effects of climate change will substantially impact marine species through a variety of environmental factors, e.g. increase seawater temperature or decreased pH and salinity. In lab-based experiments, simulated climate change often elicits a species-specific response, highlighting the importance of research that investigates interspecific interactions, such as competition, predation, and parasitism. Parasitism is especially vulnerable to altered interspecific interactions, as the life cycle of many parasites cannot be completed without a particular combination of host species, i.e. obligate parasites. However, designing experiments that expose all species in a parasite's life cycle to multiple abiotic factors would be logistically challenging to say the least. One alternative to creating such nightmarishly complex experimental set-ups, is to use naturally occurring systems that possess gradients in some or all of the abiotic parameters of interest, e.g. CO_2 vents that create pH gradients are used by ocean acidification researchers. This presentation describes my work on a trematode parasite found throughout the Strait of Georgia, British Columbia, and suggests that this body of water could be used as a natural laboratory to study host-parasite interactions in the context of climate change. *Maritrema gratiosum* (syn. *M. arenaria*) uses the acorn barnacle, *Balanus glandula*, as one of three host species to complete its life cycle. *Balanus glandula* is a ubiquitous component of intertidal ecosystems, and is abundant along naturally occurring gradients in pH, temperature, and salinity in the Strait of Georgia - the climate change hat-trick!. Consequently, the distribution of *M. gratiosum* within barnacle host populations along these gradients may provide invaluable insight not only into how climate change will alter parasite dispersal, transmission, and development, but also how the combined effects of infection and climate change will impact host species from an individual to population level.

The Integrin Activator Talin is Highly Expressed in Myometrium During Late Pregnancy and Labour and Distinctly Localized to Focal Adhesions

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Abstract: During pregnancy the myometrium undergoes growth and adaptation into a powerful, contractile tissue at labour. As uterine muscle cells transform into contractile cells, clusters of integrin receptors and associated proteins called focal adhesions form within the cells to interact and connect with the extracellular matrix (ECM). The cytoplasmic protein Talin binds to the subunits of integrin heterodimers and changes their affinity for the ECM to modulate cell behaviour. We hypothesized that Talin would also be highly expressed in the myometrium during late pregnancy and labour when clustered and activated focal adhesions would be required to facilitate myometrial contraction. For immunoblot (IB) and immunofluorescence (IF) analyses, samples of rat uterus (n=4 per timepoint) were acquired from non-pregnant animals as well as from pregnant animals on day (D) 6 to D23 (labour) as well as post-partum. IB data were subjected to a one-way ANOVA and Tukey multiple comparisons tests (P<0.05). For IF analysis, uterine tissue sections underwent heat-induced epitope retrieval prior to immunostaining and imaging with an Olympus IX83 microscope. Focal adhesions were quantified using Olympus CellSens software following deconvolution. IB analyses showed that Talin expression was markedly increased at day (D) 22, 23 and post-partum compared to D12, D15 and D19. IF showed Talin became highly detectable at late pregnancy and labour and co-localized with another integrin activator named FERMT2 (kindlin-2) in myometrial cell membranes. Deconvolution of IF images also revealed that Talin-containing focal adhesions became more distinct and prominent during late pregnancy and labour. The number of talin-containing focal adhesions was significantly greater at D23 compared to D6. The elevated expression of Talin in myometrium during late pregnancy and labour as well as prominent detection in focal adhesions with FERMT2 indicate the activation of integrin-mediated signaling in myometrium at this time.

Integrin-linked Kinase is Highly Expressed in Myometrium and Localized to Distinct Focal Adhesions during Late Pregnancy and Labour

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Abstract: During pregnancy the myometrium undergoes growth and adaptation into a powerful, contractile tissue at labour. As uterine muscle cells transform into contractile cells, clusters of integrin receptors and associated proteins called focal adhesions form within the cells to interact and connect with the extracellular matrix. The cytoplasmic adapter protein ILK is a member of the consensus integrin adhesome complex connecting the actin cytoskeleton with integrins and aiding integrin-mediated functions. We hypothesized that ILK would be highly expressed in the

myometrium during late pregnancy and labour when clustered and activated focal adhesions would be required to facilitate myometrial contraction. For immunoblot (IB) and immunofluorescence (IF) analyses, samples of rat uterus (n=4 per timepoint) were acquired from non-pregnant animals as well as from pregnant animals on day (D) 6 to D23 (labour) as well as post-partum. Data from IB analysis were subjected to a one-way ANOVA and Tukey multiple comparisons tests. For IF analysis, uterine tissue sections underwent heat-induced epitope retrieval prior to immunostaining and imaging with an Olympus IX83 microscope. IB analyses revealed that ILK expression was markedly increased (P<0.05) at D22, D23 and post-partum compared to D12 and D15. Expression on D22 was also significantly elevated compared to D17, D19 and D21. IF analysis demonstrated that ILK was predominantly cytoplasmic at early time points in both circular and longitudinal muscle layers and became progressively localized to punctate focal adhesions from D15 onwards. The elevated expression of ILK in myometrium during late pregnancy and labour as well as prominent detection in focal adhesions adds to evidence of activation of integrin-mediated signaling in myometrium at this time.

Dynamics of changes in apical cell area during sex comb rotation in *Drosophila melanogaster*

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Abstract: Epithelia are highly dynamic tissues displaying various types of tissue rearrangements However, despite the rapid progress in this field, it is still unknown how epithelia coordinate and buffer tissue rearrangement, while maintaining its functions as a physical barrier. An excellent model system for studying the dynamics of tissue rearrangement is sex comb rotation in *Drosophila melanogaster* The sex comb is a row of leg bristles which rotates during morphogenesis Here, we describe the dynamics of changes in apical cell area (ACA) in an epithelial system displaying tissue rearrangement resulting in sex comb rotation on the forelegs of male *Drosophila melanogaster*. We quantified the ACA in the region proximal to the developing sex comb by tracing apical cell boundaries using ImageJ in pupal first leg imaginal discs. We found that cells display intricate irregular oscillations in size as the comb rotates. However, the net changes in ACA within most of the cells studied are subtle, only 0 to +/-15%. Our current working hypothesis suggests these irregular oscillations confer flexibility during tissue rearrangement and can be an important mechanism for tissue homeostasis.

Prevalence, diversity, and intensity of *Trichinella* spp. in wildlife from northwestern North America

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Abstract: A new species of the zoonotic nematode of the genus *Trichinella, T. chanchalensis* (T13), was recently discovered in wolverines in the Northwest Territories and Yukon. While wolverine are generally not harvested for human consumption, resident and Indigenous harvesters consume bears and lynx, which can cause human infection with sylvatic *Trichinella* spp. (*T. nativa* and T6). Larvae of these species remain viable in naturally and artificially frozen carcasses, greatly increasing the chance of transmission via scavenging and to people relying on freezing to inactivate parasites. It is not known if the new species, *T. chanchalensis* (T13), can infect wildlife species other than wolverine. To better characterize the geographic and host range of *T. nativa*, T6, and *T. chanchalensis*, we collect tongue, diaphragm, and/or forelimb from terrestrial carnivores (fox, wolf, wolverine, coyote, lynx, and bears) from Alaska, the Yukon, the Northwest Territories, and Nunavut. Larvae are recovered and quantified using the double separatory funnel tissue digestion method. DNA will be extracted from the isolated larvae and sequenced using an Illumina MiSeq, for the first time using a metabarcoding approach to characterize co-infections of larvae in muscles. Newer methods of molecular characterization will allow differentiation of *T. chanchalensis* from *T. nativa* (previously not possible), as well as T6, *T. spiralis, T. pseudospiralis,* and *T. murrelli*, all of which have been identified in wildlife in northwestern Canada and may signify spillover from domestic animals and migratory wildlife. We hypothesize that T13 will be detected in species other than wolverine, that T6 dominates in terrestrial carnivores in the western North American Arctic, and that T2 will also be present to a lesser extent. Research into host-specific differences in prevalence, intensity, and diversity of *Trichinella* is essential to assess the risk for food safety in Indigenous communities, especially in wildlife species consumed by people.

A Heartwarming Study of Zebrafish: Investigating the Functional and Histological Response of the Zebrafish (*Danio rerio*) Heart to Re-Warming Following Cold Acclimation

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Abstract: As ectotherms, the physiological temperature of fish is that of their environment. As a result, a seasonal change in environmental temperature represents a significant challenge for the animals. For example, cardiac function is impaired by a decrease in water temperature. Some effects of a reduction in temperature on the heart include

increased myocardial stiffness, increased cardiac load, and reduced force-generating capacity. However, previous studies have demonstrated that zebrafish remain active at seasonally low temperatures and have the capacity to modify the structure and function of their heart. For example, cold acclimation has been found to decrease the thickness of the compact myocardium and the amount of collagen in the heart. In the current study, we cold acclimated adult zebrafish to 20^{o}C from 27^{o}C, and then after 17 weeks, we re-warmed these individuals back 27^{o}C for 6 weeks. To evaluate the capacity of the heart to return to the control phenotype, we used high frequency cardiac ultrasound to measure heart rate, stroke volume, and chamber areas at weeks 11, 19, and 23. We also used histological analysis to quantify collagen content, the cross-sectional area of the ventricle, and compact myocardium thickness at 23 weeks. Our results indicate that cold acclimation causes a reduction in cardiac output, ventricle size, and collagen content, but that these return to control values upon re-warming. This is the first experiment to demonstrate that cold-induced cardiac remodeling is reversible with re-warming. There does, however, appear to be an energetic consequence associated with these repeated remodeling events, as the condition factor of re-warmed fish is lower than that of control fish.

Assessing the Temporal and Spatial Variation of Gastropod Hosts to Predict Risk of Brainworm (*Parelaphostrongylus tenuis*) Transmission to Moose (*Alces alces*)

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Abstract: Brainworm (*Parelaphostrongylus tenuis*) is one of the parasites that may be contributing to moose (*Alces alces*) population decline across North America. Brainworm is transmitted to moose through the ingestion of gastropods infected with the L3 stage. Thus, gastropod infection, presence, and abundance can be used to estimate the risk of brainworm transmission to moose. It is unknown exactly where and when moose encounter and consume infected gastropods. We hypothesized that the presence and abundance of gastropod hosts would be affected by temporal variation in climate and soil chemistry. We predicted that soil chemistry would have the greatest influence, with gastropod presence and abundance increasing during late summer, in areas with neutral soil (pH = 7.0) that is high in calcium. From June-August 2022, gastropods were surveyed from six locations in Eastern Manitoba where moose have recently declined. Using transect sampling, we collected gastropods and measured aspects of their microhabitat including proportion of land cover type, humidity, temperature, soil moisture, pH, and calcium. In total, 193 gastropods were collected from six species, four of which are known brainworm hosts. Infection prevalence was 0.5%, which is low, but consistent with results from surveys conducted in North American moose habitat. Preliminary statistical analysis revealed that gastropod presence varied temporally, with their occurrence being most likely in mid-late summer and as soil increased in pH from 3.6-7.4. In addition, we will discuss how microhabitat influences gastropod abundance to further understand the temporal and spatial variation of gastropods. My results will indicate the microhabitat factors influencing when and where brainworm is transmitted to moose. This information can be used by conservation initiatives to prevent brainworm transmission from gastropods to moose by employing land management interventions to reduce the probability that the two hosts encounter each other.

Embryo Incubation Temperature Influences Aerobic Swimming Performance in Juvenile Lake Whitefish, but not Yellow Perch

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Abstract: Climate change, industrial thermal effluents, clearing of riparian zones and urban centers act as heat islands that can increase water temperature and its variability particularly in near shore and shallow waters. Mobility to avoid local fluctuations or increases in water temperature is limited in embryonic and larval fishes and these life stages are particularly vulnerable to temperature changes in part owing to the fact that they are undergoing rapid development and growth. To help understand the long-term effects of elevated incubation temperatures associated with anthropogenic activity, Lake whitefish and Yellow perch embryos were incubated at control (~ optimal) and elevated (3°C above optimal) temperatures until hatch, and then reared at common garden temperatures until their aerobic swimming performance at different acclimation temperatures was measured. We selected Lake whitefish and Yellow perch because of their economic, social and cultural importance and their contrasting reproductive strategies. Lake whitefish spawn in the late fall or early winter with embryos developing slowly at near freezing temperatures. Yellow perch spawn in the spring and embryos develop more rapidly at warmer temperatures. Embryo incubation temperature did not influence aerobic swim performance in Yellow perch, but maximum metabolic rate and aerobic scope were higher at the two higher acclimation temperatures. In Lake whitefish, embryos incubated at elevated temperatures had lower critical swimming speeds at the preferred, 15°C acclimation temperature and seemed to suppress the expected rise in routine metabolic rate at the higher 19°C acclimation temperature. Aerobic scope and critical swimming speed were also higher in Lake whitefish at the warmer acclimation temperature, whereas in Yellow perch only aerobic scope increased. Collectively, these data suggest that moderate increases in embryo incubation temperature may alter fish fitness, as measured by aerobic swim performance, with slow developing embryos likely at greater risk than more rapidly developing spring spawning species.

Local Adaptation in the Plasticity of Cold Hardiness in the Eastern Spruce Budworm

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Abstract: The eastern spruce budworm is the most destructive insect pest of Canada's boreal forest. Temperature affects many aspects of eastern spruce budworm (SBW) fitness, including survival and development; therefore, there we hypothesized there would be strong selection for local adaptation of cold tolerance and the capacity to increase it We know that regional populations are capable of plastic responses to stressful low temperature conditions, however we

have limited knowledge of the biochemical mechanisms underlying these responses. Cold tolerance is closely tied to glycerol production as glycerol is the most prevalent cryoprotectant in SBW. Yet glycerol synthesis must come at the cost of glycogen reserves, which also form the only overwintering energy store. We hypothesized that local adaptation for increased glycerol synthesis has occurred in SBW and predicted that fluctuating temperatures (which increase glycerol synthesis) would induce increased enzyme activity in the pathway responsible for glycerol synthesis, and that more northerly populations would have higher enzyme activity than more southerly populations. We developed enzyme activity assays for three key enzymes in the SBW glycerol synthesis pathway: glucose-6-phosphate dehydrogenase (G6PDH), phosphoglucoisomerase (PGI) and phosphofructokinase (PFK). We then assayed the activity of these enzymes in SBW from five populations (NWT, Alberta, New Brunswick, Quebec, and IPQL) reared in common garden conditions and then following fluctuating cold exposure to assess enzymatic reaction rate (Vmax) and enzyme substrate affinity (Km) to characterize changes in potential glycerol synthesis. While enzyme activity showed clear patterns of activation by fluctuating cold, gene expression did not. Understanding the importance of fluctuating cold temperatures is helping us build better models of eastern spruce budworm population growth, allowing us to better predict which areas of the forest are at risk for future outbreaks.

Antimicrobial Resistance and Genomic Characterization of *Melissococcus plutonius* Isolates

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Abstract: *Melissococcus plutonius* is a major bacterial pathogen of honey bee larvae, acting as the causative agent of European foulbrood (EFB) disease. In North America, antimicrobials are widely used to treat and control bacterial brood diseases, including EFB and American foulbrood. Thus, antimicrobial resistance (AMR) poses an imminent threat to North American apiaries, especially for cases of EFB disease since only a single antibiotic, oxytetracycline (OTC), is approved. Additionally, western Canada has seen an emergence of EFB disease despite OTC application. Little is known about the resistance profiles of *M. plutonius* and how these are related to the ability of drugs to clear infection in honey bee larvae. The objective of this study was to determine the AMR profiles of North American *M. plutonius* isolates through minimum inhibitory concentration (MIC) determination. Additionally, one sensitive and three resistant isolates were chosen to compare the utility of OTC, tylosin (TYL), and lincomycin (LMC) as alternative treatments for EFB using an *in vitro* larval infection model. Finally, pangenome analysis was performed for 71 *M. plutonius* isolates to evaluate AMR genes and assess relatedness. We found that 67% of isolates were resistant to OTC (MIC ≥16 μg/mL) while 100% of isolates remained sensitive to TYL and LMC (MIC ≤4 μg/mL). We demonstrated that the efficacy of antimicrobial treatments in honey bee larvae were concordant with *in vitro* MIC measurements. Moreover, survival outcomes of larvae were not dependent on *M. plutonius* clearance, but on a strain-dependent reduction of their numbers within larvae. In

the tested isolates, we did not find any genetic determinant of OTC-resistance, nor did phylogenetic analysis reveal patterns of genetic relatedness to explain resistance phenotypes. Overall, we demonstrated a high incidence of OTC-resistance in North American *M. plutonius* isolates, and that TYL and LMC may act as alternatives for EFB treatment *in vitro.*

Assessing the Mechanism of Toxicity from Naphthenic Acids Isolated From Oil Sands Process-Affected Water in *Daphnia magna*

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Abstract: The waste by-products of oil and gas extraction have the potential to impact the surrounding environment. Oil sands process-affected water (OSPW) is generated from the extraction of bitumen from surface mining activities in the oil sands region of Northern Alberta. OSPW is stored in large human-made tailings ponds that contain complex chemical mixtures, including naphthenic acids (NAs), herein defined as the O_2 acid-extractable organics. The NA fraction has been identified as one of the primary toxic components, and there is little understanding of the chronic effects of these slowly degrading compounds. It is hypothesized that chronic exposure to NAs will impact growth, survival and reproduction in the bioindicator species, *Daphnia magna*. Acute 48-hour lethal concentration studies were assessed to understand the sensitivity of *D. magna* to NAs isolated from an OSPW sample. The calculated LC50 was 70.4 mg/L (95% CI 42.2-100). A chronic 21-day exposure to environmentally relevant concentrations of NAs (0.5, 1 and 5 mg/L) was conducted. A significant increase in body length and reproductive effort was observed at 5 mg/L. Multigenerational studies are proposed to follow using similarly environmentally relevant concentrations of NAs, which may occur from tailings pond breaches, as well as from unintentional seepage or release. Overall, it is expected that exposure to NAs will perturb growth and reproduction in *D. magna* through alteration of key proteins needed for oxidative metabolism. Molecular analyses (qPCR and proteomics) will be conducted to identify the mechanisms of toxicity. This research will help inform remediation strategies, priorities, and policy pertaining to tailings pond management while providing insight into potential ecosystem recovery.

Parasites as Stressors of Cervid Populations in Boreal Saskatchewan

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Abstract: In boreal regions of Canada, many obstacles threaten stable, healthy woodland caribou (*Rangifer tarandus*) and moose (*Alces alces*) populations, which are important for conservation as well as indigenous and subsistence

harvest. In addition to anthropogenic impacts, diseases and parasites serve as stressors in wildlife populations, especially newly endemic or invading pathogens. Monitoring parasites circulating in cervid populations can provide insight into cervid health for current and future populations. Through fecal, necropsy, and molecular analysis, we describe gastrointestinal parasite prevalence, intensity and diversity in woodland caribou and moose in Saskatchewan. As well, we are mapping the distribution of meningeal worm (*Parelaphostrongylus tenuis*), a parasite of white-tail deer (*Odocoileus virginianus*) in Manitoba which can have detrimental impacts on caribou and moose populations, as well as other protostrongylids in deer and caribou in Saskatchewan. Preliminary results show that Saskatchewan caribou populations host multiple gastrointestinal parasites including nematodes in the trichostrongyle family (such as *Teladorsagia*) and *Nematodirus spp.,* coccidian parasites *Eimeria spp.,* and cestodes *Moniezia spp*.. Additionally, we detected larvae morphologically similar to the protostrongylid lungworm *Orthostrongylus macrotus* not previously reported in caribou, that may have host-switched from mule deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), or moose. For the first time, we report the presence of adult meningeal worms in brains of white-tail deer from north-central Saskatchewan, previously restricted to eastern zones adjacent to the Manitoba border. Our findings suggest that several parasitic species may act as stressors on cervid populations within boreal Saskatchewan and that meningeal worm poses an increasing risk to caribou and moose populations in central Saskatchewan. This work, in conjunction with previous and concurrent studies, can be used to better inform wildlife management officials of the potential threats facing cervid populations in northwestern Canada.

The Anti-Inflammatory Effects of INSL5 in a DSS-Induced Murine Model of Ulcerative Colitis

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Abstract: Inflammatory bowel disease (IBD) is a chronic inflammatory disease characterized by an imbalance between pro and anti-inflammatory cytokines. The two main kinds of IBD include Crohn's disease (CD) and ulcerative colitis (UC). UC affects the colon and rectum, which have a higher microbial density than the small intestine. The intestinal microbiome is a major factor in the expression of Insulin like peptide 5 (INSL5). INSL5 is a novel peptide hormone most highly expressed in the L cells of the distal colon. The cognate receptor for INSL5 is the G protein-coupled receptor Relaxin family peptide 4 (RXFP4). RXFP4 is expressed on all murine immune organs and some innate immune cells, particularly bone-marrow derived macrophages (BMDM) splenic dendritic cells (sDCs), and diverse cell subsets in the intestine with immune functions.. Our lab has found that mouse macrophages incubated with INSL5 exhibited reduction in M1 macrophage markers, including pro-inflammatory cytokines. The objective of my study is to determine if pre-incubation of murine splenic dendritic cells with INSL5 reduces pro-inflammatory signaling and to examine the potential downstream role of the JAK2/STAT3 and NF-kB signalling pathways. UC was induced by adding 2% DSS to the drinking water for seven days. Spleens were collected from eight UC and eight control mice upon euthanasia, and macrophage enriched splenocytes were cultured and then subjected to one of five treatments: control, 100 nM INSL5 for 24h, 1.0 ug LPS for 24h, INSL5/LPS cotreatment for 24h, and 12h INSL5 pre-treatment followed by LPS for 24h. RNA and proteins

were extracted for quantitative PCR and immunoblot analyses. We expect that INSL5 will reduce M1 macrophage markers, either via activation of STAT3 or inhibition of NF-kB.

A Longitudinal Snail-Trematode Survey at Reclaimed Wetlands in Central Alberta

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**Presenting Author*

Abstract: Every animal in an aquatic ecosystem can be parasitized by a digenean trematode. The ubiquity of digenean trematodes allows them to be employed as biodiversity indicator organisms due to their connections to other organisms in the environment. Even if their host is no longer present in the area, trematodes can act as a record of their presence. For the last decade, the trematode community of Alberta, Canada has been extensively studied. Prior to this study, 79 trematode species had been identified in the province. It is one of the most studied trematode communities in the world, making it ideal to study the relationship between biodiversity and disease, as well as the impact of biodiversity on host specialist and host generalist trematodes. Over the last four years, we employed trematode/snail sampling and traditional biodiversity survey methods to characterize the host-parasite communities at eight reclaimed wetland sites that differ in age but share the same geographic area. Snail collections occurred biweekly from June to September. Cercariae emerging from the collected snails were identified using DNA barcoding. Traditional biodiversity monitoring tools included benthic kick-netting, water sampling, field cameras and birdsong recorders. 1 979 of 22 396 snails were found to be infected with a digenean trematode (8.84%). Fifty-six species of trematodes from nine families have been identified at these sites, including 12 species that were not previously identified from Alberta, bringing the province-wide total to 91 species. We have also identified 96 species of vertebrate potential hosts and 59 species of potential invertebrate hosts. By combining the database of known trematodes in Alberta with the trematode and host data collected during this study, we can examine the relationship between host biodiversity and trematode species richness on a large scale and fill gaps in trematode lifecycles.

Diluted Bitumen, UV Radiation, and Embryo Fathead Minnows: Evaluating Photo-Enhanced Toxicity Through Gene Expression and Deformity Analysis From the IISD-ELA Freshwater Oil Spill Remediation Study (FOReSt).

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Abstract: Canada is one of the top oil producers with approximately 10% of the world's oil reserves. Diluted bitumen (dilbit) is the major product of the Canadian oil sands region and comprises a mixture of bitumen with natural gas condensates to lower viscosity. Dilbit spills have declined over the past decade, however, many pipelines and proposed pipelines cross freshwater, and more knowledge is required about the impact of oil in these systems. Previous studies have evaluated the toxicity of dilbit and the polycyclic aromatic compounds it contains. However, many overlook the potential for photo-enhanced toxicity, which is the synergistic interaction between the toxicity of a contaminant and UV radiation, potentially increasing the toxicity anywhere between 2 to 1000 times. The Freshwater Oil Spill Remediation Study (FOReSt) at the IISD-Experimental Lakes Area was designed to study the effects of spills in a shoreline environment and compare non-invasive methods to remediate freshwater environments. These remediation measures included engineered floating wetlands, nutrient enhanced monitored natural recovery, and COREXIT EC9580A shoreline cleaner. To examine the photo-enhanced toxicity of dilbit in fish after remediation methods are applied, a separate study was conducted where fathead minnow embryos were exposed to water accommodated fractions of dilbit. The test was then duplicated to expose individuals to low (~15%) and high (~90%) UV exposures. Mortality was documented throughout a 7-day exposure, and photographs of living individuals were taken at the end. Using the photos, a deformity analysis was conducted to identify and categorize sub-lethal impacts of dilbit and photo-enhanced toxicity. Gene expression was assessed using qPCR, analyzing a known biomarker of oil exposure (*cyp1A*) and markers of thyroid signaling (*dio2*, *dio3*, *thrα*, and *thrβ*) to evaluate impacts to growth and development. Overall, dilbit exhibited photo-enhanced toxicity to embryo fathead minnows as indicated by increased *cyp1A* expression, deformities, and mortality.

Behavioural Responses to Various Stimuli in Japanese Medaka

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Abstract: Prey species have developed several different mechanisms to warn conspecifics of incoming danger. Fishes have adapted an alarm cue involving olfactory responses that allows for individuals to sense predation through the release of chemical or pheromone cues when a conspecific is injured, allowing them to respond accordingly. The objectives of this study are to determine behavioural responses to synthesized alarm cues from a Fathead Minnow and a natural alarm cue derived from donor fish. To test the behavioural responses, 60 lab-reared Japanese Medaka (*Oryzias latipes*) were split evenly between a control and alarm cue treatment and exposed for 10 minutes. Behavioural responses were measured by recording 30-minute videos and monitoring distance travelled via video analysis. Pre-statistical analysis suggests that fish exposed to alarm cues had a change in behaviour compared to control fish.

Environmental DNA: A Successful Tool for Monitoring a Species of Special Concern

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Abstract: Environmental DNA (eDNA) is an innovative, non-invasive technology for the biomonitoring of aquatic species. To detect a species of interest, genetic material (DNA) is collected from the environment, filtered, extracted, and quantitatively measured. eDNA is a highly sensitive and specific method for determining the distribution, migration, or spawning patterns of a species. In the summer of 2021, we field tested our laboratory-validated eDNA assay to detect an iconic sport fish species, Arctic grayling, across two watersheds in Alberta. Arctic grayling eDNA signal was detected in at least one replicate at 27 of the 28 sites sampled and was highly correlated with traditional surveying techniques. Of particular interest to Provincial Wildlife managers, was a signal found in a historically populated spawning area, which suggested a suitable target location for rehabilitation efforts in the watershed. This initiated a comprehensive follow-up field study of the Pembina River watershed in the summer of 2022. Of the 36 sites sampled, 24 had positive Arctic grayling eDNA signal. The 12 sites without signal suggest the areas are rarely, if ever, used as Arctic grayling habitat. As a result, wildlife managers have used the eDNA data to guide their site selection, optimizing time and resources on the most suitable habitats. This assay is an effective biomonitoring tool, especially in conjunction with traditional surveying techniques and will continue to be used to guide conservation efforts on Arctic grayling. This assay also provides important insight into the practicality of using eDNA as a biomonitoring tool for aquatic species.

Anatomy of the Head-trunk Interface Across the Origin of Vertebrate Jaw

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Abstract: The spectacular success of jawed vertebrates is set in a stark contrast with their mysterious origin. Here we shed light on critical transitions in the anatomy of the head-trunk interface across this node, with a high-resolution 3D reconstruction of an extinct jawless fish belonging to Osteostraci, the sister group of jawed vertebrates. Osteostracans have long occupied this phylogenetic position. This canonical view sets osteostracans in a mosaic of derived gnathostome traits (such as cellular bone) against the background of lamprey-like overall morphology (such as a blind nasohypophyseal canal). Extensive studies of their perichondrally ossified endoskeletons have provided support for this interpretation. However, few CT scans have been undertaken for osteostracan internal anatomy. We present preliminary results from a synchrotron X-ray tomography scan of the osteostracan *Norselaspis*, and reveal surprisingly

derived gnathostome traits in this osteostracan exemplar. Contrary to the earlier interpretations, the inner ear of *Norselaspis* has anatomical traits otherwise only known among jawed vertebrates. The pericardial chamber is closed dorsally, which precludes the lamprey-like single midline Cuvierian duct. There is no articular facet in the pectoral cavity, which suggests an entirely fleshy base of the pectoral fin. Our three-dimensional model also enhances key findings from the previous reconstruction, including: configurations of the extraocular muscles and associated motor nerves, exogenious infillings in the labyrinth, and brachial plexus derived of the most anterior spinal projections. These findings both refine and revise the stem-to-crown continuum of gnathostome characters. *Norselaspis* has derived gnathostome conditions in the inner ear and circulatory system. However, we present evidence that endoskeletal joints emerge with the evolutionary origin of jaws; in this respect, *Norselapis* remains resolutely plesiomorphic. Interestingly, an apparent lack of the crown-like hypobranchial system in Norselaspis implies its independent and divergent evolution in cyclostomes and gnathostomes.

The Interactive Effects of Salinity and Dissolved Organic Carbon on Diffusive Water Flux, Oxygen Consumption, and Nitrogenous Waste Excretion Rates, as well as the Electrical Properties of the Gill in Pacific Sanddab (*Citharichtys sordidus*)

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Abstract: Several flatfish species are euryhaline, including the Pacific sanddab (*Citharichthys sordidus*) as they spawn and feed in highly dynamic estuaries ranging from full seawater (30 ppt) to very low salinities. It is very likely that in these estuaries, flatfish are exposed to freshwater sources of dissolved organic carbon (DOC). Diffusive water flux (a proxy for transcellular water permeability), oxygen consumption, ammonia excretion and urea-N excretion rates, and transepithelial potential (TEP, the electrical gradient across the gills between the fish and the external water) were measured in the presence (1h) and absence of DOCs at low salinity (7.5 ppt) and full strength seawater (30 ppt). In the absence of DOC, a reduction in salinity resulted in an increase in diffusive water flux and ammonia excretion rates, TEP changed from positive to negative, whereas oxygen consumption and urea-N excretion rates remained constant. The complexity and heterogeneity of DOCs make them particularly difficult to study in a mechanistic context. Therefore, two natural freshwater DOCs [Luther Marsh (allochthonous) and Lake Ontario (autochthonous)] and three model compounds [tannic acid, sodium dodecyl sulphate and bovine serum albumin [BSA]) of known chemical structure were used. Diffusive water flux increased significantly in response to Luther Marsh DOC at 30 ppt and sodium dodecyl sulphate, at both salinities. Ammonia excretion increased in response to Lake Ontario DOC at 30 ppt, and decreased in response to tannic acid at 7.5 ppt. Significant changes in urea-N excretion rates occurred upon exposure to both natural DOCs at 30 ppt, Lake Ontario DOC at 7.5 ppt and BSA at 30 ppt. Finally, there were significant reductions in TEP in response to Lake Ontario DOC and BSA at 30 ppt only. Our results demonstrate that marine flatfish alter gill function in the presence of

DOC as a function of salinity and that specific chemical moieties resembling DOC characteristics likely play a role in this response. (NSERC Discovery).

Seafood Mislabeling in Calgary Reveals the Sale of Species of Conservation Concern

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Abstract: Invertebrate (n =115) and vertebrate (n = 312) seafood products were sampled by university students in Calgary, Alberta between 2014 and 2020. Product names were compared to the Canadian Food Inspection Agency's Fish List in order to determine the possible species that could be marketed under those product names. Samples were DNA barcoded using cytochrome c oxidase I (COI) to determine the species identity of each sample, and this barcoded species identity was compared to the Fish List identity – any disagreement between these identities was considered an instance of mislabeling. Approximately 34% of invertebrate and 33% of vertebrate samples were mislabeled. Six products sold as invertebrates were identified as species of fish, including a sample of cuttlefish balls that had 100% sequence identity with the endangered Threadfin Porgy (*Evynnis cardinalis*). International Union for the Conservation of Nature (IUCN) Red List statuses were largely unknown for invertebrates, but when known (n = 37) were always Data Deficient or Least Concern. Among fish products, ~74% of mislabeled samples had barcode identities that included species of conservation concern, compared to 40% of correctly labeled samples. Furthermore, seven invertebrate and three vertebrate samples were identified as species not found on the Fish List, including the critically endangered European Eel (*Anguilla anguilla*). This study adds to the growing awareness that food mislabeling can prevent consumers from making ethical decisions about the products they purchase, by masking species of conservation concern under the names of other species.

Farms, Food and Avifauna: Rethinking Agricultural Production to Enhance Sustainability

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Abstract: Over the last 60 years, agriculture has undergone dramatic changes worldwide, where intensification has created simplified landscapes, increases in agrochemical use, resulting in consequent loss of biodiversity and ecosystem services (Dudley and Alexander 2017). Agricultural lands make up 7% of Canada and 40% of the US land use, providing habitats for farmland and migratory birds that are sensitive indicators of ecosystem health. Analysis of trends in bird populations associated with farmlands and grasslands in North America have revealed ongoing declines with 57 of 77 (74%) species exhibiting decreases from 1966 to 2013. Evidence suggests insecticides are one of the strongest drivers of

these bird declines through mortality and sublethal effects and indirectly through reduced insect prey. We have found that insecticides, such as the neonicotinoids, are routinely found in blood samples of songbirds such as Barn swallows, Tree swallows, and White-crowned Sparrows in Saskatchewan. Controlled dosing experiments confirm that these chemicals exert rapid toxic effects causing reduction in food consumption and body mass. However, a key solution is to create more diversified heterogeneous landscapes, whereby agricultural croplands support higher biodiversity across trophic levels and enhance ecosystem services. In 2019-22, we conducted a study with local farmers to restore marginal areas on 20 crop fields by planting them to mixed grasses and compared these fields to matched control fields. Acoustic recording units (ARUs) recorded over 3000 hours of soundscapes. We found bioacoustic indices, which are a proxy for avian biodiversity, showed increases over time, a measurable increase in the treatment fields relative to controls, and no appreciable effect on yields or profits. Collectively, this work suggests that restoring marginal areas within croplands is a nature-based solution that has significant promise to reduce agrochemical use and damage, enhance avian biodiversity while providing environmental, socio-economic and agronomic benefits to promote sustainability and one-health.

The Relationship Between Cardiac Pump Capacity and Cerebral Perfusion in Rats

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Abstract: Myocardial infarctions (MIs) often reduce cardiac pump capacity, which may lead to cerebral hypoperfusion. Whether MI-induced cerebral hypoperfusion is preventable with exercise training (EX) remains unknown. This study tested two hypotheses: (1) that cerebral perfusion would be reduced in post-MI rats, and (2) that 12 weeks of EX would improve cerebral perfusion in post-MI rats. To further explore the effects of cardiac pump capacity on cerebral perfusion, the effects of dobutamine (beta-1 adrenergic agonist) infusion were assessed. Male Sprague Dawley rats underwent either a sham surgery (n=8) or a left anterior descending coronary artery ligation (experimental model of MI; n=10). Subsequently, MI rats were subdivided into sedentary (MI-SED, n=5) or EX (MI-EX; n=5) groups. EX began approximately 10-days post-ligation and consisted of progressive treadmill running three days per week. Twelve weeks post-surgery, internal carotid artery blood flow (iCBF; perivascular flow probe), mean arterial pressure (arterial catheter line) and cardiac output (CO, echocardiography) were measured at rest and during dobutamine infusion (5 µg/kg/min). iCBF was significantly different between groups (ANOVA p=0.01) with post-hoc analysis revealing significantly greater blood flow in MI-EX rats (p=0.03). Internal carotid artery vascular conductance was significantly different between groups (ANOVA p=0.02) with post-hoc analysis revealing significantly greater conductance in MI-EX rats (p=0.04). There was a positive relationship between iCBF and CO (Pearson's R=0.43; p=0.04), indicating that increased cardiac pump capacity is related to increased cerebral perfusion. Dobutamine infusion increased iCBF relative to baseline (main effect of dose; p<0.01), providing evidence that augmenting cardiac pump capacity improves cerebral perfusion. These preliminary findings indicate that cardiac pump capacity and cerebral perfusion are related and that EX improves cerebral perfusion in rats with experimental MI.

A Spatiotemporal Analysis of the Causative Agent of White-nose Syndrome and Intestinal Macroparasites Based on DNA from Nova Scotia Bat Guano

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Abstract: Invasive species can have devastating effects on native wildlife. In 2006, Pseudogymnoascus destructans (Pd), causative agent of white-nose syndrome (WNS), was introduced from Europe to immunologically naïve bats in a New York hibernaculum. Pd has since spread across North America, causing extensive mortalities in multiple bat species. In Nova Scotia, Pd has caused a >90% decline in Myotis lucifugus, historically the most abundant bat species in the province. While Pd principally infects bats during hibernation, Pd spores carried to summer roosting sites could assist spread of the pathogen. Bat guano may provide a substrate for tracking Pd at these summer roost sites and reveal how the temporal movements of cave-hibernating bats relates to spread and exposure. Pd has not yet been formally detected in every county in Nova Scotia. However, because declines are province-wide, this is highly likely. Additionally, little is known about bat intestinal parasites in Nova Scotia, and most endoparasite data on bats have been based on dissections. There are also no studies on associations between bat endoparasite communities and WNS. This study quantified the spatiotemporal distribution of Pd in Nova Scotia summer maternity roosts using genetic testing of guano samples. Pd was recorded for the first time in three Nova Scotia counties. This study is also testing three newly designed primers on DNA extracted from bat guano to trial molecular identification of endoparasite diversity in Nova Scotia.

Corticosterone and Metabolomics as a Multimodal Approach to Studying Stress in Waterfowl

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Abstract: Waterfowl populations may decline because of anthropogenic and environmental changes (stressors). In response to stressors, the hypothalamic-pituitary-adrenal (HPA) axis releases corticosterone (CORT), initiating physiological processes to provide energy, restore homeostasis and increase survival. Corticosterone has been used to monitor the impacts of stress, but CORT fluctuates amongst life-history stages and may not be a reliable indicator of adverse conditions. A better approach may be to examine CORT and metabolic responses to identify physiological changes associated with stress. We examined the relationship between CORT and metabolite changes in ducks and predicted that fecal CORT and metabolomics could be used to differentiate ducks subjected to increased CORT from control ducks. We surgically implanted CORT (n=15) or placebo (n=10) pellets into mallard ducks (*Anas platyrhynchos*). Fecal samples were collected prior to (Days -1, 0) and after implantation (Days 1, 2, 3, 5, 5, 7, 10, 15). H^1 Nuclear Magnetic Resonance (NMR) spectroscopy was used to analyze metabolites, and CORT was analyzed

by radioimmunoassay. Corticosterone concentrations were elevated after CORT implantation (Friedman Test P>0.001). Post-implantation, CORT concentration peaked on day 1 and 2 then decreased over time. This decline indicates a negative feedback response of the HPA axis to elevated exogenous CORT. Metabolomic profiles did not differ between groups during the baseline period (Days -1, 0). Fecal metabolite profiles began to differ after implantation (Days 1-3), but PCA (Principal Component Analysis) score plots show almost complete separation in the metabolite profile separation at Day 4. Metabolites (fructose, sorbitol, ribose, and galactonate) were higher in the CORT implanted ducks and are associated with energy production. Metabolites associated with growth and energy maintenance were higher in the placebo ducks. Fecal metabolomics shows promise as a non-invasive novel tool and may be useful in identifying and characterizing physiological responses associated with large-scale environmental changes in wild birds.

Pulmonary Ionocytes Contribute to Regulating Airway Surface Liquid Secretion and pH in Swine

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Abstract: Ionocytes, also known as chloride cells, are mitochondrion-rich cells that have been well studied in vertebrates, where they play an important role in osmoregulation. In 2018, ionocytes were first described in murine and human lungs using single cell RNA-seq analysis based on their gene expression profile. Pulmonary ionocytes showed very high levels CFTR mRNA, the gene causative for the lethal disease cystic fibrosis (CF). Moreover, pulmonary ionocyte dysfunction in genetically modified mice triggered phenotypes resembling CF disease. Thus, it has been proposed that pulmonary ionocytes are the site of CF lung disease. More recent publications confirmed the existence of ionocytes but their significance for CF lung disease was put in doubt. We used ion-selective self-electrode technique to measure ion transport activity in a single cell level in the small distal airways of pig lungs to characterize the their activity. The results suggest that ionocyte activity in the swine small distal airway transport Na^+, and a counter anion, basolaterally after stimulation of CFTR with forskolin+IBMX. Simultaneously the tissue triggers an alkalinization of the lumen which is disrupted by treatment with the carbonic anhydrase blocker acetazolamide, suggesting that forskolin+IBMX trigger bicarbonate transport into the lumen. Blocking the Epithelial Na^+ Channel (ENaC) does not influence forskolin+IBMX-stimulated transport. The results suggest that pulmonary ionocytes play a significant role on the production of airway surface liquid responsible for mucociliary clearance and for regulating its pH. Both processes are altered in CF airway, suggesting that ionocytes may contribute to pathogenesis. Further analyzes of the behaviour of ionocytes in the airway will contribute to reveal the cellular basis of CF airway disease and identify specific cell population that must be targeted for transformation using gene therapies.

Suppressive Action of Nesfatin-1 and Nesfatin-1-like Peptide on Cortisol Synthesis in Human Adrenal Cortex Cells

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Abstract: Nucleobindin-derived peptides, nesfatin-1 (NESF-1) and nesfatin-1-like-peptide (NLP) have diverse biological roles in regulating metabolism. While both peptides showed a stimulatory effect on the synthesis of POMC, the ACTH precursor in mouse corticotrophs, whether NESF-1 and NLP have any direct effect on glucocorticoid (GC) synthesis in the adrenal cortex remains unknown. Therefore, the main aim of this study was to determine if NESF-1 and/or NLP act directly on adrenal cortex cells to regulate cortisol synthesis *in vitro*. Whether NLP injection affects stress-hormone gene expression in the adrenal gland and pituitary *in vivo* in mice was assessed. In addition, cortisol synthetic pathway in *Nucb1* knockout (KO) mice was also studied. H295R cells showed immunoreactivity for both NUCB1/NLP and NUCB2/NESF-1 using immunohistochemistry. NLP and NESF-1 decreased the expression of steroidogenic enzymes, cortisol synthesis and release through the AC/PKA/CREB pathway in H295R cells. Similarly, intraperitoneal injection of NLP in mice decreased the expression of enzymes involved in GC synthesis in the adrenal gland while increasing the expression of *Pomc*, *Pcsk1* and *Crhr1* in the pituitary. Moreover, the *Mc2r* mRNA level was enhanced in the adrenal gland samples of NLP injected mice. However, the global genetic disruption in *Nucb1* did not affect most steroidogenic enzyme mRNAs and *Pomc*, *Pcsk2* and *Crhr1* in mice adrenal gland and pituitary, respectively. Collectively, these data provide the first evidence that NLP and NESF-1 directly decreased cortisol synthesis and secretion *in vitro.* NUCB peptides still might play its stimulatory effect on GC synthesis and secretion through their positive effects on ACTH-MC2R pathway in the pituitary.

Hypoxia-acclimation does not Improve Recovery from Exhaustive Exercise in a Marine Teleost

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Abstract: Red drum, *Sciaenops ocellatus*, are a marine teleost native to the Gulf of Mexico that routinely experiences periods of low oxygen (hypoxia). Recent work has demonstrated this species has the capacity to improve aerobic performance in hypoxia through respiratory acclimation. However, it remains unknown how hypoxia acclimation impacts anaerobic metabolism in red drum, and the consequences of exhaustive exercise and recovery. Juvenile fish were acclimated to normoxia (n=15, DO 90.4±6.42%) or hypoxia (n=15, DO 33.6±7.2%) then sampled at one of three time points: at rest, immediately following exercise, and 3 h post-exercise (recovery). Whole blood, red muscle, white muscle, and heart tissues were sampled for metabolites and enzyme activity. As expected, exhaustive exercise increased muscle lactate, and decreased phosphocreatine and ATP with no effect of hypoxia acclimation. Blood pH_e and pH_i decreased

with exercise and increased with recovery in both acclimation treatments, with hypoxia-acclimated pH_i experiencing a smaller effect of exercise. Red muscle showed decreased ATP and phosphocreatine in hypoxia-acclimated fish *versus* normoxic fish at rest, after exercise, and during recovery. Hypoxia acclimation caused an increase in white muscle ATP and hexokinase activity, a glycolytic enzyme, and increases in lactate dehydrogenase activity in the red muscle. To further investigate these biochemical changes in the muscle of hypoxia-acclimated fish we used a gait-transition swim test. Hypoxia acclimated and non-acclimated controls recruited anaerobic-powered burst swimming at similar low speeds when swam in hypoxia. In normoxic trials hypoxia acclimated fish recruited burst swimming at lower speeds compared to non-acclimated controls. These data provide some evidence that hypoxia-acclimation increases anaerobic metabolism differentially in muscle tissues, and does not benefit recovery from exhaustive exercise.

Direct Action of Glucagon-like Peptide-1 (GLP-1) on Zebrafish Testicular Function

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Abstract: Regulation of testis function and development is a complex process involving pituitary gonadotropins, gonadal and other peripheral hormones. Recent evidence suggests that the gut hormone, glucagon-like peptide-1 (GLP-1), may also play a role in the multifactorial regulation of gonadal function. In addition to its metabolic function, GLP-1 has been shown to regulate appetite and induce anorexigenic effects in fish. As energy plays a crucial role in reproductive function, GLP-1 may indirectly affect reproduction. Recent studies in humans and rodents, however, suggested that GLP-1 may directly influence testicular function. In this study, we investigated the direct effects of GLP-1 on spermatogenesis using zebrafish as a model organism. Our results demonstrate the expression of GLP-1 receptor and GLP-1 prohormone variants in the zebrafish testis. Using ex-vivo culture of the testis, we tested the direct actions of GLP-1 on spermatogonial stem cell renewal as well as the early and late stages of spermatogenesis. Treatment with GLP-1 was found to significantly alter basal and gonadotropin-induced type-A spermatogonia production. This suggests that GLP-1 may be involved in energy partitioning and may in part regulate energy investment in spermatogenesis during the period of starvation. The findings provide new insights into the role of GLP-1 in the spermatogenic cycle in zebrafish and other vertebrate species.

Regulation of Glucose Metabolism in Naked Mole-rats During Hypoxia

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Abstract: African naked mole-rats (NMRs) are one of the most hypoxia-tolerant mammals and they reduce their metabolic rate significantly in hypoxia. We hypothesized that NMRs would rapidly mobilize glucose in hypoxia to help maintain carbohydrate energy metabolism through anaerobic pathways. Furthermore, we predicted that glucose mobilization would be similar across developmental stages in NMRs, indicating retention of neotenic traits into

adulthood. To test our predictions, we treated pups, juveniles, and adult subordinates and queens in 1 hr of normoxia (21% O_2) or hypoxia (7%, 5% or 3% O_2) and measured metabolic rate (via respirometry), blood [glucose] and body temperature (T_b). We also challenged NMRs with a glucose, insulin, or insulin growth factor-1 (IGF-1) injections in normoxia or hypoxia and evaluated blood [glucose] for up to 5 h. We found that: 1) all age groups similarly reduced metabolic rate and T_b in all levels of hypoxia, 2) queens metabolized carbohydrates in all conditions, whereas subordinates and juveniles switched from lipids to carbohydrates in hypoxia, 3) blood [glucose] increased in 3% O_2 in all developmental stages, and glucose tolerance was similar between developmental stages in normoxia. Conversely, glucose clearance times during hypoxia were 2-3 fold longer in juveniles and subordinates than in queens, but the rate of glucose uptake from blood increased rapidly upon reoxygenation in subordinates. Mechanistically, subordinates were sensitive to insulin in all conditions, but queens were only sensitive to insulin in normoxia. In hypoxia, subordinates and queens were sensitive to IGF-1 in both normoxia and hypoxia. Our findings suggest that glucose management in NMRs is divergent across ontogeny, countering the neotenic theory of hypoxia-tolerance.

Is Metabolism Driving the Difference in Sublethal Copper Toxicity as a Function of Sociality in Threespine Stickleback (*Gasterosteus aculeatus*)?

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Abstract: Many animals benefit from group living. Advantages include greater protection and foraging, in addition to social buffering (i.e., reduced stress concurrent with exposure to members of the same species). However, sociality is not the only variable fish encounter on a daily or seasonal basis; changes in environmental parameters due to climate change and anthropogenic pollutants are damaging aquatic ecosystems. To date, minimal research has been performed to expand our understanding of the nexus between sociality and toxicity. Therefore, we tested the effects of copper (Cu), a potent aquatic toxicant, on the Swedish marine threespine stickleback (*Gasterosteus aculeatus*) under different social contexts. In particular, we sought to understand whether the social stress of isolation would increase the resting and maximal oxygen uptake rate ($\dot{M}O_2$) of stickleback leading to greater accumulation of Cu. We also tested whether Cu exposure and social stress would influence maximal thermal tolerance (CT_{max}). After capture from the wild, fish were assigned to either $\dot{M}O_2$ or CT_{max}. To determine resting MO_2, fish were placed in respirometry chambers and randomly assigned to isolation (one fish) or pairs (two fish) and submerged in control (no added Cu) or Cu (nominal 300 µg/L) water. Fish totalled 40 hours in respirometers with chasing occurring after 22 hours for maximal $\dot{M}O_2$. Remaining fish were measured for CT_{max} after a 24-hour exposure to control (no added Cu) or Cu (nominal 300 µg/L) water in isolation (one fish) or pairs (two fish). All fish were then euthanized and dissected with the gill, intestine, liver, and carcass assayed for Cu accumulation. The importance of understanding how sociality in fish alters the response to a given toxicant is

rarely if ever investigated, but the social structure, and its role in fish metabolic rate and homeostasis cannot be dismissed.

Mitochondrial Nanotunnel Networks in Naked Mole-rat Brain

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Abstract: Nanotunnels are a recently identified ultrastructural component of mitochondrial networks. These structures are thought to expand to support mitochondrial metabolism during periods of hypometabolism, but they have not been examined in the brain of any species, nor in any organ from a hypoxia-tolerant species. We hypothesized that mitochondrial nanotunnel networks are more extensive in the brain of hypoxia-tolerant naked mole-rats (NMRs, *Heterocephalus glaber*) than in hypoxia-intolerant CD1 mice (*mus musculus*). To test this, we used serial block face electron microscopy (SBEM) to examine mitochondrial nanotunnel networks in the brains of NMRs and mice treated for 4 h in normoxia (21% O_2) or hypoxia (7% O_2). We also examined changes in protein markers associated with nanotunnel remodelling using western blot. We identified nanotunnel networks in the brains of both rodents. Approximately 20% of NMR brain mitochondria but < 10% of mouse mitochondria were connected by nanotunnels in normoxia, and nanotunnels were ~ 3-fold longer in NMR brain. Following *in vivo* hypoxia, the percentage of mitochondria connected by nanotunnels and the average nanotunnel length increased 5- to 6-fold in mouse brain but was unchanged in NMR brain. Changes in mouse brain were accompanied by upregulation of Miro1, PGAM5, and FUNDC1, which are associated with nanotunnel remodelling in other tissues. Conversely, these markers were unchanged or reduced in NMR brain. Taken together, our results suggest that NMR brain endogenously expresses an extensive nanotunnel network, potentially as a preparation for periods of hypometabolism.

Bumblebee Mouthparts Exhibit Poor Acuity For The Detection Of Pesticides In Nectar

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Abstract: Bees are important pollinators of agricultural crops, but their populations are at risk when pesticides are used. One of the largest risks bees face is poisoning of floral nectar and pollen by insecticides. Studies of bee detection of neonicotinoids have reported contradictory evidence about whether bees can taste these pesticides in sucrose solutions and hence avoid them. Here, we use an assay for the detection of food aversion combined with single-sensillum electrophysiology to test whether the mouthparts of the buff-tailed bumblebee (*Bombus terrestris*) detect the presence

of pesticides in a solution that mimicked the nectar of oilseed rape (*Brassica napus*). Bees did not avoid consuming solutions containing concentrations of imidacloprid, thiamethoxam, clothianidin, or sulfoxaflor spanning six orders of magnitude, even when these solutions contained lethal doses. Only extremely high concentrations of the pesticides altered spiking in gustatory neurons through a slight reduction in firing rate or change in the rate of adaptation. These data provide strong evidence that bumblebees cannot detect or avoid field-relevant concentrations of pesticides using information from their mouthparts. As bees rarely contact floral nectar with other body parts, we predict that they are at high risk of unwittingly consuming pesticides in the nectar of pesticide-treated crops.

Fish on Antidepressants: The Troubling Tale of Venlafaxine Pollution in Our Waterways

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Abstract: Many wastewater treatment plants (WWTPs) are not equipped to eliminate pharmaceutically active compounds (PhACs) like antidepressants. These bioactive compounds are of growing concern due to the measurable concentrations found in surface waters, leading to chronically exposed aquatic species. Some species will be more resistant than others, due to interspecific differences including energy metabolism, and their response to oxidative stress. PhAC exposure may induce oxidative stress through the manifestation of reactive oxygen species (ROS) causing cellular damage. This can reduce fitness, negatively impacting population dynamics and species richness. The objective of my thesis is to leverage the variability of stress tolerance of four darter species (rainbow darter, johnny darter, fantail darter, and greenside darter) through comparative genetics, tissue specific metabolic analysis, and gene expression characterization, in the attempt to elucidate the impacts of chronically exposed fish populations to PhACs. PhAC contaminant profiles were characterized on water collected up and downstream of the Waterloo Municipal WWTP effluent outfall site, revealing Venlafaxine (VFX) to be the most concentrated PhAC downstream. Whole transcriptomes were sequenced form RNA extracted of liver tissue from up and downstream populations, followed by bioinformatic analysis. Findings suggest that chronic exposure to venlafaxine has multifaceted deleterious effects, including metabolic dysregulation, immunotoxic affects, and dysregulation of lipid transport and remodelling. Rainbow darters exposed to VFX demonstrated a varied enzymatic response related to ROS scavengers in the brain. However, more conclusive evidence for brain damage attributed to ROS generation was found through increased lipid peroxidation and DNA damage. This study emphasizes using transcriptomics to more comprehensively understand the impacts of contaminants on biological systems. We underscore the need for direct biomarkers to accurately quantify cellular damage rather than less definitive proxies. We also provide evidence that VFX exposure can have negative impacts on aquatic ecosystems, and advocate for improved wastewater treatment measures.

Uncertainty about old information results in differential predator memory in tadpoles Preagola, A.[1]*, Crane, A. [1,2], Achtymichuk, G[1,2]., Rivera-Hernandez, I.[1], Thapa, H.[1], Ferrari, M. C. O.[2]

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Abstract: As information ages, it may become less accurate, resulting in increased uncertainty for decision-makers. For example, chemical alarm cues are a source of public information about a nearby predator attack, and these cues can become spatially inaccurate over time. These cues can also degrade quickly under natural conditions, and cue receivers are sensitive to such degradation. Although numerous studies have documented predator-recognition learning from fresh alarm cues, no studies have explored learning from aged alarm cues and whether the uncertainty associated with this older information contributes to shortening the retention of learned responses (i.e., the 'memory window'). Here, we found that wood frog tadpoles, *Lithobates sylvaticus*, learned to recognize a novel odour as a predator from alarm cues that were aged under natural conditions for up to one hour. However, only tadpoles conditioned with fresh alarm cues were found to retain this learned response when tested 9 days after conditioning. These results support the hypothesis that the memory window is shortened by the uncertainty associated with older information, preventing the long-term costs of a learned association that was erroneous.

Mating induced metabolic changes in female Drosophila is regulated by dNUCB1's expression in the nervous system

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Abstract: In the common fruit fly, the sex peptide is an accessory protein that is transferred from the male to the female during copulation. Sex peptide binding to its receptor in the female initiates post-mating response (PMR), which includes increased food intake and other physiological changes to support the increased metabolic demand of reproduction. Recently, our lab discovered that the highly conserved *Drosophila* nucleobindin-1 (dNUCB1) has anti-hunger properties, as observed in several mammalian systems. We hypothesize that dNUCB1 is involved in post-mating food intake increase. First, we confirmed that mating changes dNUCB1 mRNA level within the first 2 hours of mating and the change is dependent on sex peptide signaling. Then, we evaluated the effect of pan-neuronal knockdown of dNUCB1 on food intake in virgin and mated females using a dye-based assay. We observed that pan-neuronal knockdown of dNUCB1 increased food intake only in mated females. Our immediate future goal is to identify the specific dNUCB1-expressing neurons that are responsible for this mating-induced feeding response.

Transgenerational Neurobehavioural Effects of Ancestral Dietary Arsenic Exposure in Zebrafish (*Danio rerio*)

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Abstract: The present study examined the transgenerational neurobehavioral effects of ancestral arsenic (As) exposure in zebrafish. Adult zebrafish (F_0; 6 months old) were exposed to 3 different dietary concentrations of As (30, 60 and 100 µg/g dry weight; as arsenite) for 60 days in addition to the control. The cognitive behaviour of fish was then examined using a latent learning paradigm in a complex maze, which showed a dose-dependent impairment of As on cognitive performance. This was associated with increased As accumulation, dopamine level, and oxidative stress in the brain relative to that in the control. Moreover, marked alterations of several genes involved in dopamine signaling, which regulates essential fish behaviours including learning and memory, were also observed in the brain of As-exposed fish. Following behavioural assessments, As-exposed females from each treatment were mated with control males, and vice versa, to produce F_1 progenies that were maternally (ME) and paternally (PE) exposed to As, respectively. Subsequently, adult males and females from each F_1 progeny of ME and PE groups were bred again to produce F_2 progenies of the respective ancestral As treatment groups. Both F_1 and F_2 generations were raised to adulthood in clean water and diet before assessing their cognitive performance as described above. Cognitive deficits were observed in both F_1 and F_2 progenies from the maternal lineage of ancestral As exposure, even at the lowest dose. Cognitive impairment was also recorded in the F_1 and F_2 progenies from the paternal lineage of ancestral As exposure, but only at the highest dose. Increased oxidative stress and dopaminergic dysregulation were also observed in the brain of F_1 and F_2 generations of fish irrespective of the maternal or paternal lineage of ancestral As exposure. Our study indicates that ancestral As exposure causes transgenerational neurobehavioural effects in zebrafish, likely via epigenetic alterations.

From Ants to Cartilaginous Fish: Retention and Reactivation of Ancestral Developmental Potentials

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Abstract: Recurrent phenotypes are widespread throughout nature as a result of convergence. We have begun to accumulate a great deal of knowledge regarding the ecological context and selective forces involved in facilitating these evolutionary patterns. Nevertheless, the extent to which these patterns are the product of novel developmental processes or the redeployment of ancient developmental programs remains poorly understood. Here we describe

examples, ranging from invertebrates (ants) to vertebrates (cartilaginous fish), where the potential for the reactivation of latent developmental programs can be retained for millions of years. First, we show that the hyperdiverse ant genus Pheidole has re-evolved an ancient worker subcaste called the supersoldier through the reactivation of dormant hormonal thresholds and modulated by developing structures long thought to be rudiments. In parallel, we show that cartilaginous fish, which are thought to have secondarily lost bone, have retained the capacity to express the bone gene regulatory network (GRN) underlying vestigial calcification in their vertebra. Furthermore, this GRN can be further reactivated using in vitro and in vivo methods. Together, ancestral developmental potentials can be retained throughout the animal kingdom and may more generally facilitate the parallel and adaptive evolution of phenotypes.

Tissue-specific Modulation of Growth- and gluco-regulatory Factor mRNA Abundance by Nesfatin-1 and Nesfatin-1 Like Peptide in Goldfish

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Abstract: Nesfatin-1 and nesfatin-1 like peptides (NLP) are derived from nucleobindin-2 and -1, two calcium and DNA binding proteins. Both nesfatin-1 and NLP have hormone-like functions in mammals and fish, mainly in regulating metabolism and the endocrine system. These functions include insulinotropic effects of nesfatin-1 and NLP seen in mouse, and their growth hormone suppressive actions reported in goldfish. We hypothesized that nesfatin-1 and NLP modulate growth hormone-insulin-insulin-like growth factor and glucose transporters in goldfish in a tissue specific manner. To test this, goldfish were intraperitoneally injected with either nesfatin-1 or NLP (50 ng/g BW) or saline alone (control) and sampled at 1-hour post-injection. Transcript (mRNA) abundance data from the adipose tissue suggest that both nesfatin-1 and NLP significantly upregulate the abundance of preproinsulin, insulin receptor, and *pcsk1* and pcsk*2* mRNAs. Meanwhile, the abundance of preproglucagon mRNA in the adipose tissue was significantly downregulated in both groups. These results agree with the insulinotropic and glucagonostatic roles for nesfatin-1 and NLP reported in rodents. Interestingly, transcript abundance of growth (*igf1*, *igf2a*, and *ghra*) and glucose transporters (*slc2a2* and *slc5a1*) were upregulated in the muscle, while an opposite effect on these mRNAs was found in liver of goldfish following nesfatin-1 and NLP administration. Our results suggest that both nesfatin-1 and NLP have tissue-specific regulatory roles on growth and glucoregulatory elements in the liver and muscle of goldfish. This agrees with our previous studies that showed a suppressive action of nesfatin-1 on growth hormone in goldfish. Together, this research, while only measured mRNA abundance, provide strong supportive/confirmatory evidence for tissue-specific insulinotropic, gluco- and growth- regulatory actions of nesfatin-1 and NLP in goldfish.

Corticotropin-releasing Hormone Receptor 1 Modulates the Acute Heat Shock Response in Zebrafish Larvae

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Abstract: The zebrafish (*Danio rerio*) larvae (4-6 dpf) show a very distinct locomotory activity to alternating pulse of light and dark; freeze in bright light and hyperactive in the dark. However, when these larvae were subjected to a 1-min physical stressor they responded with hyperactivity to bright light (HBL) compared to the unstressed larvae. This HBL was recently characterized as an acute stress-related behaviour mediated by CRHR1, as this behavioural response was completely abolished in the CRHR1 knockout (CRHR1KO) zebrafish larvae. In addition to CRHR1, we also showed that both the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) play a key modulatory role in prolonging the HBL for several hours post-the physical stressor, even after cortisol levels had returned to basal levels. Here we tested whether the CRHRI-mediated HBL response was stressor-specific, by subjecting the larvae (5 dpf) to a heat shock (+5^{o}C above ambient) for 60 min and modulated by chronic cortisol stimulation. The heat shock exposure for 15 or 60 min led to HBL in the wildtype larvae, and this response was completely abolished in the CRHR1KO larvae. As the CRHR1KO larvae failed to elicit a heat shock-induced cortisol elevation, we tested whether chronic cortisol elevation by waterborne exposure of this steroid (5 ug/ml for 16 h) offsets the lack of HBL in the mutants. Indeed, chronic cortisol treatment not only raised the HBL in the wildtype larvae but also overcame the HBL deficit observed in the CRHR1KO larvae. The molecular responses indicate a role for CRHR1 in modulating the cortisol-induced heat shock proteins responses in zebrafish larvae. This along with changes in some of the GR- and MR-responsive genes in fish lacking CRHR1 suggest an important role for CRH-CRHR1 signalling in fine tuning the effects of cortisol on the cellular and organismal stress response in zebrafish larvae.

Detecting Sea Lamprey Attacks on Lake Trout with Biologgers

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Abstract: Sea lamprey (*Petromyzon marinus)* remain problematic to the restoration of lake trout (*Salvelinus namaycush*) in the Greats Lakes. Estimates of sea lamprey-induced mortality help to assess the efficacy of lake trout rehabilitation programs; however, estimates can only be derived from sea lamprey wounding rates which is an inadequate predictor of mortality. Direct field observations are needed to better assess sea lamprey attack rates and attack lethality in wild

lake trout. Advancements in implantable tags are improving our ability to examine fine-scale behaviour in fish and may be useful in detecting sea lamprey attachments. Therefore, we experimentally implanted lake trout with biologgers that record heart rate and/or acceleration then observed their response to sea lamprey attack. The end goal of this research is to develop predictive models for field deployments. Therefore, the data collected was modified to simulate the resolution of acoustic tags and the number of model predictors was limited to four. Two methods were used to determine the top predictors: (1) by calculating the percent decrease in model accuracy when the variable was not included (i.e., mean decrease accuracy) and (2) using a method which selects variables based on optimal model fit and the fewest number of variables. The top predictors, in general, included variables related to roll, pitch, and heart rate. These predictors were used in random forests fit with 1000 trees using training datasets. These models were then applied to test datasets and model fit metrics were calculated. Models proved to be effective at predicting sea lamprey attachment in test datasets and false positive predictions were rare. Specifics related to model accuracy will be discussed. The application of these models in future deployments of tagged lake trout could yield the first field-based estimates of sea-lamprey attack rates and attack lethality in lake trout.

The Winter Behaviour and Cardiac Responses of Largemouth Bass in a Small Lake

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Abstract: The winter behaviour of most fish remains elusive due to difficulties associated with collected measurements under ice resulting in an incomplete understanding of their winter ecology. The use of electronic tags shows promise for studying fish in the winter as they can be deployed prior to ice formation, and continuously record measurements over the winter. Tag calibrations can also be performed which permit the estimation of free-swimming metabolic rates from tag recordings, expanding their utility. Using implantable accelerometers and heart rate loggers that also record depth and temperature, we investigated the behaviour of largemouth bass (LMB; *Micropterus salmoides*) over two winters. In the fall or early winter, LMB were collected via angling on Warner Lake (Elgin, ON), surgically implanted with loggers, then released to overwinter (n = 31 winter 2020/2021; n = 15 winter 2021/2022). The following spring LMB were recaptured (n = 35), and the loggers removed. Activity following surgery was unchanged, however LMB remained shallower for several days post-surgery. During the winter, LMB used deeper waters and exhibited reduced activity, then moved shallower and increased activity with increasing temperature. Diel cycles of activity and depth use were absent, though activity appears to be highest during twilight. Heart rate data were poor quality and required manual calculation but adjusted methods are encouraging for later deployments. Nonetheless, heart rate showed a strong positive relationship with temperature (from 5 – 98 bpm between 2°C and 28°C) and thermal sensitivity calculations of heart rate indicate that metabolic rate depression was not involved during their overwinter periods (assuming that there is a strong relationship between metabolism and heart rate in LMB). Calibration studies are ongoing that will soon facilitate

the quantification of free-swimming energy expenditure in LMB. These measures will be used in bioenergetics models to better understand their responses to changing winter conditions.

The Effects of INSL5 on Splenic Dendritic Cell Function: an Investigation Into DSS-induced Colitis and Spleen Hypofunction

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Abstract: Inflammatory bowel disease (IBD) is characterized by chronic intestinal inflammation caused by aberrant host-microbial interactions due to impaired mucosal barrier function. The two forms of IBD, Crohn's disease and ulcerative colitis, differ in the area they affect, the extent of inflammation, and in disease-specific pathophysiology. In addition to dysregulated inflammatory responses, IBD is associated with reduced splenic function in human patients. The spleen is a secondary lymphoid organ, and its disruption impacts an individual's ability to clear infections. Recently, the novel peptide hormone, insulin-like peptide 5 (INSL5), and its cognate receptor, relaxin family peptide receptor 4, have demonstrated wide expression in immune tissues and cells, particularly splenic dendritic cells (sDCs). The hormone-receptor pair exhibited anti-inflammatory properties in a murine macrophage cell line by reducing the expression of pro-inflammatory mediators and cell proliferation. This study aimed to determine whether dextran sulfate sodium (DSS)-induced colitis can be used as a model for hyposplenism and investigated the role of INSL5 in modulating immune responses of sDCs. Colitis was induced in mice using 2% DSS for seven days before harvesting spleens and sampling blood. Splenocytes were isolated and enriched for sDCs in control and DSS-treated mice and subjected to one of five treatments; 1) control, 2) INSL5, 3) LPS, 4) INSL5/LPS cotreatment, and 5) INSL5 pretreatment followed by LPS stimulation. Blood samples were differentially stained to examine abnormalities in circulating erythrocytes, and histological analyses were performed on splenic tissue. Expression analysis was performed on whole-spleen and sDCs to examine changes in immune mediators. We find abnormalities in blood cells and alterations in the architecture of the spleen in DSS-treated mice and predict that INSL5 treatment will reduce the expression of pro-inflammatory mediators in sDCs. Since DCs play a role in initiating inflammation in IBD, this study may contribute to cell-specific therapeutics.

The Time Course of Cardiac Thermal Acclimation in a Eurythermal Fish, the Mummichog (*Fundulus heteroclitus*)

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**Presenting Author*

Abstract: Environmental temperature can vary greatly in aquatic habitats over time scales from hours to months. In fishes, the heart is an important mediator of the effects that high temperatures have on whole-animal performance. During acute warming, fishes can meet rising oxygen demands by accelerating heart rate but only until a maximum temperature at which a peak heart rate is reached. If warming continues, the heart can become arrhythmic, which is

closely associated with the upper thermal survival limit. Warm acclimation in fishes commonly includes cardiac plasticity where the cardiac pacemaker rate and the temperature at which peak heart rate and cardiac arrythmia occurs is shifted upward. This improvement of cardiac heat tolerance and resetting of heart rate can occur remarkably rapidly in salmonids (within 24-72 hrs). We do not know, however, whether rapid cardiac thermal acclimation is found widely among fishes or to what degree the speed or magnitude of the acclimation response varies depending on species' thermal niche. Using ECG and mRNA analysis, I am investigating the time scale and scope of cardiac thermal acclimation and underlying mechanisms in the eurythermal estuarine species, the mummichog (*Fundulus heteroclitus*); mummichog experience rapid and substantial temperature fluctuations with the tides as well as large seasonal thermal variation. In response to an increase in acclimation temperature from 15 to 30℃ for up to 4 weeks, my emerging results indicate mummichog undergo among the fastest cardiac thermal acclimation known in fishes (within 12 hours).

Using Mucus to Assess Gene Expression in Fathead Minnows Exposed to Graded Nutrient Loadings

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Abstract: A rising demand for nutritious and low-fat foods has resulted in the intensification of both the wild rice and finfish aquaculture industries. Wild rice (*Zizania palustris)* requires large amounts of phosphorus and ammonia-based nitrogen to achieve high yields. Whereas finfish aquaculture operations produce wastewater high in phosphorus from uneaten feed, and ammoniacal nitrogen from nitrogenous excretions. Therefore, using aquaculture wastewater as a fertilizer for wild rice presents a sustainable system of recycling wastewater while supporting optimal plant growth. Yet, adding excess nutrients to an ecosystem presents significant ecological risks, including the addition of ammonia, which is toxic at nominal concentrations, posing a threat to aquatic life. Furthermore, increasing phosphorus loadings may lead to eutrophication, where aquatic ecosystems can be depleted of oxygen from the proliferation of algae and aquatic macrophytes. We assessed the ecotoxicological risks of adding aquaculture wastewater to an aquatic ecosystem by measuring both gene and protein expression in the mucus of fathead minnows (*Pimephales promelas*) chronically exposed to incremental additions of aquaculture wastewater. Our objective is to develop minimally invasive techniques to evaluate gene and protein expression patterns in fish using dermal mucus. We aim to validate the use of mucus against liver tissue as a non-lethal alternative for the EcoToxChip, and for proteomic analysis. This study may further advance the use of mucus in genetic analyses and limit lethal sampling requirements in ecotoxicological research.

Using RNA Interference to Determine the Role of Catalase in the Freeze Tolerant Spring Field Cricket *Gryllus veletis*

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Abstract: Many organisms overwinter in temperate climates. Oxidative stress is one of the many challenges faced by animals that must tolerate freezing conditions. The spring field cricket, *Gryllus veletis*, can survive freezing and has high abundance of the mRNA encoding catalase, an antioxidant enzyme that can help combat oxidative stress. To test whether catalase is important for freeze tolerance, we have used RNA interference (RNAi) to knock down production of the catalase protein in freeze-tolerant *G. veletis*. However, this RNAi seems to be temperature-dependent, and is only effective at relatively warm (c. 20°C) temperatures that cause *G. veletis* to lose its freeze tolerance. We are currently determining whether RNAi can succeed at lower temperatures with some protocol modifications. This work will help elucidate the relative importance of one antioxidant enzyme in freeze tolerance, with implications for future work that aims to understand the interaction between animals and their complex environmental stressors.

Does Kisspeptin-10 Affect Lysozyme Activity in Female Sterlet Sturgeon, Acipenser Stellatus?

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Abstract: Kisspeptin is a major endocrine regulator of reproductive hormones and reproduction in vertebrates. In the current study, we tested whether peripheral kisspeptin administration affect non-specific immune responses in vitellogenic-stage Sterlet sturgeon, *Acipenser ruthenus*. The sturgeon decapeptide kisspeptin-10 (kp-10) was synthesized, then Sterlet (initial average weight of 1295.6 ± 277.6 g; mean ± S.D.) was intraperitoneally (i.p) injected and implanted with 250 ng/kg body weight. Lysozyme (Lys) activity within blood plasma and epithelium surface was determined in vehicle controls and kp-10 treated sturgeons. Lys activity was assayed a day post-i.p. injection, four-week post-i.p. injection, and four-weeks after implantation. Based on our results, Lys activity in plasma was significantly increased in kp-10 treated sturgeon ($P < 0.05$), and multiple comparisons showed it considerably increased 24 hr. post injection. Meanwhile, there was no significant difference in plasma Lys activity between kp-10 and control groups at four-week post injection ($P > 0.05$). Epithelial surface showed very-low levels of Lys after four-week implantation. Lys activity of epithelium significantly increased over slow-releasing of kp-10 ($P < 0.05$). Overall, our results show that peripheral administration of kp-10 increases Lys activity in female Sterlet sturgeon. This supports an immunomodulatory role for kp-10 in sturgeon, and warrants further studies to gain a deeper knowledge on this topic.

Packing on the Pounds: How do Hummingbirds Fatten for Migration?

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Abstract: One of the most important roles we have as scientists is communicating our research to the public in effective and accessible ways. Here, I use my recent postdoctoral research as a case study to explore diverse ways in which we can communicate research with the public, particularly aspiring young scientists. My research focuses on the role of the leptin in facilitating migratory fattening in ruby-throated hummingbirds (*Archilochus colubris*). In mammals, leptin is an important energy homeostasis hormone produced by adipose tissue. Circulating leptin concentrations correlate positively with fat mass and act in a negative feedback fashion to inhibit food intake and increase energy expenditure, thereby preventing excessive fat gain. For some species, leptin resistance is advantageous during times of year where fat gain is necessary (e.g., prior to hibernation). Although the function of leptin in birds remains controversial, we tested the hypothesis that leptin resistance promotes fattening in hummingbirds prior to migration. We predicted that during the migratory fattening period, leptin levels should correlate positively with fat mass but should not inhibit food intake or increase energy expenditure, resulting in excess fattening. We tracked the body (fat) mass, urine leptin concentration, and food intake of 12 captive hummingbirds from August 2021 to January 2022. In a subset of hummingbirds, we also quantified voluntary physical activity as a proxy for energy expenditure. We found remarkable age-related variation in fattening strategies, with juveniles doubling their body mass by mid-September and adults exhibiting only a 50% increase. Changes in fat mass were strongly associated with increased food intake and reduced voluntary activity. However, we found no correlation between leptin concentration and fat mass, food intake, voluntary activity. Overall, our findings suggest that leptin may not act as an 'adipostat' in hummingbirds, nor does leptin resistance regulate how hummingbirds fatten excessively prior to migration.

The Significance of Variation in the C-start Response Among Fishes With Differing Overwintering Strategies

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Abstract: During winter, certain fish species remain active while others become dormant. We hypothesized that winter dormancy is a survival strategy that arises in poleward species that experience severe, uncompensated constraints of cold on physiological performance. However, to date we have found that cunner (*Tautogolabrus adspersus*), a winter-dormant fish, shows partial cold compensation of nearly all metabolic and exercise performance metrics studied, similar to winter-active species. Only responsiveness to stimuli during the C-start escape response was greatly constrained in the cold even after acclimation, suggesting a sensory limitation that may help explain the need to become dormant. To explore this idea, we measured the C-start escape performance of 6 phylogenetically diverse species along the spectrum

of overwintering strategies from winter-dormant to winter-lethargic to winter-active, including cunner, goldsinny wrasse (*Ctenolabrus rupestris*), pumpkinseed sunfish (*Lepomis gibbosus*), common killifish (*Fundulus heteroclitus*), brook char (*Salvelinus fontinalis*), and Atlantic cod (*Gadus morhua*). Warm-acclimated fish (~15°C) were exposed to either acute thermal change to ~9°C or ~3°C (winter transition and cold winter temperatures, respectively) or acclimated (3-5 weeks) to ~15 or ~3°C and then assessed for C-start performance using high-speed videos to measure maximum burst velocity and acceleration, response latency, and responsiveness to stimuli. As we predicted, winter-active cod responsiveness was similarly high (~90%) across all temperatures, as were the moderately winter-lethargic killifish (~65% responsive across all temperatures). Winter-lethargic pumpkinseed sunfish and winter-dormant goldsinny wrasse exhibited similarly constrained responsiveness to cunner with cooling below ~9°C, though goldsinny responsiveness increased following acclimation to ~3°C. We are currently analyzing other parameters and will be investigating an emerging possibility that photoperiod may influence whether or not species partially compensate their responsiveness. Our evidence to date suggests that severely impaired responsiveness to C-start stimuli among winter-dormant fishes may indeed be a weak thermal link driving dormancy as an overwintering strategy.

Emerging Antimicrobial Resistance Hiding in Plain site

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Abstract: Antimicrobial resistance genes (ARGs) that are shared between disparate biological niches pose significant risks to the practice of human and veterinary medicine. Moreover, AMR surveillance systems are incomplete: unreported ARGs that have been disseminated between environmental and animal, including human, reservoirs exist. The discovery of these known unknowns remains essential to better inform AMR surveillance, stewardship programs and clinical practice. As part of our recent AMR surveillance efforts at beef cattle feedlots in western Canada, we isolated a multidrug resistant bacterium and identified that it carried a plasmid-borne cluster of known ARGs (*tetX*, *floR* and *erm35*) that included an uncharacterized α/β-hydrolase. A search for homologs of this α/β-hydrolase showed that the orphaned gene has a cosmopolitan distribution in animal microbiomes and pathogen genomes. Notably, it had also been routinely reported alongside *tet(X4)*, an emergent tigecycline resistance gene that has been the focus of several One Health studies. By performing a phenotypic screen of AMR using a panel of feedlot-specific antibiotics, macrolides were identified as the substrates for this previously unstudied enzyme. Recombinant gene expression and production of the enzyme, which we named EstT, demonstrated that it is an esterase capable of hydrolyzing the macrocyclic lactone rings of tylosin and related antibiotics. Our discovery of EstT and our work to re-identify and characterize a second member of the a/b-hydrolase superfamily that has been overlooked as an ARG for decades will be discussed in the context of current and future antimicrobial use.

Mitochondrial Physiology in Cardiac Muscle of Deer mice Native to high Altitude

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Abstract: High-altitude environments are characterized by cold temperatures and low O_2 levels (hypoxia). Small mammals at high altitude thus face the metabolic challenge of maintaining thermogenesis to cope with cold in a hypoxic environment that can constrain aerobic ATP supply. Circulatory O_2 delivery by the heart is essential for supporting tissue O_2 demands, but it is unclear whether evolved or plastic changes in cardiac mitochondria help overcome constraints on thermogenesis in high-altitude environments. We examined this issue in deer mice (*Peromyscus maniculatus*). Mice from populations native to high altitude and low altitude were born and raised in captivity, and adults were acclimated to warm (25°C) normoxia or cold (5°C) hypoxia (~12 kPa O_2 for 5-6 weeks) in a full-factorial design. Mitochondrial function was studied by high-resolution respirometry and fluorometry in permeabilized tissue from left and right ventricles and was complemented by assays of several metabolic and antioxidant enzymes. Mitochondrial capacities for oxidative phosphorylation and electron transport were similar between populations and were unaffected by acclimation to cold hypoxia, as were activities of citrate synthase and cytochrome oxidase. However, exposure to cold hypoxia increased activities of lactate dehydrogenase, which were also greater in highlanders than in lowlanders, likely to augment capacities for lactate oxidation. Furthermore, mitochondrial emission of reactive oxygen species (ROS) was lower in highlanders than in lowlanders across environments, associated with lower levels of lipid peroxidation. Therefore, phenotypic plasticity and evolved changes in cardiac mitochondria help deer mice cope with metabolic challenges at high altitude.

Neural Encoding of Motion Parallax in Avian Pretectal Neurons of Zebra Finches (*Taeniopygia guttata*)

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Abstract: Optic flow is the global image motion that results from self-motion and is experienced by locomotory animals. Humans experience optic flow through pathways in the midbrain and cerebellum, which is critical for maintaining posture, locomotion, and navigation. Motion parallax is a subset of optic flow produced during an animal's self-motion. Motion parallax occurs when an animal moving at a constant speed perceives objects that are closer to themselves as moving at a greater speed than objects that are further away. In avian species, the midbrain cerebellum is considered to be a homolog for encoding self-motion as in humans. Small birds such as zebra finches (*Taeniopygia guttata*) can execute rapid and robust visuomotor transformations. Visual processing in avian species occurs in the pretectal nucleus, lentiformis mesencephali (LM), of the midbrain which receives input from the retina. The LM in birds is known to be highly conserved within all vertebrates that detect translational and rotational visual flows. Recent studies conducted using pigeons (*Columba livia*) have resulted in identifying LM neurons that encode motion parallax. We hypothesize that

in zebra finches, there will be localization of motion parallax activated neurons within the LM. We presented anesthetized zebra finches with random dot stimuli containing motion parallax. The theoretical framework in the midbrain pathway integration in detecting motion parallax will contribute to the growing knowledge of the avian brain structures and how vision is encoded within neural pathways. Understanding neural connections in anesthetized birds could unlock insights into how birds contend in dynamic, three-dimensional natural environments.

How do Frozen Crickets Maintain Mitochondrial Function?

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Abstract: Frozen cells are rarely viable, likely because organelles such as mitochondria are susceptible to freeze-injury. However, the freeze-tolerant spring field cricket, *Gryllus veletis*, has normal – or even increased – whole organism metabolic rate after freezing. Do freeze-tolerant crickets protect their cellular metabolic machinery from freeze-injury? We measured mitochondrial function from Malpighian tubules, a highly metabolically active tissue, and evaluated changes in mitochondrial ultrastructure after freezing with EM. We compared acclimated (*i.e.*, freeze-tolerant) and unacclimated (*i.e.,* freeze-intolerant) crickets to identify changes associated with freeze tolerance acquisition. We found that acclimated crickets had lower state 3 respiration than their unacclimated counterparts, indicating that freeze-tolerant crickets have lower mitochondrial activity. We compared frozen freeze-intolerant crickets with frozen freeze-tolerant crickets to identify potential mechanisms that permit survival from freezing. We also compared unfrozen cold-exposed crickets with frozen crickets to disentangle the effects of cold and ice formation *per se*. Freezing does not appear to damage the outer mitochondrial membrane, but frozen freeze-intolerant crickets had evidence of damage to the inner mitochondrial membrane. These damaged crickets had increased proton leak and decreased state 3 respiration, and the inner membrane was permeable to NADH. TEM showed swollen mitochondria with fewer cristae in freeze-intolerant frozen crickets. Thus, freeze-tolerant crickets appear to protect their inner mitochondrial membrane from freeze-injury, allowing them to fuel the metabolic demands of recovery, whereas insects that cannot survive freezing incur mitochondrial damage.

What's the worm? Characterizing the Equine Nemabiome in Saskatchewan Horses

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Abstract: Drug resistance is increasing in many equine nematode parasites, including the small strongyles (cyathostomes) and ascarids. Current diagnostic methods are based on the detection and quantification of parasite eggs recovered from fecal samples. With over 53 species of strongyles infecting horses, all having nearly identical eggs and varying degrees of pathogenicity, this method is flawed and outdated. The aim of this project is to use a curated sequence database with DNA metabarcoding and next generation sequencing to characterize the nemabiome of domestic horses in Saskatchewan, Canada.

Fecal samples were collected from horses in fall and spring and fecal egg counts (FECs) were performed on samples before and after owner-administered treatment. Information was collected from owners through online surveys. Third-stage larvae were cultured from samples that contained > 300 strongyle eggs per gram (EPG). DNA was extracted from pooled L3s, and assayed using high-throughput, parallel sequencing of the PCR-amplified internal transcribed spacer 2 (ITS2) region. Sequences were pushed through two different bioinformatics pipelines, compared to an equine GI nematode database, and taxonomically classified. A total of 231 horses were sampled for FEC and 95 samples were sequenced. 34 total species were identified. Three samples were sequenced after anthelmintic treatment for evidence of lack of efficacy. *Cyathostomum catinatum, Cylicocyclus nassatus*, and *Cylicostephanus longibursatus* were the most prevalent species. Differences existed in the nemabiomes of domestic vs wild horses. *Strongylus spp.,* known for their increased pathogenicity, were only seen in 3 of our samples, accounting for 0.15% of fall and 0% of spring ASVs. This greatly contrasts results from the Sable Island feral horse population, where 86% of all ASVs belonged to *Strongylus spp.* Bioinformatics methods used greatly affected the resulting species found in samples, with our pipeline showing species never having been identified in previous studies.

Investigation of Hormonal Control of Energy Balance During the Stress Response in an Elasmobranch fish

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Abstract: The vertebrate stress axis is largely conserved across taxa and is fundamental in maintaining energy balance following a stress event. Corticosteroids, specifically glucocorticoids, secreted from the interrenal gland in teleosts and elasmobranchs, act to regulate 1) energy mobilization primarily from the liver and 2) use by extrahepatic tissues, with the objective of reestablishing energy balance. Elasmobranchs are a phylogenetically ancient group that may represent a primitive stress phenotype, but our understanding of the endocrine regulation of energy balance is complicated by their reliance on ketone bodies (e.g., ß-hydroxybutyrate; ß-HB) as compared to fatty acids, and the uncertain actions of corticosteroids. Therefore, to investigate the role of prospective glucocorticoids in energy metabolism in elasmobranchs, a representative species, the North Pacific spiny dogfish (*Squalus suckleyi*), was first subject to surgical cannulation, air exposure, and confinement to initiate the stress response; serial blood samples were taken over 24hrs and analyzed for blood metabolites involved in energy balance (e.g., glucose, ß-HB) and corticosteroids. While both metabolites increased following the series of stressors, plasma corticosteroids were only weakly correlated with

measured metabolites. Next, tissues from air-exposed dogfish were sampled for enzyme activity and mRNA transcript abundance of genes associated with metabolite balance. The glucocorticoid receptor and presumptive ketone body transporter transcripts in the liver decreased 1hr post-air exposure, which corresponded to a trending decrease in ß-HB dehydrogenase activity (ß-HB production). Further, *glut1* and *glut4* (glucose transport) transcripts increased 30min post-air exposure in white muscle, possibly indicating a reduction in translation from mRNA to protein to reduce glucose transport into myocytes. However, none of these metrics were correlated with measured plasma corticosteroids and plasma metabolites did not change post-air exposure. Finally, in both studies plasma metabolite concentrations were inversely related, suggesting preferential fuel use during these stress events; potentially, a reduction in hepatic ketone body mobilization.

Mass Spectrometry Suggests Time-Dependent Regulation of Stretch Signaling, cell Membrane Homeostasis, and Myocardial Tension During Cold Acclimation in Zebrafish (*Danio rerio*)

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Abstract: Zebrafish (*Danio rerio*) in their natural environment remain active across a range of temperatures that vary seasonally by at least 10°C. This is impressive considering the effects of temperature on biological processes. The thermal range of ectothermic fish is determined by the capacity of the heart to function at different temperatures. Our experiment evaluated the changes in the cardiac proteome and phosphoproteome during the first 6 weeks after transferring adult zebrafish from 27°C to 20°C. We used mass spectrometry to identify the differences in protein abundance and phosphorylation between cold-acclimated fish and controls held at 27°C. Our results indicate that, within the first week, cold acclimation increases the abundance and phosphorylation of stretch-sensitive and phospholipid biosynthetic proteins. There is also increased phosphorylation of proteins involved with tension activation in the myofilament and with cytoskeletal organization. By two weeks, there are decreases in the abundance of proteins involved with protein and lipid biosynthesis and in mitochondrial structure and function and increases in cellular and myocardial remodeling proteins. By six weeks, the decreases in abundance of biosynthetic proteins persist while there is also an increase in abundance of proteins involved with interspecific interactions and decreased abundance of remodeling proteins. These proteomic changes occur concurrently with decreased metabolic rate but maintenance of body condition and appetite, suggesting that cold-acclimated zebrafish can satisfy energetic demand and delivery despite metabolic depression. The results of this study, suggest that the maintenance of cell membrane homeostasis and stretch signaling are important early in the cold response while changes in the abundance and activity of proteins associated with myocardial tension activation are significant throughout. This work was funded by NSERC.

CRHR1 Modulates the Organismal Stress Response Independent of Cortisol

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Abstract: Temperature changes are an important stressor and fishes respond by increasing their stress axis activity. Corticotropin-releasing hormone receptor 1 (CRHR1) is the primary step in this stress axis activation leading to cortisol release. Recently, we showed that CRHR1, independent of cortisol stimulation, was also important for eliciting the acute stress-related behavioural changes in zebrafish (*Danio rerio*) larvae. Here, we investigated whether temperature shifts may affect the locomotor activity of adult zebrafish by modulating CRHR1 signaling. We used a ubiquitous CRHR1 knockout (CRHR1KO) zebrafish and subjected them to a heat shock (HS; +5°C for 60 min). The wildtype (WT) fish responded to HS by increasing cortisol production while CRHR1KO fish failed to elicit a cortisol response to HS. The WT fish showed greater swimming activity and higher metabolic rate during HS, but this was absent in the CRHR1KO fish. We tested whether elevated cortisol may be involved in the higher activity and metabolic rate in the WT fish exposed to HS. We used metyrapone to suppress the cortisol response, but this did not reduce the HS-induced activity and metabolic rate. This raised the possibility that CRHR1, independent of cortisol, may also be affecting HS-mediated metabolism, and this was tested by exposing WT and CRHR1KO zebrafish to HS. The muscle molecular (transcript abundance of a suite of metabolic genes) and biochemical responses (metabolites and enzyme activities) did not vary between the WT and CRHR1KO fish in response to HS. This lack of HS response may be associated with the shorter duration (60 min) of the HS exposure. We are currently also assessing the HS response in the liver and brain of WT and CRHR1KO fish. Overall, CRHR1 may modulate the cellular and acute organismal stress response to HS, and this may be independent of its role in activating the cortisol stress response.

Wing Deformations in Steady and Maneuvering Hummingbird Flight

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Abstract: Animals exhibit rich diversity in body shapes, but the functional complexity of soft tissues is only hinted at by static measurements. How do we define the shape of a tissue whose function and evolution depend on its deformations? In fluidic locomotion, deformations of fins, wings, and tails (propulsors) are key elements contributing to properties ranging from propulsive efficiency to environmental navigation. Our notions of what constitutes propulsor shapes and how to measure them are typically derived from two-dimensional posed specimens, possibly motivated by theoretical considerations based on rigid, engineered craft. However, it is unclear whether such measurements are sufficient for capturing real biological shape variation and differences between steady and maneuvering motor patterns. I present work on hummingbird wing shape variation in different flight contexts, studied through high-speed marker tracking. Modal analyses (e.g., singular value decomposition) are highly effective at explaining principal axes of shape

variation throughout the flapping stroke cycle, and moreover are strongly linked to many typical aspects of shape variation, such as wing twisting and cambering. More importantly, shape modes distinguish spatiotemporal signatures of shape variation associated with elements of the stroke cycle, such as the mid-half strokes and stroke reversals. Through these functions, we can identify temporal intervals associated with specific shape changes among distinct flight behaviours. Our approach offers a basis for studying dynamic shape variation and its contribution to behavioural diversification, especially the evolution of agility and fine motor control.

A Circumpolar Parasite: Evidence of a Cryptic Undescribed Species of Sucking Louse, *Linognathus* sp., Collected from Arctic Foxes, *Vulpes lagopus*, in Nunavut (Canada) and Svalbard (Norway).

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Abstract: The North has experienced unprecedented rates of warming over the past few decades, impacting the survival and development of insects and the pathogens that they carry. Since 2019, Arctic foxes from Canada (Nunavut) have been observed with fur loss inconsistent with natural shedding of fur. Adult lice were collected from Arctic foxes from Nunavut and Svalbard (Norway) and were identified as sucking lice (suborder Anoplura). Using conventional PCR targeting the mitochondrial cytochrome c oxidase subunit 1 gene (cox1), lice from Canada and Svalbard were 100% similar, indicating that there is gene flow between ectoparasites on Scandinavian and North American Arctic fox populations. The cox1 sequences of Arctic fox lice and dog sucking lice (*Linognathus setosus*) had significant differences (87% identity), suggesting that foxes may harbour a cryptic species that has not previously been recognized. Conventional PCR targeting the gltA gene for *Bartonella* bacteria amplified DNA from an unknown gammaproteobacteria from two pooled louse samples collected from Svalbard foxes. The amplified sequences were 100% identical to each other but were only 78% like *Proteus mirabilis* reported in GenBank (CP053614), suggesting that lice on Arctic foxes may carry unique microorganisms that have yet to be described.

Dermacentor Ticks in Saskatchewan: Molecular Barcodes, Sympatric Populations, *Rickettsia* Prevalence and Abundance in dog Parks — not so Boring After all!

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**Presenting Author*

Abstract:

Ticks are haematophagous ectoparasites of vertebrates and effective vectors of pathogens. While *Dermacentor* species are not associated with Lyme disease, they are the dominant ticks in western Canada and can transmit the causative agents of Rocky Mountain spotted fever, Tularaemia and Colorado tick fever. Additionally, *D. andersoni* (Rocky Mountain wood tick) is associated with tick paralysis. Until recently, only three species of *Dermacentor* ticks were thought to occur in Canada: *Dermacentor albipictus* – the winter tick – in boreal regions, while *D. andersoni* occurs westward and *D. variabilis* (American dog tick) eastward from Saskatchewan. A fourth species, *D. similis*, is now recognized in western Canada and the USA. Off-leash dog parks, nature conservancies and an agricultural site in Saskatchewan were surveyed using standard tick dragging/flagging methodologies. Three widely used molecular markers – 16S, COX1 and ITS2 – were capable of distinguishing among all four species, and molecular and morphological identification had perfect congruence. Tick abundance was, surprisingly, lower in periurban dog parks when compared to conservancies and agricultural land. Two newly discovered sympatric populations of *D. andersoni* and *D. variabilis* are reported here, including the first record of *D. andersoni* east of Regina. No tick hybridisation was detected. No pathogenic *Rickettsia* species were detected using a PCR targeting ompA. DNA of known endosymbionts *Rickettsia peacockii* was detected in all *D. andersoni* specimens, while *R. montanensis* was detected in only 3.2% of *D. variabilis*. Since ticks generally host only one *Rickettsia* species, *D. variabilis* are deemed more colonizable by pathogenic *Rickettsia* species. This work shows that frequent use of environments by dogs may alter the abundance and diversity of ticks, that *D. variabilis* has higher potential to vector pathogenic *Rickettsia*, and that pockets of *D. andersoni* remain in sympatry with *D. variabilis* at the interface of their distributions in Canada.

Seasonal Patterns in Metabolism and Energetics in Earth's most Northerly Distributed Freshwater fish, the Arctic char (*Salvelinus alpinus*)

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**Presenting Author*

Abstract: Arctic fishes, such as Arctic char (*Salvelinus alpinus*), spend a vast majority of their lives under extreme winter conditions including frigid temperatures and limited food availability. Adult anadromous Arctic char feed in the ocean during summer, but then return to freshwater to avoid freezing over winter and do not feed (10 months/year). We examined the seasonality of Arctic char metabolic requirements and energy saving mechanisms by measuring seasonal changes in activity, organ somatic indices and tissue composition, and metabolic rate in wild anadromous Arctic char in Nunavut's Kitikmeot region. Arctic char nearly ceased activity over winter. After winter, Arctic char had markedly decreased digestive organ masses but conserved heart mass compared with end of summer fish. Standard metabolic rate was lower after winter compared with end of summer, probably a result of fasting overwinter. However, maximum aerobic metabolic rate and aerobic metabolic scope was conserved even after the long winter, consistent with the maintained cardiac mass. We simulated these overwintering conditions in the laboratory and found that prolonged

fasting resulted in a substantial reduction in protein synthesis rates, and specific organ masses, and a substantial reduction in standard metabolic rate while maximum metabolic rate was conserved. The marked similarity of our field and lab results illustrates the usefulness of careful lab-based simulation of environmental change, and the decoupling of changes in maximum and resting metabolic rates during fasting may be an important strategy to save energy while retaining performance. Overall, the passive slowing effects of cooling, activity suppression, and the decreased resting metabolism and selective tissue atrophy during fasting over winter are likely crucial energy conservation strategies used by Arctic char to facilitate their extraordinary feast and famine lifestyle in extreme cold.

Costs of two Larval Trematode Stages to the life History Traits of two Freshwater Snail Species

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**Presenting Author*

Abstract: Density-dependent prophylaxis predicts hosts will invest more in immune function in response to increased population density, as infection risk is expected to increase with host population density. This hypothesis assumes that investment in immune defense to avoid or minimize infection is costly for hosts, but prophylaxis is net positive when infection risk is high. Freshwater snails are common second intermediate hosts for trematode parasites, and they could be used as a model system to better understand the role of density-dependent immunity in aquatic invertebrate-trematode interactions. However, the assumption that trematode cercariae (penetrating stage) and metacercariae (encysted stage) are costly to snails is unclear as the effects could vary by life history trait, host species, host age, and parasite dose. To investigate the costs of larval trematode infection, we exposed adult and juvenile lab raised *Stagnicola elodes* (native) and *Planorbella duryi* (non-native) snails to *Echinostoma trivolvis* lineage A cercariae at dosages of 0, 50, and 100 cercariae. We assessed growth, feeding, fecundity, and survival for 14 days post-exposure, as well as penetration and encystment success. We will discuss the results of generalized linear modelling that we used to determine if parasitism affected these life history traits and whether costs differed between native and non-native hosts. Although parasitism is defined in part by the negative consequences that hosts experience as part of this type of symbiosis, many times those consequences are unknown or unclear. Determining the costliness of these two larval trematode stages may help to validate the assumptions of density dependent prophylaxis and other phenomenon associated with host-parasite interactions.

Assessing Aquatic Invertebrate Nickel Sensitivity Under Arctic Exposure Scenarios

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Abstract: Nickel (Ni) contamination in aquatic ecosystems is increasing as demand for this economically important metal rises. Nickel production in Northern Canada has led to contamination of surrounding environments, with both Arctic freshwater and marine ecosystems receiving substantial Ni input. Freshwater Ni toxicity is influenced by abiotic water characteristics (hardness, pH, DOC), with the low ion content of many Arctic waters predicted to increase risk. Marine water composition is typically stable, but heterogeneity in Arctic seawaters may lead to site-specific increases in bioavailability. Consequently, this work aimed to evaluate Ni toxicity to sensitive invertebrates in both Arctic freshwater and marine systems. Freshwater Ni toxicity was evaluated with acute and chronic Ni exposures in the Arctic relevant cladoceran *Daphnia pulex*. Alteration of a single hardness cation to Arctic relevant levels (0.5-10 mg/L magnesium and 5-50 mg/L calcium) reduced 48-hour median lethal concentrations (LC_{50}) for *D. pulex* neonates from 4.90 mg/L in control waters to 3.76 mg/L and 1.57 mg/L, respectively. Co-modification of both ions to Arctic relevant concentrations further increased toxicity yielding LC_{50} values as low as 1.04 mg/L. Chronic exposure to 30 µg/L Ni in Arctic relevant waters slightly altered molt frequency and time to first brood, with no impacts on other reproduction endpoints or metabolic rate. This suggests *D. pulex* are somewhat resilient to low concentration Ni exposure, but acute lethality risk under environmentally relevant conditions persists. Nickel risk in Arctic marine waters was evaluated with green sea urchin (*Strongylocentrotus droebachiensis*) embryos, indicating they are highly sensitive to Ni contamination, with a 96-hour effect concentration (EC_{50}) of 1.38 µg/L for induction of developmental abnormalities. These results taken together provide insight into the mechanism and extent of Ni toxicity in fresh and marine waters under Arctic-specific exposure scenarios, furthering the understanding of the potential risks of Ni in these unique ecosystems.

Effects of Diluted Bitumen Exposure on Hypothalamus-Pituitary-Thyroid (HPT) axis and Olfactory Epithelium in Coho Salmon (*Oncorhynchus kisutch*)

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Abstract: Bitumen, extracted from the Alberta oil sands, is diluted (dilbit) and then transported via pipelines to tidal water in BC. These pipelines cross endangered Pacific salmon habitats and with the recent expansion of the

Transmountain Pipeline, there is an increased risk of spill. There is some evidence that exposure of fish to components of crude oil can disrupt the hypothalamus-pituitary-thyroid (HPT) axis and alter the physiology of the olfactory epithelium. Similar effects of dilbit on Pacific salmon could impair olfactory imprinting, which is essential for the anadromous life history of these fish. We examined the effects of environmentally relevant concentrations of dilbit on the HPT-axis and the olfactory epithelium in early life stages of coho salmon (*Oncorhynchus kisutch*). Coho fry and parr were exposed to uncontaminated water (control), or to one of two concentrations of the water-soluble fraction of dilbit (2.9 and 6.3 µg/L) for 48 h or 4 wk. The expression of several genes related to thyroid hormone activity in the liver were quantified as indicators of HPT axis disruption, including the thyroxine transporter (*oatp1c1*), deiodination enzymes (*dio2, dio3*) and target genes of thyroid hormone signaling (*igf-1, igf-2*). After 48 h of dilbit exposure, there was an increase in the expression of *igf-1*, *igf-2*, and *dio2* in the livers of fry exposed to 6.3 µg/L of dilbit and *dio2* was also elevated in parr with exposure to 6.3 µg/L of dilbit relative to their respective controls. After 4 wk of dilbit exposure, liver *dio2* expression was lower in fry exposed to 2.9 µg/L of dilbit and there was a decrease in expression of *dio3* and *oatp1c1* in fry and parr exposed to 2.9 µg/L of dilbit relative to their respective controls. Plasma thyroxine was elevated by approximately 50% in parr exposed to the 6.3 µg/L of dilbit for 48 h relative to unexposed parr. The olfactory epithelium from these fish is currently being assessed for indications of exposure using histological approaches. Taken together, these findings suggest that the HPT axis of early life stages of Pacific salmon is altered by exposure to sublethal concentrations of dilbit.

Barcoding the Biters: Standard and Molecular Surveillance of Mosquitoes and Disease Potential in Nova Scotia

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Abstract: Shorter, warmer winters linked to climate change in Nova Scotia are inviting the northward expansion of non-native mosquito species and the diseases that they vector. To mitigate the health impacts of mosquito-borne disease, and to understand the ecosystem impacts of these invasions, we must establish regular monitoring of mosquito populations. Using traditional and molecular surveillance methods, alongside vector prediction tools such as disease presence, seasonality, proximity to human populations, temperature, humidity, rainfall, and weather patterns, we can identify at-risk areas for vector-borne disease spread. To assess the current risk from mosquitoes in Nova Scotia, we sampled adult mosquitoes from 50 CDC light trap locations and collected mosquito larvae from 232 water sources across nine ecozones from May 1st to October 25th, in 2021 and 2022. To determine if we could detect invasive mosquitoes solely by the presence of trace DNA in the environment, we collected water from 61 of our larvae sampling sites and used PCR to amplify mini-barcode regions specific to Culicidae using eDNA metabarcoding methods. Of the 6,180 mosquitoes collected using traditional trapping methods, we identified 33 species including seven species not previously recorded in the province: *Aedes decticus*, *Aedes pionips*, *Aedes riparius, Aedes sticticus*, *Culiseta minnesotae*, *Culiseta melanura* (an important vector of Eastern Equine Encephalitis Virus and West Nile Virus), and *Culex salinarius* (a known vector of Eastern Equine Encephalitis Virus and West Nile Virus). We have also observed the province-wide expansion of *Aedes japonicus* since the initial detection of the species in Nova Scotia in 2008. Overall, the diversity of mosquitoes

in Nova Scotia appears to be increasing, highlighting the potential for increases in vector-borne disease transmission as climate change progresses, furthering the need for continued monitoring.

Peripheral Effects of Serotonin Transporter (Sert) Knockout in Zebrafish, *Danio rerio*

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Abstract: In zebrafish, two serotonin transporters, Serta (Slc6a4a) and Sertb (Slc6a4b), function in reuptake of the neurotransmitter serotonin from the synapse to the presynaptic neuron, and in the uptake of serotonin into peripheral tissues. The present study used gene editing technology (CRISPR-Cas9) to develop Serta and Sertb knockout (KO) zebrafish lines to investigate the role of serotonin in stress axis function. For both *serta* and *sertb*-KO, an early stop codon within the third exon was confirmed by Sanger sequencing. Assessment of transcript abundances between wildtype and *serta*-KO fish of targets within the serotonergic system revealed elevated *sertb* in *serta*-KO larvae but not adult fish. Serotonin receptor *5-ht1aa* and *5-ht1ab* were elevated in both larvae and adult zebrafish brain whereas expression of the serotonin degradation enzyme monoamine oxidase (*mao)* decreased in larvae but increased in adult brains. Additionally, transcript abundances of *serta* and *sertb* were measured across a range of tissues in wildtype adult fish. Transcript abundances of *sertb* were low in all peripheral tissues relative to the brain, whereas *serta* exhibited higher and similar transcript abundances in heart and gill tissues respectively relative to the brain. Cortisol levels were measured under baseline conditions and following exposure to a stressor in larvae and adult wildtype and *serta*-KO fish. Collectively, these findings suggest that Serta and Sertb have tissue-specific roles within the peripheral system. Ongoing studies are investigating the roles of Serta and Sertb in the periphery.

Effect of Background risk on Associative Learning Performance of Zebrafish (*Danio rerio*)

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Abstract: Repeated exposure to high-risk environments is known to induce high-risk phenotypic expression in aquatic prey. These high-risk phenotypes display antipredator response to any novel odour they encounter as well as improved ability to learn and retain predator-related information. We conducted two experiments where we induced high-risk phenotype in zebrafish by exposing them to risk for five consecutive days (three times a day) via injections of damaged conspecific skin (chemical alarm cues) which reliably indicates a predator risk. Then, high-risk and control individuals (exposed to water) were subjected to multiple training trials in a plus-shaped maze to associate a visual cue (red card)

with a social reward (a group of conspecifics).We gave eight and twenty training trails to zebrafish during the first and second experiment, respectively. During the test trial for each experiment, we evaluated their learning efficiency through latency to approach the rewarded arm, the number of correct and incorrect arm entries, and time spent in the correct and the incorrect arm. The high-risk individuals were able to associate the visual cue with the reward; however, individuals in the control group could not establish the association after eight training sessions. On the other hand,low-risk individuals showed associative learning performance after 20 trials. These results suggest improved associative learning ability of high-risk phenotypes with social reward.

Transcriptional, Physiological, and Behavioural Responses of lake Sturgeon Exposed to Interacting pCO_2 and Temperature During Development

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Abstract: The effects offreshwater acidification areless understood than those in the marine environment, butare likely to have important ecological consequences for fishes worldwide with current trendsin climate change. The lake sturgeon (*Acipenser fulvescens*) was used to study transcriptional, physiological, and behavioural responses to the interacting effects ofsustained exposure to elevatedpCO_2 and temperature, particularly in a vulnerable post-overwintering period in age-0 individuals. We conducted a long-term exposure experiment during the first year of lifeto determine the impacts on tolerance to an acute change in pCO_2, mimicking a spring melt.Experimental groups includeda control (15 °C, 1,000 µatm pCO_2), increased temperature (22 °C), increased pCO_2 (2,500 µatm pCO_2), and the combination of increased temperature and pCO_2 (22 °C, 2,500 µatm pCO_2). Each group was reared in their respective environmentsfor approximately 7 months, followed by2 months of simulated overwintering at 3 °C. Following the overwintering period, all fish were exposed to acute hypercapnia of 10,000 µatm pCO_2, also at 3 °C.Messenger RNA sequencing revealed diverging patterns of transcription, with responses related to protein synthesis and cell death especially prominent in the combined temperature and pCO_2 group. Na^+/K^+ ATPase activity increased in temperature and control groups, but not in groups exposed to pCO_2 throughout development, indicating potential compensatory mechanisms. Routine and maximum metabolic rate were slightly lower in the combined temperature and pCO_2 group, relative to the pCO_2-only group at 720 hours post-acute hypercapnia. Behavioural assays revealed no effect of rearing environment on cue response or boldness, but groups exposed to acute pCO_2-only exhibited decreased activity. These

data highlight the utility of using gill transcriptional activity with physiological and behavioural data to identify responses to key climate variables during early life history, with implications for conservation of the endangered lake sturgeon.

Does mild Hypoxia Affect Olfaction in a Marine fish?

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Abstract: Due to changes in land use practices, hypoxia is becoming a growing concern in the ocean, especially in coastal areas. Other climate change conditions such as ocean acidification have been shown to impair the sensory systems of marine fish, however the effects of hypoxia on sensory systems is greatly understudied. Olfaction is an important sense, allowing fish to sense over long distances, helping them to locate food, and avoid predators. To determine if hypoxia affects olfaction in a marine fish, threespine stickleback (*Gasterosteus aculeatus*)were exposed to either control (8.5 mg/L O_2, ~90% saturation) ormild hypoxia for one week (4.0 mg/L O_2, ~50% saturation), and their behavioural response to shark bile, a predator cue, was assessed using a choice flume. In response to shark bile, fish in control conditions did not avoid the odorant (p=0.48), but they significantly increased the amount of time spent freezing (p=0.02), another form of anti-predator behaviour. A combined predator response score, consisting of the amount of time avoiding the odorant or freezing in response was also calculated.The control fish had a strong response to the odorant (p=0.01) showing that sticklebacks responded to the predator odour. There was no significant difference in avoidance behaviour between control and hypoxia exposed fish (p=0.98). However, there was a significant difference in freezing behaviour between the groups withhypoxia exposed fish spending significantly less time freezing in response to a predator cue (p=0.04). This shows that hypoxia had a direct effect on the ability of stickleback to detect and respond to an ecologically relevant odorant. This has implications for how the increased prevalence of coastal hypoxia may impact the sensory physiology of marine fishes and how they respond to predators. Future studies will investigate whether more severe hypoxia would have more severe effects on olfaction in sticklebacks.

Hypoxia-Mediated Changes in Uncoupling Protein Expression in Naked mole-rats

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Abstract: Animals that live in intermittently hypoxic environments often experience deleterious bursts of reactive oxygen species (ROS), which can cause oxidative damage and cell death. Uncoupling proteins 1-5 (UCP1-5) are a group of pore-forming inner mitochondrial membrane proteins whose activation uncouples the electron transport chain (ETC), reducing the mitochondrial membrane potential and the generation of ROS. However, uncoupling the ETC also reduces the efficiency of mitochondrial oxidative phosphorylation, and thus aerobic ATP production. We hypothesized that, upregulation of UCP expression during hypoxia may be an adaptive strategy to reduce hypoxia-induced ROS bursts, whereas downregulation of UCP expression would increase the efficiency of oxidative phosphorylation. To better

understand the role of UCPs in hypoxia, we exposed hypoxia-tolerant naked mole-rats to 4h of *in vivo* normoxia or hypoxia (7% O_2; n = 6 each) and measured the expression of UCP4 and UCP5. We found that following hypoxia, UCP5 expression increased in liver, decreased in kidney, and was unchanged in muscle and heart. Furthermore, UCP4 expression decreased in brain and kidney but increased in muscle and heart. Our findings suggest that naked mole-rats alter UCP expression in hypoxia, presumably to meet the specific needs of individual tissues.

How do Overwintering Insects keep Track of time? The Impacts of Temperature on Apple Maggot fly Diapause

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Abstract: The apple maggot fly *Rhagoletis pomonella* relies on seasonally available resources (e.g. apple fruits), and must regulate their life history timing to coincide with availability of that resource.Like most temperate insects, *R. pomonella* overwinter in diapause, a dormant state that helps regulate life history timing. In many diapausing species, chilling is assumed to be important for diapause completion, after which insects remain in quiescence until temperatures increase and morphogenesis (morphological development) can resume. The relationship between this post-diapause morphogenesis and temperature is often used to predict when insects will reach a certain developmental stage (e.g. in degree days). Although described as a type of dormancy, diapause is a dynamic developmental process, and we therefore expect temperatureto impact the rate and duration of diapause development.In this study, we exposed groups of *R. pomonella*diapause pupae to different combinations of chilling (4°C) and warming (21°C) to test how temperature impacts diapause duration. We showed that chilling was necessary in early diapause for most individuals, but that diapause development rates were higher (diapause proceeded more quickly) at warm temperaturesin late diapause development. Although there was high interindividual variation in rates of early diapause development, synchronization of development time after prolonged chilling occurred in the absence of quiescence. Rather, low interindividual variation in late diapause development rates appeared to drive this synchronization. Accurate predictions of insect pest emergence therefore do not solely depend on the relationship between temperature and post-diapause morphogenesis; diapause thermal sensitivity must also be considered.

Transhydrogenation Through time: Mitochondrial Peroxide Metabolism Through a lens of Endosymbiosis

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Abstract: The energy-linked nicotinamide nucleotide transhydrogenase (NNT) is common across Eukaryotic and Prokaryotic aerobic organisms. The NNT catalyzed reaction is held heavily towards NADPH formation because this

direction is linked to the favourable influx of free protons driven by the protonmotive force. Thus both aerobic bacteria and mitochondria scavenge from the protonmotive force to maintain NADP more reduced than the internal pool of NAD. Many extant bacteria use hydrogen peroxide, a reactive oxygen species, as a metabolically derived chemical signal for interindividual communication. Mitochondria can both produce and consume hydrogen peroxide; however, isolated mitochondria can act as intrinsic regulators of steady-state hydrogen peroxide levels. This regulation by mitochondria appears to rely on the preferential reduction of NADP to support consumption of hydrogen peroxide. Here I review parallels between energy transformation processes and reactive oxygen species metabolism between mitochondria and bacteria. If mitochondria evolved from free-living single celled aerobes by endosymbiosis, then these parallels in metabolic organization could be seen as relics of the initial endosymbiont(s).

Post-Exercise Oxygen Consumption and Recovery of Post-metamorphic Juvenile Sea Lamprey Following a Prolonged Non-Trophic Period

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Abstract: The life cycle of the sea lamprey (*Petromyzon marinus*) includes a prolonged (3–7 years), filter-feeding larval (ammocoete) stage followed by a complex metamorphosis lasting 3–4 months. Changes include reorganization of the intestinal tract, switching to tidal ventilation of the gills, and development of an oral disc and rasping tongue to facilitate parasitic feeding on teleost fishes. Post-metamorphic sea lamprey may go months without feeding (pre-feeding juvenile, PFJ) which could lower energy stores and compromise their ability to migrate downstream, pursue hosts, and to evade predators. To better understand their metabolic capacity to perform and recover from burst exercise, PFJs were vigorously chased for 5 minutes, followed by measurements of post-exercise O2 consumption ($\dot{M}_{O2}$), and muscle energy stores and metabolites. Resting PFJ had muscle glycogen reserves of 16.8 µmol/g wet weight (ww) and phosphocreatine (PCr) concentrations of 3.4 µmol/g ww, much lower than concentrations found in larvae and fed juveniles. Following exercise, $\dot{M}_{O2}$ increased5-fold to 7.8µmol/g.h., returning to resting rates after 2h.Muscle glycogen decreased 33%, accompanied by 5-fold increases in muscle pyruvate, however lactate did not peak until 1h post-exercise, only reaching concentrations of 2.9 µmol/g ww after pyruvate concentrations returned to resting levels. PCr decreased less than 1 µmol/g ww, returning to resting concentrations along with muscle glycogen after 4h recovery, while lactate concentrations remained elevated. We conclude that the short-term burst exercise capacity of non-trophic juvenile sea lampreys is lowfollowing metamorphosis, and exacerbated if parasitic feeding is delayed. Post-metamorphic juvenile sea lamprey in the Great Lakes are not thought to feed prior to their downstream migration, potentially leaving themvulnerable to predation,this may also explain why migration coincides with periods of high-water flow in rivers during the fall and winter, which could minimize the energetic costs of dispersal.

Brain-Derived Neurotrophic Factor (BDNF) Increases Reproductive Hormone mRNA Abundance in Zebrafish Brain and Gonads *in Vivo* and i*n Vitro*

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Abstract: Brain-derived neurotrophic factor (BDNF) is a neurotrophin that functions primarily in promoting the growth and survival of neurons. In addition to its well-known neuroprotective role, emerging evidence suggests its involvement in activities outside the central nervous system. These include BDNFregulation ofappetite, glucose, and lipid metabolism, and energy balance, whichhave been widely studied in mammals. We recently demonstrated that BDNF stimulates feed intake and regulates energy balance in zebrafish.Since reproduction and energy balance are closely associated processes,we hypothesized thatBDNF stimulatesreproductive hormones in zebrafish. The present study aimed to test this hypothesis. BDNF-like immunoreactivity was detected in the testis of zebrafish, suggesting its local production and possible action of the peptide within the zebrafish testis, while no BDNF-like immunoreactivity was detected in the zebrafish ovary. Intraperitoneal (IP) injection of 1, 10, or 100 ng/g bodyweight BDNF caused a significant increase in brain and gonadal reproductive hormones/their receptor mRNAs in both male and female zebrafish. BDNF enhanced the abundance of two isoforms of gonadotropin-releasing hormone (salmon/*sgnrh* and chicken/*cgnrh-II*), kisspeptin (*kiss1*), kisspeptin receptor (*gpr54a*), and brain aromatase (*cyp19a1b*) mRNAs in the brain of both male and female zebrafish at 60 minutes post-injection. Kisspeptin receptor(*gpr54a*), androgen receptor (*ar*), estrogen receptor (*er*), as well as steroidogenic enzyme mRNAs,were significantly increasedin the gonads of zebrafish 1 h post-IP injectionof BDNF. Similar changes in brain and gonadal hormone mRNAs were obtained when zebrafish brain and gonads were incubated in BDNF *in vitro*for 60 minutes. Collectively, these results support our hypothesis that BDNF is a positive regulator of reproduction in zebrafish. Our current researchfocuses on identifying the potential effects of BDNF onvitellogenesis, gametogenesis, and steroidogenesis in zebrafish. **Funding:** NSERC Discovery grant (2022-2027) and Centennial Enhancement Chair funding to Dr. Suraj Unniappan, and Devolved scholarship to Chinelo Uju from the Department of Veterinary Biomedical Sciences.

Positive Actions of Fibroblast Growth Factor 21 (FGF21) on male and Female Zebrafish Reproductive axis

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**Presenting Author*

Abstract: Fibroblast growth factor 21 (FGF21) is a member of the endocrine subfamily of fibroblast growth factors. Its role in regulating glucose and lipid metabolism and energy balance has been widely studied in mammals. Research in our lab recently demonstrated that FGF21 stimulates feed intake and regulates energy balance in zebrafish. Reproduction and energy balance are tightly interlinked processes. As a first step to extend our original findings on FGF21

and energy balance, this research aimed to determine whether FGF21 regulates reproductive hormones in zebrafish. Our results demonstrate FGF21-like immunoreactivity in the testis of zebrafish, suggesting local production and possible action of the peptide within the zebrafish testis. Meanwhile, no FGF21-like immunoreactivitywas detected in the zebrafish ovary. Single intraperitoneal (IP) injection of 1, 10, or 100 ng/g bodyweight FGF21 caused a significant upregulation of key brain and gonadal hormone/its receptor mRNAs that are critical for reproductive success in both male and female zebrafish. FGF21 induced the abundance of two isoforms of gonadotropin-releasing hormone (salmon/*sgnrh* and chicken *gnrh*-II/*cgnrh-II*), kisspeptin (*kiss1*), kisspeptin receptor (*gpr54a*) and brain aromatase (*cyp19a1b*) mRNAs in the brain of both male and female zebrafish at 1 h post-administration. Similarly, the gonadotropin receptors (*fshr* and *lhr*), androgen receptor (*ar*), estrogen receptor (*er*), and kisspeptin receptor(*gpr54a*) as well as steroidogenic enzymes were upregulated in the gonads of zebrafish 1 h post-IP administration of FGF21. *In vitro* incubation of the zebrafish brain and gonads with FGF21 at 1 h showed a very similar upregulation of mRNAs involved in zebrafish reproduction. The results so far support a very strong positive role for FGF21 in the reproductive endocrine axis of zebrafish. Research is in progress to elucidate the potential roles of FGF21 on gametogenesis and steroidogenesis in zebrafish. **Funding:** NSERC Discovery grant (2022-2027) and Centennial Enhancement Chair funding to Dr. Suraj Unniappan, and Devolved scholarship to Chinelo Uju from the Department of Veterinary Biomedical Sciences.

The Effect of Abiotic Stimuli on the Predation Efficiency of Water Tigers (*Dytiscus alaskanus*)

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Abstract: Predation efficiency is subject to experiential learning via repeated exposures to prey items in a similar environment. Water tigers – a common name given to the larvae of the predaceous diving beetle – are notably voracious predators that have significant influences on seasonal amphibians and aquatic invertebrate populations. Natural and anthropogenic abiotic changes to the larvae's environment may reduce predation efficiency via sensory interference. Indeed, water tigers use chemical, vibrational, and visual cues to successfully attack their prey. To assess the effect of abiotic alterations on the predation efficiency of the water tigers, I exposed larvae and their prey items to control (no), low, and high ranges of wind, vibration, or turbidity stimuli during 10-minute survival trials. During these trials, larvae and prey were assessed for speed and distance moved as well as additional behavioural events that were reduced into different predatory behaviours via Principal Component Analysis. Our results show evidence of context-dependent foraging behaviour when faced with natural stimuli of wind and turbidity, and additional evidence of abiotic novelty causing ineffective predatory behaviour. These findings support necessary actions to limit anthropogenic encroachment on native wetlands and provide future frameworks for similar abiotic interference experiments in insects and other arthropods.

Nucleobindin-1 Disruption Affects Feeding, Metabolism, and Glucose Homeostasis in mice in an age-, sex-, diet- and Light Cycle-Dependent Manner

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Abstract: Nesfatin-1, processed from the calcium and DNA binding protein nucleobindin 2 (NUCB2) is a bioactive ligand with metabolic effects. Recently, our lab provided evidence for a NESF-1-like peptide (NLP) processed from a NUCB2-related precursor, NUCB1. This research aimed to determine whether endogenous NUCB1 is critical for energy homeostasis. Global genetic disruption of *Nucb1* (*Nucb1* knockout/KO mice) led to increased food intake in chow-fed male and female mice across different points of light and dark phases. White adipose tissue weight was significantly increased in male and female *Nucb1* KO mice. Dark phase total activity was increased in male *Nucb1* KO mice, while it was decreased in female *Nucb1* KO mice compared to wildtype littermates. Energy derived from fat was significantly decreased in both male and female *Nucb1* KO mice. Male *Nucb1* KO mice handled glucose better during an oral glucose tolerance test, while the opposite effect was found in an intraperitoneal glucose tolerance test. A significant two-way interaction between mice group and time was observed on weekly food intake of male and female *Nucb1* KO mice fed control fat diet, but not in 60% fat-fed group. Handling of blood glucose during IPGTT was better in male *Nucb1* KO mice fed both diets, while such an effect was not observed in female KO mice. A significant two-way interaction of mice group and time on food and water intake value in 24 h was observed for male *Nucb1* KO mice fed 10% fat diet. Adiposity increased in male *Nucb1* KO mice fed a high fat diet. Our results indicate that the disruption of *Nucb1* leads to metabolic changes *in vivo*. The phenotype depends on sex, age, diet- and light/dark cycle. In conclusion, these outcomes furnish important evidence supporting significant roles for endogenous NUCB1 in energy homeostasis.

Metabolic Myokine Irisin is a Secreted Peptide Detectable in Domestic Animal Circulation

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Abstract: Irisin is a 112-amino acid peptide hormone that is cleaved from fibronectin type III domain-containing protein 5 (FNDC5), a type I transmembrane protein abundantly found in muscle tissue. Irisin is a putative mediator of the benefits of exercise, neuroprotection, bone growth, and cardiac health. Yet, few studies have focused on irisin in domestic animals. To address this, we first conducted an *in silico* survey of the irisin (peptide) sequences from domestic animals. The irisin sequence is identical between humans, mice, rats, cows, dogs, and horses. To detect the presence of irisin in tissues, total RNA and protein were extracted from skeletal muscle samples of pigs (n = 5) and ducks (n = 10). RT-PCR analysis found FNDC5 mRNA in all pig and duck skeletal muscle samples. An approximately 25 kDa band representing FNDC5/irisin was detected in both pig and duck skeletal muscle. Fluorescence immunohistochemistry using a rabbit

monoclonal FNDC5/irisin primary antibody and a goat polyclonal anti-rabbit secondary antibody found FNDC5/irisin-like immunoreactivity in both the glandular and muscular regions of pig, dog and cat stomach tissue sections. We also detected circulating levels of irisin in horse, pig, and duck serum using a multispecies ELISA kit. Our results provide important information to support the presence of irisin in several domestic animals, which helps pursue irisin as a biomarker or a therapeutic agent for diseases in veterinary medicine.

Nucleobindin-Derived Nesfatin-1 and Nesfatin-1-like Peptide Modulate Hepatic Lipid Accumulation *in Vitro* and *in vivo*

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Abstract: Fat-influencing protein, Nesfatin-1 (NESF-1) has shown to modulate lipid metabolism. However, whether Nesfatin-1-like-peptide (NLP), a peptide closely related to NESF-1, has any effects on hepatic lipid metabolism remains unknown. Therefore, the main goals of this study were to determine if NLP and/or NESF-1 regulate lipid accumulation *in vitro*, and test if the disruption of Nucb1 and Nucb2 affect genes involved in hepatic lipid metabolism *in vivo* in mice. HepG2/C3A cells express NUCB1/NLP and NUCB2/NESF-1. These peptides significantly reduced lipogenic genes and enhanced beta-oxidation genes mRNAs. These alternations were accompanied by decreased lipid contents in oleic acid-induced HepG2/C3A. The stimulatory effect of these peptides on cellular lipid reduction was blocked by compound C. Furthermore, the genetic disruption of Nucb genes affected lipid metabolism-related enzymes and endogenous Nucb mRNAs and AMPK phosphorylation in a diet- and sex-specific manner. Our studies demonstrate that NLP and NESF-1 attenuated lipid storage in hepatocytes through AMPK-pathway.

The Role of Cytoskeletal Structure in Freeze-Tolerant Crickets

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Abstract: Animals that survive cold stress are hypothesized to protect cytoskeletal structureduring cold stress or repair cytoskeletal structure after cold stress. The spring field cricket, *Gryllus veletis,* is freeze-tolerant if acclimated to fall-like conditions.To test whether these crickets modify cytoskeletal structure during acclimation, we dissected multiple tissues from crickets at three time points during acclimation. To visualize the cytoskeleton in these tissues, we stained them with fluorescent markers for actin and tubulin cytoskeletal proteins followed by imaging with confocal microscopy. Actin abundance increased in some tissue types during acclimation, while tubulin abundance was relatively stable. We also examined cytoskeletal structure after *G. veletis* were frozen or chilled to similar temperatures (-8°C), and found that cytoskeletal structure was well-maintained in tissues from freeze-tolerant crickets.Our results suggest that modifications to cytoskeletal structure may be an important mechanism for protecting cells in freeze-tolerant insects.

Effects of Water Temperature on Personality Traits in Crayfish: Implications for Invasiveness

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Abstract: Consistent interindividual differences in animal behaviour (animal personality) have been shown to exist across many taxa. Current work seeks to understand the ecological significance of personality, including the influence that personality of introduced individuals has on the probability of an introduced population becoming invasive. The environment to which the individuals are introduced may also impact their ability to spread. Personality traits, such as boldness and exploratory activity, are believed to be important behaviours associated with invasiveness in crayfish. Additionally, water temperature is known to impact movement of aquatic invertebrates. We measured behavioural traits across two temperature treatments in *Faxonius virilis*, a crayfish that has become invasive across many watersheds. Individuals were assessed on their emergence time from shelter (boldness) and in a separate assay on their time spent moving in a novel tank (exploration). Preliminary results indicate that the effect of temperature on boldness and exploration was dependent on treatment order in one group of individuals (collected in spring) but not in a second group (collected in late summer). Boldness and exploration scores were correlated within individuals, indicating a boldness-exploration behavioural syndrome. The difference in reaction norms between the groups in response to temperature may be explained by the reproductive status of the crayfish. Individuals in breeding condition may be less risk-adverse than those in a non-breeding state. Our work suggests that the reproductive status of the individuals, as well as water temperature, may impact their behaviour and therefore the ability of an introduced population to spread.

Effects of Microplastics on Vector Physiology and on Susceptibility of *Hepatozoon clamatae* in *Culex territans*

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Abstract: Microplastics are ingested by the aquatic larval stages of mosquitoes, and recent reports show that they are retained in Malpighian tubules of the terrestrial adults. The ontogenic transference of microplastics from larval to adult stages may affect the vectoring capabilities of adult mosquitoes and in turn influence disease transmission. *Hepatozoon clamatae* is a highly prevalent protozoan parasite of Green Frogs *(Rana clamitans)* that develops in the Malpighian tubules of *Culex territans*. The retention of microplastics in Malpighian tubules, and the concurrent development of *Hepatozoon clamatae* in these organs, provides an opportunity to investigate the interactions of microplastics and parasites within a mosquito host. The objectives of this study, therefore, were to determine the effect of microplastic contamination on physiological aspects of *C. territans*, and to investigate if this contamination affects their response to *H. clamatae*. Wild-caught larvae of *C. territans* were reared in beakers containing different levels of microplastic beads, including control (0 beads/mL), low (20 beads/mL), high (200 beads/mL), and ultra (2000 beads/mL) conditions.

Physiological traits of *C. territans*, including larval, pupal, and adult mortality, developmental rate, body size, and post-infection mortality were monitored among the four treatment groups. Exposure to low, high, and ultra levels of microplastics did not significantly affect any of these aspects of vector physiology. To investigate potential interactions of microplastics and parasites, the intensity of *H. clamatae* in infected mosquitoes under different microplastic treatments is currently being quantified using qPCR.

Oxygen Consumption and Transcriptomic Response to Acute Thermal Stress in the Truncate Soft-shell Clam, *Mya truncata*, in Northern and Southeastern Populations in Canada

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Abstract: Rising temperature associated with climate change has led to various responses and consequences across aquatic ectotherms. Temperature governs physiological and biochemical processes, and rising temperature causes an increase in oxygen consumption to meet an increase in energy demand in ectotherms. However, at a critical sub-lethal temperature (Tcrit), the organism has an inability to supply sufficient oxygen to meet demand at the tissues, leading to a reliance on anaerobic metabolism. The heat shock response (HSR) is a primary mechanism driving differences in thermal tolerances of ectotherms through the induction of heat shock proteins (HSPs). Each species has different onset temperature (Ton) where the HSR is activated and there is increased HSP production. The HSP levels increase with rising temperatures and eventually reaches maximal capacity (Tpeak). The limit of HSP induction constrains species thermal tolerance. The HSR costs energy, thus, ATP energy demand increases under a thermal stress event, which leads to a further increase in oxygen consumption. This study aimed to 1) determine the Tcrit based on oxygen consumption using respirometry, 2) define Ton and Tpeak, and 3) evaluate the relationship between Ton and Tpeak with oxygen consumption and Tcrit in short-term temperature exposure on the truncate softshell clam, *Mya truncata* in two geographically different populations (the St. Lawrence Estuary, Quebec and Frobisher Bay, Iqaluit, Nunavut, Canada). We acutely exposed clams to one of six temperature treatments (10, 13, 16, 19, 22°C, and 7°C as control) for 2 h in a respirometry chamber. Oxygen consumption was measured at 7°C (before temperature ramping) and at the target temperatures (after ramping with a rate of 0.1°C.min^{-1}). We found that, oxygen consumption increased with the increasing temperatures and decreased at 16°C in both populations. After the temperature treatments, target mRNA transcripts related to thermal stress (including HSP, apoptosis, and antioxidant genes) in gill tissues was estimated using qPCR.

Cross-talk Inhibition between Serotoninergic Type 3 Receptor (5HT3R) and Nicotinic Receptor (nAChR) in Mouse Sympathetic Neurons

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Abstract: Nicotinic acetylcholine receptors (nAChRs) and serotoninergic type 3 receptors (5HT3Rs) are members of the Cys-loop family of ligand-gated ion channels, which also includes GABAA and glycine receptors. All members of this family are widely expressed in the central and peripheral nervous systems, where they mostly participate in fast synaptic transmission. In autonomic ganglia, nAChRs are located postsynaptically, from where they drive cholinergic synapses. Although 5HT3Rs are also strongly expressed in autonomic ganglia, their function remains not well understood. Unpublished observation from the Campanucci's lab on neurons from the superior cervical ganglion (SCG) revealed that co-stimulation of 5HT3Rs and nAChRs in autonomic neurons generated non-additive currents suggesting cross-talk inhibition, and co-immunoprecipitation revealed physical interaction between the receptors. In the current study, we concentrated on further characterizing the interaction between these receptors by investigating its effect on the generation of action potentials (APs) and their localization at the plasma membrane. Our findings revealed that while APs were generated by the independent effects of either ACh or 5HT, their combined application did not result in increased AP frequency, consistent with cross-talk inhibition between the receptors. The latter was further supported by immunohistochemistry experiments revealing that these receptors colocalized at the plasma membrane in SCG neurons. Taken together, our data unveiled a new physiological role of the 5HT3R at autonomic synapses, by regulating nicotinic fast synaptic transmission through cross-talk inhibition.

Does Chronic Hypoxia Elicit a Whole-animal Ketogenic Response in Brook Trout (*Salvelinus fontinalis)?*

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**Presenting Author*

Abstract: As a result of climate change, hypoxic events are becoming more common and lasting longer. Exposure to hypoxia can result in poor ATP production as oxygen is the final electron acceptor in the electron transport system (ETS). Hypoxia can also lead to cell damage due to the accumulation of reactive oxygen species (ROS). Lipids and glucose are the preferred substrate of most vertebrates during normoxia. But, when species encounter acute hypoxia, they usually limit lipid metabolism to prevent ROS accumulation, and glucose becomes the predominant substrate to make ATP through anerobic glycolysis. However, stored glucose can be limiting if exposure persists, and they may need to rely on other substrates such as ketone bodies. In mammals, ketone body oxidation during hypoxia has been shown to have fewer negative effects in the mitochondria and to be beneficial for ATP production. Therefore, we hypothesize that ketone body oxidation during hypoxia may benefit other species as well. We predict that chronic hypoxia exposure will elicit a ketogenic response in brook trout (*Salvelinus fontinalis*) and the use of ketone bodies as a mitochondrial

substrate will impact their function. To test this hypothesis, we investigated the efficiency and function of the ETS and key enzymes involved in ketone metabolism after a 7-day hypoxia exposure. Mitochondria were isolated from red muscle, heart, and brain, and we measured mitochondrial respiration and ATP/O_2 with ketone body substrates. Key enzymes in ketone metabolism, namely HOAD, BHBDH, and SCOT as well as SOD, were used as an indicator of how fish adapted to hypoxia. Our results indicate that chronic hypoxia elicits a whole-animal ketogenic response, and that ketone body oxidation impacts mitochondrial function and efficiency. Ketone body metabolism has largely been ignored in teleost fish; however, our results show that ketone body metabolism may be important during chronic hypoxia exposures.

Effect of Acute Exposure to Elevated Carbon Dioxide on Early life Stages of Medaka (*Oryzias latipes*)

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Abstract: Elevated dissolved CO_2 is an environmental stressor for fish and can influence physiology, behaviour, and development. The objective of this study was to quantify the effects of acute (60 min) exposure to various elevated CO_2 levels in lab-reared medaka (*Oryzias latipes*). We focused on eggs and larvae as they may be more sensitive to elevated CO_2 due to limited energy stores to handle associated changes in acid-base regulation. It was hypothesized that with increased CO_2 concentrations the rate of egg hatching and physiological processes would decline. It was also predicted that that behaviour in both embryo and larvae would be altered (possibly lower activity to conserve energy or greater activity to escape the stressor). Medaka eggs (72 hpf) and larvae (10 dpf) were exposed to CO_2 levels of 500 (control), 2200, 3400 5500 and 10500 μatm and examined. There were multiple changes in embryo physiology as CO_2 concentration increased to 10500 μatm, including a significant decrease in survival rate (1±0 to 0.5090±0.174) and heart rate (139.8±7.502 bpm to 76.78±6.469 bpm). Behaviour was examined by burst activity in embryos and distance moved (cm) and velocity (cm/s) in larvae. Although there was a significant difference in distance moved in the 2200 (373.0±150.7 cm) and 5500 (199.6±115 cm) μatm treatments, there were no other behavioural trends observed in embryos or larval fish. Future studies should explore if longer CO_2 exposures and exposures at these early developmental stages would cause more serious physiological and behavioural effects.

Seasonal and Site-specific Effects of Biofilm on the Fatty Acid Composition of Migrating Shorebirds

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Abstract: Western Sandpipers (Calidris mauri) stop at mudflats along the Pacific Flyway where mudflat surface biofilm is thought to be a major component of their diet. Omega-3 (n3) long-chain polyunsaturated fatty acids (LC-PUFAs) within

biofilm diatoms have been hypothesized to improve the physiological condition and migration performance of marine-associated birds. These LC-PUFAs are abundant in aquatic environments, with longer forms such as eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3) being strongly tied to marine sources. Shorebirds evolving in the presence of n3 LC-PUFAs likely need to acquire the LCPUFAs directly from diet and these may assist in cognition and endurance flight. The fatty acid composition of biofilm diatoms is expected to vary considerably among mudflats due to different environmental conditions tied to location and season. This variation is expected to confer advantages at specific locations during migration stopover, such as at Robert's Bank where the spring snowmelt freshet of the Fraser River Delta (FRD) is suspected to induce n3 LC-PUFA proliferation in biofilm diatoms. We investigated whether shorebirds on stopover differed significantly in the assimilated total lipid composition of their plasma depending on mudflat location and season. In 2020 and 2021, plasma samples were obtained from Western Sandpipers captured at Robert's Bank and two other FRD mudflats farther from the river outflow, as well as the marine-dominated Tofino Mudflats. Our results reveal significant differences in assimilated LC-PUFAs by site and season, with a particularly strong seasonal shift occurring at Robert's Bank.

The Mixed Blessings of Glycolysis During Anoxia-Reperfusion in Locusts

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Abstract: Oxygen reperfusion is a major cause of tissue damage following anoxia or ischemia, as the sudden influx of oxygen can overwhelm cellular respiratory mechanisms and generate free radicals. While there has been much research on the role of glycolysis as a coping strategy for energy production during anoxia, the impact of glycolytic flux on reperfusion and recovery in the insect CNS is not well characterized. Using *Locusta migratoria* CNS treated with either glucose or the glycolytic inhibitor 2-Deoxy-Glucose (2DG), we monitored the kinetics of CNS electrical activity, whole-animal respiratory output, and the extent of neural tissue damage following anoxia-reperfusion. We found that whereas both glucose and 2DG increased the recovery rate of CNS electrical activity during reperfusion, glucose treatment was associated with more neural cell apoptosis. Additionally, the tissue damage observed in the glucose group was linked to greater respiratory CO_2 output in the initial moments of reperfusion. On the other hand, 2DG led to a bimodal response, with CO_2 output first being dampened and then gradually increasing throughout the recovery period. Our findings demonstrate that energy from glycolysis is not critical during reperfusion and may act to exacerbate reperfusion injuries in the locust CNS. We suggest that anoxia recovery is not solely dependent on energy but involves tightly regulated metabolic flux to minimize damage. However, the long-term effects of glycolytic flux during reperfusion on fitness and survival require further examination. To better understand survival strategies during anoxia, future studies should focus on metabolic regulation during reperfusion in different anoxia-tolerant species.

Use the Stable Isotope to Measure Protein Turnover in a Freshwater Snail *Planorbella duryi.*

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Abstract: Protein metabolism occupies 11-42% of total metabolism in ectotherms. This process is also closely related to growth and reproduction; hence, understanding protein turnover thoroughly can provide insight into ectotherm metabolism. The flooding dose method is widely used to estimate protein synthesis. This method relies on a single bolus injection of a labelled amino acid into the studied organism, and the incorporation of this tracer into the protein pool is used to calculate the protein synthetic rate. However, this method only applies to short-term measurement of protein synthesis because the enrichment of the isotope fails to maintain over a prolonged period due to the release of unlabelled amino acids from protein degradation. Long-term quantification of whole-body protein balance refers to the measurement of body size or weight over time, which requires weeks between measurement points to calculate. We have adapted the flooding dose approach using D_5-phenylalanine as a tracer to maintain enrichment for several days to measure both instantaneous rates of protein synthesis and estimate steady state protein turnover rates. The freshwater snail *Planorbella duryi* was placed in water containing D_5-phenylalanine for a week. The mantle foot (muscle tissues), gonads (reproductive tissues) and visceral mass (rest somatic tissues) were sampled simultaneously to verify the ability of this experiment to be widely applied to most tissue types. We show that the tracer quickly enriches the free amino acid pool and eventually the protein pool. In both cases, the isotopic enrichment reaches a plateau, supporting that this method presents the opportunity to investigate the instantaneous protein synthesis and steady-state protein turnover on a time scale of many days or even weeks.

Reversible Thermal Acclimation in 10-year-old White Sturgeon

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Abstract: With the increased frequency and duration of warm river temperatures, the ability of white sturgeon to tolerate these changes is a growing concern, especially when many populations are considered vulnerable or endangered. Phenotypic flexibility is a proposed mechanism juvenile and adult sturgeon may employ to cope with shifting temperature regimes. Previous studies have demonstrated white sturgeon increase thermal tolerance with acclimation to warm temperatures. However, it's unclear if this increased thermal tolerance is reversible or if this phenotypic change is costly to maintain. We hypothesized juvenile sturgeon utilize reversible acclimation, wherein thermal tolerance increases temporarily with warm acclimation, but can be reversed upon return to control temperatures. We also hypothesized if sturgeon demonstrate reversible acclimation, the mechanisms underlying this plasticity are associated with changes in aerobic and anaerobic capacity. To investigate this, we conducted a repeated-measures study measuring the critical thermal maxima (CTmax) of 10-year-old white sturgeon. Fish (n=16) were initially

tested at 14°C (CTmax 1), then split into either control treatment (14°C) or warm-acclimated treatment (20°C) for one month and tested again (CTmax 2). Finally, fish were returned to control treatment (14°C) for one month and all were tested (CTmax 3). After each CTmax trial, blood samples were taken to measure blood oxygen binding capacity (hemoglobin concentration and hematocrit), stress (plasma cortisol), and anaerobic end-products (plasma lactate). As predicted, CTmax significantly increased with warm acclimation (32.9±0.42°C) compared to initial CTmax (29.8±.54°C) but returned to baseline CTmax upon reacclimation to control temperature (31.1±.57°C). In addition, average CTmax was not significantly different between the three CTmax trials for control-acclimated fish. When combined with the underlying biochemical and tissue-level mechanisms, this understanding of thermal plasticity will allow us to determine more accurately the effects of climate change and rising river temperatures on white sturgeon populations.

Characterizing Darter (*Etheostoma* spp.*)* Interspecific Enzymatic Responses to Climate-Induced Temperature Changes

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Abstract: Aquatic environments are predicted to experience increases in temperature variability as the occurrence and severity of heat waves and daily maximums continue to rise. Here, we examined the impact of microhabitat temperatures on the thermal tolerance limits and energetic metabolic function of three closely related darter species: Fantail *(Etheostoma flabellare;* FTD), Rainbow (*Etheostoma caeruleum:* RBD), and Johnny darter (*Etheostoma nigrum;* JD) native to the Grand River of Southern Ontario. Brain and heart enzymatic activity of pyruvate kinase, lactate dehydrogenase, malate dehydrogenase, and citrate synthase were characterized for each species at 15° C baseline and following CT_{max} thermal challenge. Further quantification of enzyme gene transcripts will be performed to determine if observed differences in enzyme activity, either between species or treatment group, correlate with differences in expression. Significant differences were observed in the thermal tolerance limits of each species. For brain tissue, FTD appeared to have higher baseline enzymatic activity of many of the enzymes examined compared to JD and RBD, however at CT_{max}, this difference was lost as JD had significantly higher activity compared to baseline. Heart tissue exhibited no interspecific differences in enzyme activity levels at baseline. At CT_{max}, however, JD had increased activity than RBD for all enzymes, although neither JD or RBD were different from FTD. These results suggest that Fantail darters may be better equipped at responding to temperature-induced increased metabolic demands due to their higher baseline enzymatic activity, and that Johnny darters, although often having the lowest baseline activity, appear to have a plastic ability to increase enzyme activity on relatively acute scales, potentially improving their handling of thermal stress. Collectively, it appears that the enzymatic activity and CT_{max} of each darter correlates with the temperatures of their respective microhabitats, which may provide insight when predicting how climate change will affect local species.

It only Takes one: Neural Specification in the Annelids *Capitella teleta* and *Platynereis dumerilii*

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Abstract: Cell fate acquisition requires a complex mix of inherited and external signaling cues. An ideal framework to examine fate acquisition is spiral cleavage, the ancestral form of cleavage for Spiralia (~Lophotrochozoa). Animals with spiral cleavage undergo stereotypic cell divisions, and each cell (blastomere) reproducibly produces a specific set of tissues. By removing potential signaling cells via blastomere isolation we can determine if external signals are necessary for a specific developmental fate (conditional specification). If the specific end fate is still produced, this suggests that inherited signals are sufficient (autonomous specification). To better understand these processes in Spiralia, we isolated blastomeres in the annelids *Capitella teleta* (Sedentaria) and *Platynereis dumerilii* (Errantia) and assayed for neural fate. In both annelids, the first-quartet micromeres (8-cell stage) generate the ectoderm of the 'head' and brain, while the 2d micromere (16-cell stage) forms the trunk ectoderm plus ventral nerve cord (VNC). Surprisingly, we found that these micromeres generated neural tissue in isolation, suggesting that inherited factors may promote brain and VNC fate in both species. Furthermore, partial larvae arising from isolated animal-pole blastomeres (first- and second-quartet micromeres) failed to form a VNC but still developed a dorsoventral axis. This suggests that dorsoventral axis formation is decoupled from VNC specification in annelids and additional external signaling beyond inherited factors may affect neural fate. Thus far our results suggest that CNS evolution may have been more complicated than a single centralization event at the base of Bilateria or that significant changes occurred within Spiralia. Future experiments to identify the genes involved in neural specification in annelids will ultimately provide insight into CNS evolution. This research was supported an MBL Whitman Early Career Investigator Fellowship to NBW and an NSF grant to NPM.

Protection from high Cellular Ammonia Concentrations is Mediated by the Novel NH_4^+ Transporter, HIAT1

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Abstract: Ammonia is a toxic waste product from protein and nucleic acid catabolism. Accordingly, ammonia is either converted into less toxic nitrogenous compounds such as urea or uric acid (land living organism) or is excreted directly as ammonia as seen in fish and aquatic invertebrates. As basically every metabolically active cell in any given organism produces ammonia, cellular excretion is equally crucial to keep intracellular ammonia levels in a tolerable range.

Interestingly, very little is known with regard to ammonia excretion pathways in non-epithelial cells. Most recently, our group discovered that a so far not characterized membrane protein, HIAT1, cloned from crustaceans, chelicerates, and fish, acts as a novel ammonia transporter when functionally expressed in frog oocytes.

This transporter is highly conserved in the animal kingdom, sharing e.g. 65% amino acid identity of the protein between fish and decapod crustaceans. Interestingly, expression of HIAT1 is not restricted to excretory tissues, but exhibits rather a ubiquitous tissue distribution. Our studies on the green shore crab *Carcinus maenas* and the horseshoe crab, *Limulus polyphemus*, revealed that, when expressed functionally in *Xenopus* oocytes, the ammonia uptake is reduced, when compared to water injected oocytes (sham). Accordingly, oocytes preloaded with radioactive labeled methylamine, that serves as a proxy for ammonia, showed a higher excretion rate as control oocytes. Moreover, preliminary experiments suggest, that the HIAT1-mediated transport depends on the presence of Na^+, indicating that the transporter may function as an Na^+/NH_4^+ exchanger. The data further suggests that K^+ serves not as a substrate for the crab HIAT1 when expressed in oocytes. Endogenous expression of HIAT1 in the amphibian oocyte, imply crucial importance of this transporter in cellular ammonia and pH regulation even in the earliest stage of life.

Contractile Properties of the Pacific Hagfish Hindgut in Response to Microbial Dietary Fatty Acids

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Abstract: The intestinal microbiome is responsible for the production of dietary-sourced short chain fatty acids (SCFAs), including acetate and butyrate. Infusion of these SCFAs in vertebrates has been shown to alter colonic contractility with stimulation or inhibition of contractions differing depending on species. Hagfishes are intermittent feeders that, upon feeding, are known to rapidly transfer digesta to the hindgut after which they remain motionless for days while digesting. The relative proportions of endogenously produced SCFAs are unknown for this species, as is their potential effect on hindgut contractility. This study examined the effects of increasing acetic acid, butyric acid, and hydrochloric acid (0–25 mM) on the contractile properties of isolated hagfish hindgut segments. We demonstrate that acetic acid induces significant relaxation of the hindgut muscles with increasing concentration, while butyric acid and hydrochloric acid do not. Further, acetic acid nearly eliminated spontaneous contraction events, while butyric acid induced stronger contractions at higher concentrations. The strength of contraction was not altered with the addition of hydrochloric acid, indicating this effect is specific to SCFAs and not acidification. Overall, this study demonstrates that SCFAs elicit differential effects on hagfish hindgut contractility, which could alter meal processing depending on their relative production rates by the intestinal microbiota.

Western Meets Indigenous: Horse Nations in the Americas

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Abstract: The popular narrative that horses were introduced to Indigenous peoples of the Americans by Europeans is being challenged. New scientific evidence continues to emerge that suggests horses were here independent of, and prior to, European contact. Western science is finally catching up to what most Indigenous nations have always known. This paper explores these recent western "discoveries" and the corresponding Indigenous knowledges of the horse preserved in Indigenous oral histories. Study of the multidimensional roles of horses in Indigenous societies, as well as secular and sacred human-horse relations, shed deeper understanding of the place of the horse in Indigenous cosmologies and ways of knowing.

Exploring the Role of Ammonia Transporters (AMTs) in the Branchial Tissue of *Limulus polyphemus*

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Abstract: All organisms must manage ammonia as it is highly toxic and a product of many essential biochemical processes. To facilitate ammonia movement across cell membranes, organisms need to utilize transmembrane ammonia transport proteins. Ammonia transport proteins are generally sorted into three groups, ammonia transporters (AMTs), Rhesus glycoproteins (Rh proteins), and methylamine permeases (MEPs), expressed in plants, animals, and fungi, respectively. Recently, transcripts of AMTs have also been found in invertebrates, where experimental evidence suggest that they play a role in both, ammonia excretion and ammonia sensing. The marine American horseshoe crab, *Limulus polyphemus*, expresses at least 2 AMT proteins within the epithelia of their book gills which is the primary surface for ammonia excretion. In preliminary investigations these two transcripts, LpAMT-1 and LpAMT-3, show different levels of expression across different gill regions. LpAMT-3 was found most abundantly in the ammonia permeable ventral surface of the gill, particularly in the central mitochondrial rich region that has previously been implicated in ammonia excretion. LpAMT-1 however, was found more abundantly in the relatively ion impermeable dorsal side and the previously uncharacterized marginal gill setae, a group of stiff hair-like structures that lay on the lateral margins of each gill lamella. Interestingly, in a functional expression analysis in *Xenopus* oocytes, both AMTs failed to mediate the transport of radiolabeled methylamine, while transport was detected for a mammalian Rh protein (RhCG, pos. control). The ability of invertebrate AMTs to transport ammonia is still debated and they have also been found to have a sensory function e.g. in the antenna of terrestrial insects. Since ammonia transport facilitated by animal AMTs has never been shown by direct evidence, experiments are now on the way to determine whether these AMTs may have lost their NH_3 transport capability through the channel's pore but may still transport the dissociated proton.

A Proposed Mechanism of Ammonia-Induced Brain Swelling in Goldfish and Rainbow Trout

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Abstract: Goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) each experience brain swelling (cerebral edema) characterized by increased brain water content when exposed to elevated ammonia, which may arise naturally, as run-off from agricultural fields, or from municipal waste water effluent. Goldfish readily recover from 10-20% increases in brain water content following exposure to high external ammonia (HEA), whereas even relatively small increases in brain water content lead to brain damage or death in mammals experiencing hyperammonemia. However, the underlying mechanism(s) for ammonia-induced brain swelling remains unresolved. One prevailing hypothesis is that glutamine accumulation in the brain, as a means to detoxify ammonia, increases intracellular osmolarity leading to the osmotic influx of water into neurons and glial cells. A second is that ammonia disrupts transmembrane potentials leading to excess glutamate neurotransmitter release that over-activates ionotropic N-methyl-D-aspartate (NMDA) receptors, leading to increased intracellular Ca^{2+}, followed by the osmotic influx of water and brain swelling. To test these hypotheses, rainbow trout and goldfish were exposed to high external ammonia (HEA; 1mM and 5mM NH_4Cl, respectively) in the presence of the glutamine synthetase inhibitor, methionine sulfoximine (MSO), or the NMDA receptor antagonist, MK801. In both species, HEA resulted in 10-fold increases in plasma ammonia and corresponding 12-15 % increases in brain water content in saline-injected fish. MSO injection (Intraperitoneal; IP) did not prevent brain swelling in either fish at HEA. However, MK801 injection (IP) attenuated brain swelling in rainbow trout exposed to HEA, but not in goldfish. We conclude that glutamine accumulation is not the cause of brain swelling in fishes exposed to HEA. Instead, our MK801 results in trout suggests direct involvement of NMDA receptors in brain swelling in that species. The lack of effect in goldfish may because they downregulate NMDA receptor protein abundance and activity in response to elevated internal ammonia.

Seasonal Changes in the Temperature Tolerance, Sensitivity, and Regulation of Cardiac Function in Fishes

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Abstract: Temperature is a dominant environmental factor influencing most major physiological processes and, by extension whole animal performance. Many fish can experience large thermal variation over the course of their lifetime, such as the substantial temperature fluctuations that can occur seasonally in temperate zones. Heart rate is a critical function that is strongly influenced by temperature in fishes. During acute warming, fish can increase their heart rate to support increased tissue oxygen demands but only until a maximum temperature, and concomitant peak heart rate, is reached. Conversely, at cold winter temperatures, life processes such as heart rate will be slowed. Fishes will commonly

reset their heart rate, cardiac thermal sensitivity, and cardiac temperature tolerance to compensate. However, the degree to which this compensation varies among species with different thermal habitats and strategies is not well defined. Additionally, at thermal extremes, adrenergic regulation of the heart can have a significant effect on cardiac function. The extent to which any thermal acclimation of cardiac thermal sensitivity and heat tolerance are driven by changes in cardiac regulation by adrenaline in fishes also requires further exploration. Using electrocardiogram (ECG) and mRNA analysis, I am investigating seasonal changes in the temperature tolerance, sensitivity, and adrenergic regulation of cardiac function in fish, in both a laboratory setting and the wild. In particular, I will compare species with different strategies to cope with seasonal temperature changes (e.g., winter dormant vs. winter active fishes). My goal is to understand the importance of seasonal cardiac acclimation in both temperate and extreme northern environments.

Influence of Body Condition Dynamics on Food Caching in North American red Squirrels

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Abstract: Foraging for the energy required to survive, grow, and reproduce is energetically costly, yet the relationship between body condition and foraging success varies across animal systems. Capital breeders fuel reproduction through energy stored in advance of the breeding season, and thus must balance the costs of foraging with energy returns while also gathering surplus energy to be stored as fat or cached food. We aim to resolve these relationships in a wild food-caching capital breeder, the North American red squirrel (*Tamiasciurus hudsonicus*), which experiences high interannual variation in food abundance (cones produced by white spruce *Picea glauca*). Cones are available to be harvested by squirrels for only a short time period each year, and the energetic cost of caching can rival that of lactation. We hypothesized that body condition (i.e., body mass and percent body fat) facilitates foraging success, measured as cached spruce cones. We first examined the relationship between body mass and caching success across 14 years. Next, we analyzed the relationship between body mass and cache size in food-supplemented squirrels. Finally, we quantified body composition (specifically, fat mass) and body mass of squirrels before and after caching season in two years, one with a very low cone crop and the next with a very large cone crop. In the long-term dataset, males (but not females) that gained body mass between summer and autumn cached more cones, though the effect was small. Food supplementation did not influence caching success. In the two years that we quantified body composition, we found no relationship between body mass, percent body fat, or the change in either of these metrics and the number of cones cached by squirrels. In general, body mass and percent body fat neither facilitate foraging, nor appear to act as substitute energy stores in this primarily food-caching mammal.

The Physiological Consequences of a Large Meal in a Voracious Marine Fish

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Abstract: When offered a large meal of anchovies, staghorn sculpins (*Leptocottus armatus*) at 10ºC voluntarily ate 15.8% of their body weight. Gastric clearance was slow with > 60% of the meal retained in the stomach at 48h, and emptying was not complete until 84h. At approximately 12h post-feeding, pH was depressed by 3 units and $[Cl^-]$ elevated 2-fold in the stomach chyme, reflecting HCl secretion, while in all three sections of the intestine, pH was depressed by 1 pH unit but $[Cl^-]$ remained unchanged. PCO_2 and [total ammonia] were greatly elevated throughout the tract, whereas PNH_3 and $[HCO_3^-]$ were depressed, reflecting at least in part, the lower pH in chyme. However, *in vitro* gut sac preparations revealed that intestinal HCO_3^- secretion rates were also lower in fed fish. Whole animal O_2 consumption rate was elevated approximately 2-fold for 72h post-feeding, reflecting "specific dynamic action", whereas ammonia and urea-N excretion rates were elevated about 5-fold. A modest "alkaline tide" occurred in the arterial blood for about 48h, but there was negligible excretion of metabolic base to the external seawater, and arterial $PaCO_2$ and PaO_2 remained unchanged. Plasma [total amino acids] and [total lipids] were elevated about 1.5-fold for at least 48 h post-feeding, whereas small increases in plasma [total ammonia], PNH_3, and [urea-N] were quickly attenuated, and plasma [glucose] did not change. We conclude that despite the large size of the meal, its slow processing minimizes disturbances in internal homeostasis. However, discrepancies in nitrogen and HCO_3^- budgets raise interesting questions about the role of the gastro-intestinal microbiome in ammonia, urea-N, and acid-base metabolism (NSERC Discovery).

Macrophage cell Biosensor System to Detect Bioactivity of Electro- and Solar Oxidation-Treated oil Sands Process-Affected Waters

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Abstract: Alberta is well-recognized for its contributions to the oil sands industry as it has one of the top four largest oil reserves worldwide. Water is used in various oil sands processes to separate contaminants and extract bitumen with two to four barrels of water required during extraction to produce one barrel of oil. This water, termed oil sands process-affected water (OSPW), accumulates pollutants such as naphthenic acids and is required to be stored in tailings ponds until remediation. Advanced oxidation processes, such as electro- and solar oxidation treatments, target and degrade naphthenic acids which are recognised as a major source of OSPW-mediated toxicity. The unsustainable nature of the OSPW waste product necessitates a proper evaluation method for remediation procedures and we propose a macrophage cell-based *in vitro* biosensor system to detect changes in bioactivity between treated and untreated OSPW. Immune cells, such as macrophages, are equipped with various receptors to detect environmental stimuli and respond

immediately due to their innate nature. Bioactivity reflects macrophage cell activation from a baseline, basal state and is evidenced by an increase or decrease in proinflammatory gene expression. We can monitor bioactivity resulting from treated or untreated OSPW exposure by identifying changes in cytokine gene expression as cytokines are released by activated immune cells in response to various environmental stimuli to coordinate cell movement and regulate inflammation. In addition, this assay is useful in evaluating two potential remediation methods, electro- and solar oxidation, and the amount of time for each treatment. Our research demonstrates changes in cytokine gene expression after OSPW exposure and reduced bioactivity associated with longer treatment periods. Our research offers a new method to analyze contaminated waters and moving forward, may be used to evaluate potential water treatments.

Influences of Prey Type on American Lobster Agonistic Behaviours

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Abstract: Decapod crustaceans use an array of agonistic interactions with conspecifics during conflicts over food, mates, and other resources. To date, such interactions have been primarily studied in the lab. We have developeda system using baited underwater video to record the agonistic behaviours of American Lobster, *Homarus americanus*, in nature. Cameras (on custom built metal tripods) faced the substrate, recording activity in a 2 m^2 area around a fixed mesh bag containing one of a variety of possible prey. We used an ethogram to document occurrences of a range of specific agonistic behaviours, while also documenting the number of lobster present over time. To assess the influence of prey type on agonistic interactions, we compared frequencies of behaviours (both absolute frequencies and frequencies normalized to the number of lobsters) when the bait was either natural prey (mussels – *Mytilus edulis*, rock crab – *Cancer irroratus*) or commercial bait used in the lobster fishery (mackerel - *Scomber scombrus*, herring - *Clupea harengus*). A second comparison recorded agonistic activity in response to four different portions of the body of alewife (*Alosa pseudoharengus* - whole, fillets, heads, and trunk skeleton and viscera). Our data suggest lobsters do vary agonistic behaviours in response to different prey types, and that the difference is not necessarily driven by the odour molecules derived from different tissues in the prey, but rather prey-type-specific odours: few differences in agonistic behaviours were observed between the different body portions, but differences were evident between the different prey types. Overall, our findings will be useful in understanding how lobsters use odours to modulate behaviour. In addition, the data will also support efforts to develop sustainable bait options in commercial fishery.

Effects of Ocean Acidification on the Activity and Metabolism of Threespine Stickleback (*Gastrosteus aculeatus*)

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Abstract: Ocean acidification (OA) has been shown to either decrease or increase activity in marine fish. Although proposed mechanisms involved in these changes in activity is hypothesized to be reduced sensitivity of olfactory neurons or neurotransmitter function, heterogeneity of results in the literature may indicate that other physiological parameters such as muscle physiology and energy metabolism could also play a role in the altered activity. In this study, we examined the physiological mechanism that drives changes in activity of threespine sticklebacks under OA. Marine threespine sticklebacks were captured from Bamfield inlet, and exposed to current (~750 µatm; pH 8.0 representing the current level), medium (~1,500 µatm; pH 7.7 representing upwelling events) and high (~3,000 µatm; pH 7.1 representing predicted future levels) CO_2 for two weeks. Sampling was performed at one and two weeks of exposure where baseline activity, group behaviour, escape response and metabolic rate were measured. The total distance moved and average speed were higher in medium group than low at both sampling points ($p<0.05$), but not different from the high group. Inter-individual distance was higher in the medium level at one week of exposure than those exposed to the current level ($p<0.05$), but not different from the high level. Conversely, maximum escape speed was lower in the medium level than the current level at one week exposure ($p<0.05$). Routine, maximum metabolic rate and metabolic scope was lower in the high level than the current level at both sampling points albeit statistically insignificant. Our study demonstrated that exposure to elevated CO_2 may increase activity and shoal size at the cost of reduced escape response and aerobic metabolism. As we investigated the study in fully grown adults, the results imply that high CO_2 levels may impact mating and reproduction, which can further influence cohort and population dynamics under OA.

The Stress Metabolome of Juvenile Rainbow Trout is Disrupted by Exposure to Municipal Wastewater Effluent and Urban Runoff

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Abstract: Municipal wastewater effluent (MWWE) and urban runoff (stormwater) are complex contaminant mixtures that have the potential to impact aquatic biota including fish. However, our understanding of how these mixtures influence stress response, energy allocation, and metabolism is limited. We investigated whether the metabolic costs associated with detoxification of contaminants would be reflected in an altered energy metabolism in fish exposed to MWWE and stormwater. This was tested by assessing the plasma metabolome, and liver energy metabolism in response to a secondary acute stressor in rainbow trout (*Oncorhynchus mykiss*). Hatchery raised juvenile trout were caged in the Bow River at three locations (a reference site, a stormwater site and a MWWE site) for 21 days in July and August. Following which, fish at each site were subjected to an acute stressor, consisting of 1-minute air exposure followed by 60-minute confinement. Plasma and liver was sampled from these fish before and after the acute stressor exposure and the plasma metabolome assessed using hydrophilic interaction liquid chromatography. Non-metric dimensional scaling, two-factor analysis of similarity, and permutational ANOVA identified differences in metabolite profiles between the reference site and the sites exposed to MWWE and stormwater. Metabolite quantitative enrichment analysis identified metabolites that were stress-responsive. While no metabolite sets were significantly enriched following the stressor at sites upstream from MWWE, metabolites involved in more than 15 biological pathways were significantly enriched

downstream from the MWWE. Leucine, a key regulator of protein synthesis, was significantly altered by acute stress downstream from MWWE. In addition, plasma metabolite concentrations, and liver enzymatic activities suggest a higher preponderance for protein breakdown in fish caged at sites downstream from stormwater and MWWE. Overall, stormwater and MWWE exposure impacted the plasma stress metabolome and liver energy metabolism, leading to the proposal that these complex mixtures disrupt protein homeostasis in rainbow trout.

Dietary N-3 Polyunsaturated Fatty Acids Promote Mitochondrial Proton Leak in a Migratory Sandpiper

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Abstract: The acute energetic demands of migration require metabolic adjustments that promote endurance flight capacity which may be influenced by diet. Long-chain n-3 polyunsaturated fatty acids (PUFA) are more abundant in marine environments when compared with terrestrial habitats, and animals that feed in marine environments may have greater requirements for n-3 PUFA. The western sandpiper (*Calidris mauri*) is a marine-associated shorebird that feeds on biofilm, including algal primary producers of n-3 PUFA, during migration. We previously demonstrated that cultured shorebird muscle cells exhibit greater maximal respiration and proton leak associated respiration compared with mouse cells. We manipulated sandpiper diets to investigate the impact of dietary n-3 PUFA on muscle mitochondrial physiology. Mitochondrial proton leak flux, measured by O_2 consumption rate, was greater (57%) in western sandpiper muscle mitochondria fed a high n-3 PUFA diet, but oxidative phosphorylation linked respiration was not affected when provided pyruvate fuel substrate. Further, increased assay temperature increased oxidative phosphorylation linked respiration, but this did not differ between diet treatments. Additionally, muscle mitochondria from the high n-3 PUFA fed sandpipers exhibited 40% less H_2O_2 production under both oxidative phosphorylation and leak conditions. Mitochondrial membranes are less coupled in n-3 PUFA fed sandpipers, and consequently produce less reactive oxygen species (ROS) without changes in oxidative phosphorylation linked respiration. Sandpipers feeding on abundant n-3 PUFA may experience reduced ROS damage without changes in metabolic performance through unchanged oxidative phosphorylation linked O_2 consumption. Western sandpipers feeding in marine environments may require n-3 PUFA to support metabolic function by reducing cellular and tissue damage when energy expenditure increases with endurance flight and associated refuelling.

Ultrasound Imaging Provides a Non-Invasive Means of Examining the Hearts of Pacific Hagfish (*Eptatretus Stoutii*)

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Abstract: Hagfish possess many morphological and physiological characteristics that are unique among vertebrates. One such feature is the arrangement of their circulatory system. With accessory hearts, large blood sinuses, and low blood pressure it does not resemble that of any other vertebrate. Previous studies of the hagfish heart have involved the use of flow probes for *in vivo* studies, or *in vitro* heart preparations. Here we report the use of ultrasound imaging to obtain real time video of the hagfish heart with non-invasive methods. For this study we used high frequency ultrasound to characterize heart function in Pacific hagfish (*Eptatretus stoutii*). This method was successful in obtaining images of both the hagfish systemic heart and the accessory portal and caudal hearts. The systemic heart of the hagfish resembles that of more derived fish in morphology but functions at alower power output. The functional characteristics we have observed reflect this. The atrium is weakly muscled with an early peak velocity of 35 mm/s and late peak velocity of 180 mm/s. The ventricle of the systemic heart lacks the compact myocardium or coronary circulation seen in more active fish. The ventricle generated a peak velocity of 160 mm/s and ejection time of 700 ms. The peak velocity was approximately three quarters of the velocity generated by a zebrafish (*Danio rerio*) heart while the ejection time was almost five times longer. The ventricle also had an isovolumetric contraction time of 420 ms and an isovolumetric relaxation time of 340 ms. These are over five times longer than a zebrafish but the myocardial performance index was similar between the species. Hagfish hearts also possess remarkable anoxia tolerance. Using this ultrasound protocol, we will record the heart in anoxic conditions to determine how the functional parameters change in during anoxia. This work is supported by NSERC.

Effects of Substrate Choice and Anoxia Exposure on the Cardiac Mitochondria of the Pacific Hagfish (*Eptatretus stoutii*)

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Abstract: Pacific hagfish (*Eptatretus stoutii*) are marine agnathans that possess an impressive anoxia tolerance. Such an ability is rare among vertebrates, making it of particular research interest. Mitochondria, which utilize oxygen to convert chemical energy within the cell are acutely affected by its absence. Therefore, to understand the anoxia tolerance of the Pacific hagfish it is necessary to understand their mitochondria and the specific consequences anoxia has on them. We utilized a permeabilized fibre preparation to measure the oxygen consumption of cardiac mitochondria from Pacific hagfish. The mitochondria were provided with one of three substrate treatments to fuel oxidative phosphorylation (OXPHOS); malate + glutamate (activating Complex I), succinate (activating Complex II), or malate, glutamate, and succinate (activating Complex I + II). After measuring the rate of OXPHOS in normoxic conditions the mitochondria were left for one hour in either normoxia or anoxia before a second measurement. In mitochondria with only Complex II active the initial respiration was significantly reduced compared to when both Complex I and Complex II were supplied with substrate, demonstrating that Complex I makes a greater contribution to OXPHOS. Only in the Complex II fuelled mitochondria was respiration significantly decreased after one hour. Declining in both normoxic and anoxic treatments, demonstrating that anoxia was not the cause. This effect was likely due to inhibition by oxaloacetate, which accumulates through the action of malate dehydrogenase. Rotenone was added to inhibit this process but had limited effect, possibly due to non-mitochondrial factors retained in the permeabilized fibre preparation. In the other treatments there was no

significant reduction in respiration due to time or oxygen treatment. In summary, this work suggest that Pacific hagfish mitochondria are more reliant on Complex I than Complex II and are not adversely affected by one hour of anoxia exposure. This work is supported by NSERC.

Direct Action of Arginine Vasotocin on Zebrafish Spermatogenesis

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Abstract: Arginine vasotocin is a neurohypophyseal hormone known to be involved in the regulation of osmoregulation and social behavior. Vasotocin is also expressed in peripheral tissues, including male and female gonads. This study investigated the direct effects of vasotocin on spermatogenesis in zebrafish. Results demonstrate that vasotocin receptors (*avpr1aa*, *avpr2aa*, *avpr1ab*, *avpr2ab* and *avpr2l*) are expressed in the zebrafish testes, indicating that vasotocin may play a role in the regulation of testicular function. Using ex vivo culture of zebrafish testis, we investigated the direct action of three concentrations of arginine vasotocin (1nM, 10nM, 100nM) on spermatogenesis. A morphological approach was used to investigate the actions of vasotocin on spermatogonia stem cell renewal and mitotic and meiotic germ cell development over seven days. The results demonstrate that vasotocin directly increases the number of spermatozoa but decreases mitotic cell stages. Interestingly, the administration of vasotocin, in combination with gonadotropins, showed a different response in early and late germinal cells, indicating a potential role in the synchronized regulation of testicular development. Taken together, our findings will provide a framework for a better understanding of the physiological significance of vasotocin in vertebrate species and the regulation of male reproductive function.

Branchial Ammonia Excretion in the Green Crab *Carcinus maenas* is Regulated by cAMP.

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Abstract: As one of the toxic metabolic waste, ammonia, especially in high concentration, is harmful to aquatic animals. Thus, the excretion process is important for animals to maintain a tolerable range ammonia within their body fluids. Previous studies have shown that in the European green shore crab, *Carcinus maenas*, the excretion of ammonia is an active process, depending e.g. on the Na^+/K^+-ATPase, the V-type H^+-ATPase, and a functional microtubules network. In order to investigate which cellular compounds regulate this excretion process, gill perfusion experiments were conducted using SW acclimated green crabs applying a natural outwardly directed ammonia gradient, with 100 µM NH_4Cl in the perfusion solution and 0 µM NH_4Cl in the bathing solution. Preliminary data showed that enrichment of the perfusion solution with 100 µM Forskolin, an activator of the adenylyl cyclase (increase of cellular cAMP) that increases e.g. the transmembrane potential in osmoregulatory crabs, caused not an increase in ammonia excretion rate, but a retention of ammonia. This effect was also confirmed by the application of the adenylyl cyclase inhibitor, SQ22536 (10

μM), which now stimulated ammonia excretion. Interestingly, similar observations were made in an earlier study by our group employing the marine polychaete, *Eurythoe complanata*. Overall, these data suggest, that marine animals have a cAMP-dependent mechanism in place to adjust systemic ammonia concentrations, potentially for acid-base regulatory function.

The Role of 11β-Hydroxysteroid Dehydrogenase Type 2 (Hsd11b2) in Regulating Neurogenesis During Brain Development in Zebrafish (*Danio Rerio*)

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Abstract: During early life stages, neurogenesis drives development and growth of the brain, and factors that alter the rate of neurogenesis – like the hormone cortisol – could therefore induce lasting effects on brain form and function. In zebrafish, maternal stress increases the amount of cortisol deposited into the yolk, and brain cell proliferation and neural activity is altered in embryos treated with cortisol during early life stages. The effects of cortisol on neurogenesis can be mitigated by the catabolic enzyme 11β-hydroxysteroid dehydrogenase type 2 (hsd11b2) which converts cortisol to cortisone. Indeed, zebrafish embryos treated with exogenous cortisol showed an increase in the whole-body transcript level of *hsd11b2* suggesting that endogenous hsd11b2 activity may help buffer the potentially deleterious effects of elevated cortisol on brain development; however, this remains unknown. This study is testing the hypothesis that hsd11b2 regulates the capacity for cortisol to alter brain cell proliferation during zebrafish ontogeny. It is predicted that the inhibition of hsd11b2 in combination with elevated cortisol will impair brain development by inhibiting brain cell proliferation and reducing brain size. Fertilized zebrafish eggs were collected from six breeding tanks and reared in one of four treatments: 1 mg/L 18β-glycyrrhetinic acid (18β-GA; an hsd11b2 inhibitor); 100 μM cortisol; 1 mg/L 18β-GA and 100 μM cortisol; and vehicle control (0.3% DMSO) from 0-24 hours post-fertilization (hpf). This time period encompasses the majority of brain formation. A subset of embryos was collected at 0, 12, and 24 hpf to quantify cortisol levels. Changes in brain development will be assessed by comparing the brain:body length ratio of 24 hpf embryos from each treatment. In addition, differences in the rate of brain cell proliferation will be quantified at 24 hpf using the mitotic marker, 5'-bromo-2'deoxyuridine (BrdU), followed by immunohistochemistry. The number of $BrdU^+$ cells in defined brain regions will be counted and normalized to DAPI. By studying the effects of hsd11b2 on cell proliferation and brain sizes, this research will test if hsd11b2 supports brain development and help study the mechanisms regulating stress-induced changes to neurogenesis in zebrafish brains.

Population Coding of Motion-Sensitive Neurons in *Locusta migratoria*

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Abstract: Adaptive locust flight relies on rapid detection and processing of objects moving in the visual field. One identified neural pathway, comprised of the Lobula Giant Motion Detector (LGMD) and the Descending Contralateral

Motion Detector (DCMD), responds preferentially to approaching objects. LGMD receives retinotopic inputs from ipsilateral ommatidia and generates spikes in a 1:1 ratio in the DCMD, which synapses with multiple locomotion-related neurons. Other motion-sensitive neurons have also been identified in locusts but are not as well characterized. To better understand how locusts process visual information, we used multichannel neural recordings within a stimulus arena and presented various complex visual stimuli to test the effects of visual background or repeated object motion. We found multiple discriminated units that responded uniquely to visual motion and categorized the responses. More units responded to motion trajectories with a looming component, compared to translational movements across the visual field. Dynamic factor analysis of discriminated units revealed common trends that reflect the activity of neural ensembles. The numbers and types of common trends varied among motion trajectories. Compared to a simple white background, a visual flow field evoked responses in a similar number of units, yet resulted in fewer response categories. When presented with repeated looming stimuli, different units displayed unique habituation patterns, which were also affected by the presence of a flow field. These results increase our understanding of complex visual motion processing in this tractable system.

The Gut Microbiome Contributes to Thermogenesis in High-Altitude Deer Mice (*Peromyscus maniculatus*)

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Abstract: High altitude is one of the most extreme environments inhabited by endotherms, where extreme cold temperatures and low O_2 availability (hypoxia) can constrain aerobic heat production (thermogenesis) to maintain body temperature (T_b). Recent findings suggest that the gut microbiome contributes to whole-body thermogenesis, but the significance of this mechanism for coping in cold environments is unknown. We examined whether the gut microbiome contributes to adaptive variation in thermogenic performance in deer mice at high altitude. Mice from populations native to high altitude and low altitude were born and raised in common conditions. Adults from both populations were acclimated to warm (25°C) normoxia or cold (5°C) hypoxia (~12 kPa O_2 for 6 weeks) in a full factorial design, and a subset of mice in each group were treated with antibiotics to deplete the gut microbiome. Thermogenic endurance was then measured as the duration that T_b and metabolism could be maintained during acute cold challenge. In lowlanders, antibiotics had only modest effects on thermogenic endurance. In highlanders, in stark contrast, antibiotic treatment led to pronounced reductions in thermogenic endurance in both environments. These effects could not be explained by impairments in aerobic heat production by host thermogenic tissues, because antibiotic treatment had no effects on cold-induced increases in O_2 consumption or UCP-1 content of brown adipose tissue. These results suggest that the gut microbiome plays an increased role in thermogenesis in high-altitude mice. Thermogenic performance contributes to fitness at high altitude, suggesting that changes in host-microbe interactions contribute to high-altitude adaptation.