1. Exploring the Spatial-Temporal Expression of Shell Matrix Proteins in the Intertidal Gastropod, *Crepidula atrasolea*

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As the principal organ involved in shell formation, the mantle plays a fundamental role in the biomineralization of the gastropod (snail) shell. Notably, the mantle secretes a suite of shell matrix proteins (SMPs) whose diverse spatial and temporal patterns of expression are thought to be the driving force behind novel shell morphologies. The objective of this study was to use a novel technique: in situ hybridization chain reaction (HCR) to comparatively map the expression of three SMPs (*CaSMP1*, *CaSMP20*, *Ca94322*) in the mantle of *Crepidula atrasolea*, a marine gastropod with year-round reproduction and a simple unpigmented shell, throughout larval development. This work highlights the potential to develop *C. atrasolea* as a molluscan model. Despite advances in modelling shell secretion by the mantle, the exact mechanisms controlling shell secretion have not been confirmed, though some evidence suggests that shell deposition is under neural control. The optimization of an HCR protocol for the early stages of *C. atrasolea* provides a precise means of explicitly testing the neural control hypothesis. Further work will determine the expression of neural genes in correlation with biomineralization genes in the mantle throughout the early developmental stages of *C. atrasolea* to determine if the formation of the neural mantle tissue precedes the expression of biomineralization genes.

2. Neural Specific-Gene Expression in the Great Pond Snail, *Lymnaea stagnalis*

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Studies of gastropods have enhanced our understanding of how the nervous system controls animal behaviour. Immunohistochemical labelling has traditionally been the primary method used to investigate gastropod neuroanatomy, however, challenges persist regarding antibody availability and specificity. To address this, we explored a novel technique, in situ hybridization chain reaction (HCR), to label mRNA expression of neural-specific genes. This method offers higher versatility, as genetic sequence data allows for labelling a wider array of potential targets. Moreover, HCR inherently provides increased specificity by relying on genetic base-pairing, rather than the lock-and-key mechanism seen with antibodies. We conducted a comparative analysis of the techniques by parallel investigations of two enzymes associated with the synthesis of neurotransmitters in the nervous system of *Lymnaea stagnalis*. Tyrosine hydroxylase (involved in the production of catecholamines; e.g. dopamine) labelling with HCR was consistent with current and past immunohistochemical findings. However, discrepancies emerged with Tyramine Beta Hydroxylase (involved in the production of octopamine) expression in the central nervous system, suggesting possible differences in the sensitivity of the two methods and the need for further investigation. Despite the potential of HCR to offer enhanced versatility and specificity, it failed to label neural fibers (axons and dendrites) with both enzymes, limiting anatomical detail compared to immunohistochemical labelling. This highlights complementary advantages of both techniques: while HCR offers stronger evidence of specificity and greater target range, immunohistochemistry appears to provide superior visualization. Overall, the integration of both approaches could synergistically advance neuroanatomical exploration and facilitate future studies of neuron functions.

3. Wish for a fish: whole-mount in situ hybridization of mRNA in Threespine Stickleback

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In the era of phenomics, methods such as whole mount in situ hybridization (WISH) permit detection and mapping of relative timing and positioning of gene expression throughout ontogeny. While crucial for studying the genotype-phenotype map, this technique is mostly used with laboratory organisms such as zebrafish. This is owing in part to the complexity and specificity of this method and the challenges inherent in adapting the technique for non-model organisms. We describe an approach to WISH applied for the first time to the threespine stickleback fish, *Gasterosteus aculeatus*, an emerging model organism in ecology and evolutionary developmental biology. This methodology was adapted with the goal of being applicable both to wild and laboratory-housed individuals across ontogeny. We successfully applied this technique to visualize *BMP4* expression in individuals ranging from 5 to 90 days post fertilization (dpf), with staining being most efficient for individuals around 13 dpf. At this stage of development individuals were small enough to allow for thorough clearing and imaging while being large enough to manipulate. Gene expression was observed in the craniofacial region, the hypaxial and epaxial muscle blocks, and along the spinal cord. While genes involved in morphological patterning and body plans tend to be conserved in their timing and location of expression, subtle differences in regulatory genes and morphogens can produce different phenotypes in response to epistatic and environmental variation. The development of WISH for emerging model organisms serves to broaden the scope of evolutionary developmental biological inquiry.

4. Expression of *mmp25b* and its Inhibitor clu in the Developing Zebrafish Nervous System – A Novel Axon Guidance System?

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Matrix metalloproteinases (MMPs) are secreted or membrane-bound proteases best known for their roles in extracellular matrix (ECM) remodelling during development, regeneration, tissue homeostasis and in pathological contexts such as inflammation and tumour metastasis. Membrane type MMPs (MT-MMPs) also play central roles in the regulation of other MMPs, converting latent 'proMMP' into their active forms. Matrix metalloproteinase 25 (a.k.a MT6-MMP, leukolysin, or Mmp25) is a membrane-tethered MMP studied primarily in the context of leukocyte extravasation and the pathology of colon cancer, but limited research done in developmental contexts suggests Mmp25 is necessary for pathfinding/elongation of sensory axons of the trigeminal ganglion and Rohon-Beard cells. Here we replicate and refine our understanding of the expression of *mmp25b* during zebrafish development using hybridization chain reaction (HCR) and analysis of single cell RNA sequence databases. Consistent with older data, we see strong expression of *mmp25b* in peripheral sensory ganglia, including trigeminal, anterior and posterior lateral line, vagal, and glossopharyngeal ganglia; Rohon-Beard sensory neurons in the spinal cord; and leukocytes. Intriguingly, we also detect expression of clusterin, a poorly studied extracellular inhibitor of Mmp25, in the floor plate of the spinal cord, dorsal midbrain, the epiphysis in the forebrain, and generally throughout the epidermis. These findings suggest that gradients of Clusterin, specifically around the spinal cord and midbrain, may function as axon pathfinding cues by antagonizing Mmp25-mediated ECM-clearance and/or activation of latent MMPs, and further underscore the overlap between molecular mechanisms involved in inflammation and neural development.

5. Neural Role in Biomineralization: The Ontogeny of Mantle Neural Tissue in *Crepidula atrasolea* (Calyptraeidae, Gastropoda)

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While we have a good understanding of biomineralization as a chemical process, we have very little understanding of how organisms like molluscs (e.g., snails, nautilus, clams, etc.) control mineral deposition. Several studies suggest that biomineralization in molluscs is under neuronal control, however, there is currently no direct evidence to support this hypothesis. Specifically, the neural control hypothesis (NCH) proposes that neurosecretory networks signal to the mantle, the organ that secretes the shell, to control biomineralization. Our goal was to test the NCH by determining if nerves are present throughout all stages of shell formation in *Crepidula atrasolea*. The ontogeny of mantle neural anatomy was observed across shell developmental stages using the neural antibodies serotonin, FMRF-amide, and anti-tyrosinated α-tubulin. We created a spatiotemporal map of the presence and location of each neural marker in the mantle tissue. This image map determined if neural tissue is present throughout development in the shell-secreting tissue, as well as where it connects to shell-secreting cells in the mantle. This research is the first step in understanding how molluscs control shell secretion, how the process can be modified based on environmental changes, and how that process has evolved to create the vast diversity of shells.

6. The effect of movement on the development of the peripheral nervous system in zebrafish

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During development, the nervous system grows and starts to gather sensory inputs that can stimulate neurogenesis. For example, the sensory feedback generated by movement has been shown to stimulate zebrafish larvae brain development, with larvae that swim more generating more new neurons in the forebrain than those swimming less. Dorsal root ganglia (DRG) are a type of peripheral sensory cells present in most vertebrates, including zebrafish. During swimming, DRG detect movement and convey the sensory inputs to the brain. Cell populations of DRG grow during postembryonic development through neurogenesis like the brain, so it's possible that movement directly impacts the generation of DRG. I hypothesize that movement during postembryonic development affects the production of DRG. To test this, I used three different approaches: (1) I raised fish under restraint using smaller wells that reduce larval swimming; (2) I raised the fish in 6% methylcellulose, a thick media that reduces swimming; and (3) I used chrna1 fish, mutants that can't move due to the lack of synaptic transmission at the neuromuscular junction. For all approaches, I used transgenic zebrafish (*Isl2b*:*gfp*) that produce a green fluorescent protein in their DRG allowing for in vivo visualization and resampling over development to count DRG cells. All treatments reduced larval swimming compared to controls and consequently reduced the number of DRG generated. Therefore, I conclude that the amount of postembryonic movement that a zebrafish larvae experience affects DRG growth.

7. Stay grounded: hydrodynamic interactions between the ground and river stingrays

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The ocellate river stingray (*Potamotrygon motoro*) is a foil-shaped stingray that migrates along the ground throughout South American river basins. In this study, we measured lift and drag forces, and the posterior flow field using particle image velocimetry (PIV), to characterize the hydrodynamic performance of the form of river rays as a function of flow speed and distance from the ground. The experiments were conducted in a recirculating flume where a deceased ray (14cm width, W) was attached to load cell at an angle of attack of 0°, to measure forces and flow at different heights from

the substrate (0.001-0.85W) at a range of speeds (0-1.33W/s). The ray generated negative lift when positioned furthest away from the ground (>0.5W). However, lift changed to positive and increased in value as the ray was positioned closer to the ground. Furthermore, as expected, drag decreased as the ray was positioned closer to the ground. From the lift-drag ratios (L/D) we observed three distinctive regions: weak ground interaction with negative L/D due to negative lift (>0.5W), intermediate ground interaction with slightly positive and constant L/D (0.07-0.5W), and strong ground interaction with high L/D (<0.07W). Hence, river rays may benefit from hydrodynamic interactions with the substrate that inherently stabilizes the fish near the ground when swimming or station holding in flowing rivers.

8. Losing a sense of our surroundings: Assessing how the loss of social senses like touch and vision can modulate neuropeptide activity in the brain of zebrafish

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Social interactions, which rely on sensory information, are important for the well-being of social animals like humans. Chronic loss of our senses can change how we experience social interactions and can leave us feel lonelier. In zebrafish, parathyroid hormone 2 (Pth2) is an excitatory neuropeptide associated with social interactions, with pth2 levels decreasing in the brain during social isolation. Mechanical detection of conspecifics via the lateral line system contributes to the majority of pth2 levels in zebrafish, with nonfunctional lateral lines leading to decreased pth2 levels. However, pth2 levels are not completely reduced, suggesting that an additional sense contributes to social detection. With my research, I explore vision as a social sense, and if/how lateral line and visual inputs interact in the brain to affect pth2 levels in zebrafish. Furthermore, thalamic pth2 expression overlaps with the thalamic expression of an inhibitory neuropeptide called somatostatin 7 (Sst7). Excitatory and inhibitory transmitters in the brain tend to have dynamic relationships and can induce a phenomenon called neurotransmitter switching. Exploring the excitatory Pth2 and inhibitory Sst7 in tandem may uncover a dynamic switching relationship that can further illustrate the molecular consequences of sensory loss on the social brain. In my experiments, I will use both environmental and genetic approaches, along with hybridization chain reaction, to explore the relationship between social senses, pth2 and sst7. Through my research, I hope to use the zebrafish model to understand the molecular basis of how sensory loss affects social perception and brain chemistry.

9. Cold Temperature Regimes and Morphometrics through Ontogeny in the Threespine Stickleback (*Gasterosteus aculeatus*)

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The Threespine Stickleback (*Gasterosteus aculeatus*) is a model organism for parallel evolution that occupies marine habitats across North America. Temperature has been proposed as an important selective pressure in this process, but the mechanism remains unclear. In particular, the effects of temperature on morphology or the possibility of growth advantages under differing temperature regimes are unknown. We used 2D geometric morphometrics to study how cold temperatures affect shape and size from hatching to adulthood in marine stickleback obtained from the Bamfield Marine Sciences Centre. A 'control' group was held at a steady 15°C throughout the experiment. A 'cold' treatment group had their water temperature gradually dropped from 15°C to an ecologically relevant temperature of 8.5°C for 20 days at 140 days post-fertilization (dpf), simulating a shortened winter, after which temperatures were gradually raised back to 15°C. Morphometric analyses were conducted before and after this simulated winter (190 dpf), and at a third, adult timepoint (240 dpf). We found that temperature did not significantly affect either shape or size through development. Developmental

time was the only significant factor contributing to shape and size change. There were allometric changes in mouth positioning, operculum size and pelvic spine length. Together, these findings suggest that juvenile G. aculeatus are resilient to ecologically relevant cold temperatures and that such temperatures do not appear to confer a notable growth advantage. Further work is required to understand the complex relationship between temperature and adaptive evolution in this species.

10. The Effects of Chronic Hypoxia on the Lipid Metabolism of Brook Trout (*Salvelinus fontinalis*)

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Aquatic hypoxic events are becoming far more frequent, severe, and long lasting. To cope with periods of hypoxia, fish can decrease their oxygen requirements by reducing energetically costly activities such as locomotion, growth and feeding. During normal oxygen conditions fasting will typically upregulate fat metabolism, as fats can be stored in large quantities, and yield high levels of ATP per molecule. However, during periods of acute hypoxia, fat metabolism is typically downregulated as this process requires large amounts of oxygen and can produce harmful levels of reactive oxygen species. Instead, cells typically breakdown carbohydrates which use considerably less oxygen, but have limited stores. However, it is unclear how periods of chronic hypoxia may impact fat metabolism in fish when carbohydrate stores are exhausted. To determine the effects of chronic hypoxia and fasting we exposed brook trout (*Salvelinus fontinalis*) to 14 days of fasting and normoxia, or fasting and hypoxia at 45% dissolved oxygen. We measured mitochondrial respiration using both short-chain (sodium butyrate) and long-chain (palmitoyl-CoA) fatty acid,s as well as the activity of various other enzymes important in lipid metabolism in cardiac muscle tissue. We found that fish exposed to chronic hypoxia had an increased capacity for long-chain fatty acid uptake and higher oxygen consumption in the heart than control groups. This suggests that chronic hypoxia may induce fat oxidation to support cardiac function despite the potential negative side effects.

11. Taurine efflux: a novel mechanism constraining maximum cardiac function under thermal stress

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Stressors that activate anaerobic metabolism in mammalian hearts increase intracellular osmotic strength via the breakdown of glycogen and glucose and the accumulation of lactate, protons, and inorganic phosphate. This is rarely acknowledged in mammalian research and, to our knowledge, has not been studied in fish. In the face of any osmotic disturbance, regulatory volume increase and decrease mechanisms protect cardiomyocyte volume via the active and passive movement of ions and organic osmolytes, with taurine being the most important of the latter. Anaerobic stressors stimulate cardiac taurine efflux in healthy, but not taurine deficient (TD) fish, and that efflux is critical for maintaining cardiac function. Taurine efflux occurs in the absence of changes in extracellular (plasma) osmolality, suggesting it is driven by intracellular changes. We developed and validated a rapid method to assess changes in tissue osmolality and studied the effects of acute thermal stress on plasma and heart osmolality, and heart taurine and lactate content in control and TD brook char (*Salvelinus fontinalis*). Acute thermal stress caused equivalent lactate accumulation in control and TD fish. Plasma osmolality did not differ between groups, but heart osmolality was >40 mosmol/kg higher in TD fish following thermal stress. These data confirm that taurine efflux occurs in response to increases in intracellular osmolality and is required to maintain cardiac osmotic homeostasis, and thus mechanical function, during thermal stress. This represents a novel mechanism constraining maximum cardiac function under thermal stress that has important implications for our understanding of thermal tolerance across fish species.

12. Taurine supports the cardiovascular response to hypoxia in marine fish

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Climate change is altering aquatic environments through processes like eutrophication. This is generating hypoxic conditions which may have adverse consequences for aquatic species. This may be exacerbated by taurine-deficiencies occurring due to a loss of taurine-rich prey. Taurine is a non-proteogenic β-amino acid that exhibits cardioprotective properties through osmoregulation and calcium homeostasis. Taurine is necessary for supporting cardiac function under hypoxic conditions in freshwater fish but analogous studies in marine fish are lacking. The present study investigates this knowledge gap by determining the effect of taurine-deficiency on cardiac function in marine fish. Saltwater acclimated brook char (*Salvelinus fontinalis*) were used as a model marine fish to facilitate comparisons with existing data on freshwater brook char. Taurine deficiency was generated through a 5% β-alanine feed, which acts as a competitive inhibitor of tissue taurine transport. Heart rate (fh) was quantified using electrocardiograms (ECG) under normoxic or hypoxic conditions. Plasma osmolality and tissue taurine concentrations were determined to confirm taurine deficiency and understand the dynamics of taurine flux under hypoxic conditions. Results showed that control brook char exhibit a typical bradycardia in response to hypoxia with an average decrease in fh of 55%. Taurine deficient fish showed high interindividual variability and a blunted bradycardia. Additionally, control fish maintained plasma osmolality following hypoxia, while it increased by 57 mosmol/kg in taurine-deficient fish. Overall, taurine is important for the normal cardiac response to hypoxia through the maintenance of osmotic homeostasis in the heart.

13. Parental environmental and embryonic thermal history can alter thermal tolerance and the heat shock protein threshold of induction in embryos and post-hatch Lake whitefish (*Coregonus clupeaformis*)

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A warming environment and increased temperature fluctuations associated with climate change or other anthropogenic processes can detrimentally affect fish development and survival; however, protective cellular responses can attenuate the effects of these stressors. We investigated how parental environmental history (lake type) and embryonic thermal history influenced thermal tolerance and the heat shock response (HSR) in early-stage Lake whitefish (*Coregonus clupeaformis*). Embryos derived from parents of a warm shallow lake, Blackstrap Lake, were 6^{o}C more thermally tolerant than embryos from Lake Diefenbaker and Doré Lake, which are deep cool lakes. Post-hatch fish from Blackstrap Lake were also more thermally tolerant than fish from the deep, cool lakes. Daily thermal stress (1 hour at 3^{o}C above incubation temperature) for 50 days during embryogenesis reduced thermal tolerance by 2^{o}C in embryos from all three lakes. The minimum temperature for heat shock protein production (HSR induction threshold) in embryos at 80 days post-fertilization did not change with parental thermal history. However, embryonic thermal history resulted in a more variable HSR, with those exposed to daily thermal stress tending to have a higher HSR threshold induction temperature than embryos held at constant temperatures. Our data show that parental and embryonic environmental/thermal history can have lasting effects on thermal tolerance and the HSR in Lake whitefish.

14. Determining the relationship between cryoprotectants and Lyme disease infection status in *Ixodes scapularis*

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The black legged tick, *Ixodes scapularis*, is the primary carrier of *Borrelia burgdorferi* - the bacterium that causes Lyme disease. Previous research has shown that black legged ticks infected with *B. burgdorferi* exhibit higher survival after winter conditions. This research aims to determine which mechanisms are underlying this enhanced ability of infected I. scapularis to survive winter. Many organisms survive winter due to enhanced cold tolerance, which can be supported by the accumulation of small molecules known as cryoprotectants. Cryoprotectants include polyols (e.g., glycerol), small sugars (e.g., trehalose), and some amino acids (e.g., proline). We hypothesized that cryoprotectant concentrations would be higher in infected ticks compared to noninfected ticks. To test this hypothesis, we collected *I. scapularis* in spring 2023 and fall 2023 in Nova Scotia, tested for infection by *B. burgdorferi*, and used biochemical assays to measure concentrations of glycerol, trehalose, proline, and myo-inositol. By determining whether infected *I. scapularis* have higher cryoprotectant concentrations than uninfected I. scapularis, we will improve our understanding of the ticks' overwintering mechanisms, with implications for the spread of Lyme disease in Atlantic Canada.

15. Shocking worms!

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In the Anthropocene era, living organisms are confronted with numerous environmental changes that threaten their survival. The adenylate energy charge (AEC), which represents the energy status of an organism, may be a good indicator of stress due to these factors. It is therefore crucial to develop a fast and efficient method for measuring adenylates and therefore AEC. To achieve this, the muscle contraction of earthworms was electrically stimulated to generate stress. Several solvents and homogenization methods were tested in order to select those most effective in extracting ATP, ADP and AMP. Adenylates and lactate, another stress marker, were quantified by HPLC-MS/MS and AEC was calculated. The results showed that electrical stimulation did not affect AEC, but did reduce total adenylate concentration in stimulated earthworms. The experimental method developed now makes it possible to measure adenylates and AEC in a variety of organisms exposed to stressful environmental factors.

16. The Ecotoxicity of a N-Heterocyclic Carbene Species on *Dugesia dorotocephala*

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Anticorrosion materials often contain chemicals such as metals, organic solvents, or corrosive substances. When these materials are applied to surfaces exposed to water, such as bridges and pipelines, there's a risk of chemicals leaching into aquatic environments, which can lead to adverse effects, depending on the concentration and nature of the chemicals involved. Current research shows that the unique properties of N-heterocyclic carbenes (NHC) make them attractive candidates for the development of advanced anticorrosion materials; however, their effects on the toxicology of organisms found in freshwater systems remains understudied. Using brown flatworms (*Dugessia dorotocephala*) as a model, the relationship between NHC concentrations and planarian toxicity were investigated under various pH and temperature conditions over a 96-hour exposure. The greatest mortality in both experiments was found in the highest concentration of NHC. However, the

percentage and rate of mortality differed for each pH and temperature experiment. Results indicate that higher NHC concentrations lead to increased mortality rates, with abiotic factors playing a significant role in modulating toxicity. Further research into the accumulation and distribution of NHCs in aquatic organisms is required to fully assess their environmental impact and inform the development of safer anticorrosion materials. Ultimately, this study contributes valuable insights into the complex interactions between NHCs and freshwater ecosystems, highlighting the importance of considering abiotic factors in toxicity assessments and environmental risk evaluations.

17. Temperature differences in the molecular physiology of the poeciliid gill epithelium TJ complex

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Poeciliids are livebearing fishes, known for their adaptability to diverse environmental conditions, including varying temperatures. This study considered the molecular physiology of the gill epithelium tight junction (TJ) complex of the guppy (*Poecilia reticulata*), in response to water temperature. Claudin (Cldn) TJ proteins known to be abundant in the gill and responsive to environmental change were targeted. Fish were acclimated to either high (35°C), medium (control) (25°C) or low (15°C) temperature conditions for two weeks. Gill samples were collected for analysis of cldn mRNA abundance by qPCR. Data indicate that many cldns exhibit differences in mRNA abundance in response to elevated or lowered water temperature, but in all cases, a decrease in mRNA abundance was observed. In terms of trends, transcript abundance of genes encoding Cldn-8 proteins typically decreased irrespective of temperature change (i.e. elevated or reduced water temperature typically saw a decrease in *cldn-8* gene family mRNA abundance) while *cldn-3* gene family mRNA abundance only significantly decrease in low temperature conditions. In contrast, *cldn-28b* and *-30b* mRNA decreased only in high temperatures. Data suggest that temperature-induced differences in the molecular physiology of the gill TJ complex occur in fishes. By inference, alterations in mRNA abundance indicate that the gill epithelium TJ complex becomes leakier in high and low temperature conditions. But this study considered a limited number of Cldn family members at the transcriptional level only. Therefore further study will be required to tease out the TJ complex role in gill epithelium permeability following temperature change.

18. Does the parasitic wasp, *Cotesia congregata*, produce leaky brains and sticky synapses in its host?

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The parasitic wasp, *Cotesia congregata*, injects its eggs into the body of the caterpillar. The host's behaviour remains normal during wasp larval development within the host's body cavity. Once the wasps are ready to pupate, they exit through the caterpillar's body wall and form cocoons on its cuticle. After the wasps have exited the host, the caterpillar loses all self-generated behaviours (i.e. feeding and spontaneous locomotion). However, defensive behaviours remain intact, allowing the host to act as a bodyguard for the wasp cocoons.

One hypothesis for the change in host behaviour is that the wasps induce neuroinflammation, resulting in heightened immune-neural signaling. We tested whether the blood-brain-barrier becomes leaky concomitant with the host's behavioural changes. Such leakiness would produce neuroinflammation. Trypan blue injections were used to assess brain permeability. Penetrance of the dye into the supraesophageal ganglion was measured using histological images and absorbance spectroscopy. However, we found no evidence for a leaky brain barrier.

The bodyguard phenotype could also be created by reducing synaptic transmission. We increased neural activity in 'bodyguard' caterpillars by using electroshock and stressful handling. Although there

was no significant difference across stressed groups, preliminary data suggests all forms of stress triggered feeding. These results suggest that a stress response can produce partial recovery.

19. Field Testing and Validation of Clearflow Coal Mine Water Treatment Technologies

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Mining activities often lead to high levels of suspended particles (TSS) as well as other exceedances in a number of water quality guidelines for aquatic life. Clearflow Group has developed coagulating/flocculating polymers that produce rapid clarification of mine waters due to the cationic polymers binding to the mainly negatively charged TSS. However, following the clarification of mine waters, residual cationic polymers are still toxic to aquatic life, especially fish. Clearflow's neutralizing polymer ameliorates the toxicity of residual cationic polymers rendering the process of mine water treatment environmentally friendly. In addition, the polymer treatments reduce the concentrations of a number of other chemicals that typically exceed the guidelines for aquatic life in mine water effluents. This field study examines the effects of treating mine water with both cationic and neutralizing polymers on nutrient and metal contaminant removal, invertebrate communities impacts, and toxicity to fish. The first season of study demonstrated that the use of neutralizing polymers in combination with cationic polymer clarification of the mine water had no acute toxicity to trout fingerlings. Water chemistry overall was substantially improved by the polymer treatments. The invertebrate community compositions in the polymer treated mine effluent stream vs the receiving river appears to promote the abundance of mollusk species.

20. Seasonal acclimation of cardiac heat tolerance and adrenergic sensitivity in Greenland cod (Gadus ogac) in the Central Canadian Arctic

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Northern climates are characterized by extreme seasonality which can challenge critical physiological processes, including cardiac function. During warming, heart rate in fish generally increases, which supports increased tissue oxygen demands but only up to a critical temperature at which a peak heart rate is reached. Conversely, at cold winter temperatures, heart rate is slowed. Available single-species studies have revealed considerable diversity in the responses of heart rate to temperature change among fishes, but the mechanistic basis and significance of this diversity is unclear. For example, adrenaline can be critical for maintaining heart function at thermal extremes, but the extent to which thermal acclimation of cardiac temperature sensitivity is driven by changes in adrenergic regulation requires exploration. Using an electrocardiogram approach, we investigated seasonal changes in cardiac heat tolerance and adrenergic sensitivity in winter-and summer-acclimated Greenland cod in the Central Canadian Arctic. Fish were anaesthetized, fitted with subdermal electrodes, and given a drug injection to elicit either maximum or intrinsic heart rate, with the difference being indicative of adrenergic sensitivity. Fish were then acutely warmed until heart rate peaked, and cardiac arrythmia began, indicating heart failure (an upper cardiac thermal limit). We found that cardiac heat tolerance is markedly compromised in winter relative to summer-acclimated cod with only slight effects of adrenaline. The magnitude of thermal plasticity is likely a result of the significant acclimatory changes

(e.g. membrane fluidity) beyond adrenergic control of heart rate that are required to allow cod to remain functional at winter temperatures as low as -1.8°C.

21. The disruption of gastrointestinal hormonal control of digestion in Nile tilapia (*Oreochromis niloticus*) by omeprazole

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Stomach acidity is a critical aspect of the digestive system, facilitating the breakdown of protein through the activation of the protease pepsin. Gastric acid is enabled by H^+/K^+ ATPase, the gastric proton pump. Our earlier study on Nile tilapia showed that inhibiting the production of stomach acid using omeprazole, reduced growth in the species that correlated with an acceleration of the rate of gastric emptying. This contrasts with studies in mammals in which there is a delay in gastric emptying and no impacts on growth. To address the question of the mechanism behind the effects on gastric emptying in tilapia, we examined the role of cholecystokinin (CCK). CCK is secreted by enteroendocrine cells in the anterior intestine in response to acidic chyme from the stomach and acts as a brake to stomach emptying. CCK controls the rate of gastric motility as it contracts the most anterior part of the intestine to delay gastric emptying, holding food so that it can properly go through the digestive process. Post-prandial changes in tilapia fed a fixed ration of 2%BM/day of pellets either with (25mg/kg/d) or without omeprazole. Our results show lower circulating plasma CCK concentrations and lower *cck* gene expression in anterior intestine. Which together suggest a reduced release of CCK that would explain the accelerated rate of stomach emptying seen in tilapia. My study will observe the interactions of gastric acidification and its relationship to the endocrine system on a molecular level not only with CCK but other hormones that are influenced by gastric acidification, bridging the gap in knowledge towards the endocrine systems interaction with the stomach in the teleost species.

22. Cold acclimation and its impact on the protein metabolism of *Drosophila suzukii*

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Drosophila suzukii, (a major threat to agricultural productivity which was localized to tropical regions) is now found in all Canadian provinces due to its ability to survive the cold characterized by a clear phenotypic distinction between "summer morph" and "winter morph". The biological mechanisms that confer this cold hardiness to *D. suzukii* have been studied at the transcriptome level but remain poorly studied at the proteome level. Maintenance of protein conformation or degradation is an energetically costly process and might thus be a key process to understand this phenotypic switch. This project therefore aims to explore the impact of cold development and acclimation on the protein metabolism of *D. suzukii*. For this purpose, two cohorts of *D. suzukii* will be used, one control with larvae and adults developing at 25°C (summer morphs), and the other with larvae and adults developing at 10°C (winter morphs). We will first measure protein synthesis, adenylic energy charge (ATP, ADP and AMP concentrations) and mitochondrial ROS in vivo (with the ratiometric probe MitoB) by mass spectrometry (MS). The activation of regulatory proteins, such as AMPK, a kinase responsible for activating catabolic pathways and inhibiting anabolic ones to maintain the energy balance, will also be evaluated to understand the regulation of protein metabolism between morphs. Finally, the expression of genes linked to antioxidant defences and protein folding will be evaluated to understand how protein damage is controlled by the organism.

23. Simulating Season: The effects of photoperiod and temperature on thermogenesis in deer mice

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Ambient temperature is known to drive changes in the thermal physiology of mammals, such as an increase in the capacity for thermogenesis in winter. Our previous work has shown that acclimation to chronic cold increases the capacity for non-shivering thermogenesis (NST) and thermogenic capacity (cold-induced VO_2max) in deer mice (*Peromyscus maniculatus*). Daylight, or photoperiod, is an important driver of seasonal changes in physiology. In the wild, animals are attuned to seasonal changes in temperature and photoperiod. However, few studies have examined the combined effect of photoperiod and temperature on the capacity for NST in small mammals, such as the deer mouse. To address this, we acclimated deer mice to long or short photoperiods (12h or 8h light), in either thermoneutral or cold conditions (30°C vs 5°C). To simulate the fall conditions in their natural habitat (Nebraska) we gradually reduced either daylength, temperature, or both over 4 weeks and acclimated mice an additional 4 weeks at those conditions. After 8 weeks of acclimation, we determined NST and VO_2max for each mouse. Thus far, we found that the capacity for NST increases with acclimation to short photoperiod and cold conditions but cold-induced VO_2max does not significantly change.

24. Induction of sodium uptake recovery mechanisms in adult zebrafish during low-pH exposure

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Sodium homeostasis in fish gill cells is integral for maintaining proper cellular and bodily function. Sodium uptake in most freshwater fish species is conducted by electroneutral transporters, however, electroneutral transporters in fish ionocytes are thermodynamically unfavoured for forward function at reduced environmental pH (< pH 6) and low environmental ion concentrations (freshwater). Many fish species like cyprinids are known to live in these environments without ionoregulatory issues, which has created interest in the overall function and molecular mechanisms of ion transporters in these fishes. One novel transport model of sodium uptake that has been shown in zebrafish (*Danio rerio*) that uses an as yet undetermined potassium-driven sodium transporter that theoretically circumvents the thermodynamic constraints of low environmental pH and has shown to recover sodium uptake to nominal rates after chronic exposure to low pH conditions. This current study has used radiotracer-based ion flux technologies to measure sodium uptake in zebrafish over acute (<2 hours) and chronic (8 hours) exposures to pH 4, which has shown increased sodium uptake and net potassium efflux between 6-8 hours of pH 4 exposure. We are currently investigating ion flux profiles similarly in other species of known acid-tolerant fish species like cardinal tetras (*Paracheirodon axelrodi*) and fathead minnows (*Pimephales promelas*) and conducting differential gene expression analyses to find candidate genes for the unknown potassium-driven sodium transporter.

25. Total suspended solids impact on the stress response in fish

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Total suspended solids (TSS) are present in our waterways, and they are subject to daily fluctuations caused by both natural and anthropogenic events. The increases in TSS can negatively impact water quality, and also affect aquatic life. However, whether the TSS impacts the stress performance of fish is far from clear. In this study, we investigated whether 4-day exposure to TSS would compromise the stress response and alter the liver and muscle metabolism in fish. To this end, we carried out a multi-species comparison, including rainbow trout (Oncorhynchus mykiss), cutthroat trout (*Oncorhynchus clarkii*), brook trout (*Salvelinus fontinalis*), fathead minnow (*Pimephales promelas*), and longnose dace

(*Rhinichthys cataractae*) exposed to TSS concentrations ranging from 0-1000mg/L for 96 hours. We measured cortisol as a marker of the primary stress response, while glucose, glycogen, and lactate concentrations were measured to assess the metabolic consequences. Furthermore, to test their stress performance, fish in the 100 mg/L group were subjected to an additional acute stressor, which consisted of a 3-minute air exposure and sampled at 0, 60, and 120 min later. All species tested showed no significant effect of 4-day TSS exposure on the cortisol and metabolic stress response. Also, they were all able to elicit a cortisol and metabolic stress response to a secondary acute stressor despite the TSS exposure. Altogether, 4-day exposure to TSS did not affect either the steady-state cortisol and metabolite levels or the dynamic stress response, suggesting that the stress performance of fish was not compromised in the present study.
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26. If looks could chill: Characterizing visual response in freeze-tolerant crickets
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Freeze-tolerant insects can withstand internal ice formation, during which their cells experience osmotic, ionic, metabolic, and mechanical stress. Insects process visual information via modulation of ions in precisely structured, integrated sensory and nervous tissues in the compound eye, which is therefore likely vulnerable to the stresses of freeze-thaw. I aim to determine how freezing and thawing affect the eyes of the freeze-tolerant spring field cricket, *Gryllus veletis*. I use electroretinograms to measure photoreceptor potential, time-to-peak response, and critical flicker fusion frequency, which describe photoreceptor activity, laminar neuron activity, and temporal resolution, respectively. I compare acclimated freeze-tolerant crickets to non-acclimated freeze-intolerant crickets to identify how becoming freeze-tolerant changes visual performance. I also compare the effects of freezing and cold exposure on freeze-tolerant and -intolerant crickets to control for the effects of cold verses ice formation. This is the first study of eye function in freeze-tolerant insects, and will generate hypotheses about how freeze-tolerant insects protect and/or repair sensory epithelia and neurons in winter.

27. Temperature tolerance and regulation of cardiac function in lumpfish (*Cyclopterus lumpus*)
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At poleward latitudes, water temperature can fluctuate greatly over days to seasons, which can challenge the physiology and whole animal performance of ectothermic fish. Acute and acclimatory adjustments of the cardiovascular system are thought to be key to coping with thermal challenges among fishes. Heart rate will increase with warming water to meet high tissue oxygen demand up until a peak temperature at which point heart rate peaks and then becomes arrhythmic and fails with further warming. Conversely, when faced with colder water, the heart rate and metabolism is slowed. We are exploring the extent to which different species with different thermal niches and strategies can adjust their heart rate, cardiac thermal sensitivity, and cardiac temperature tolerance to compensate for thermal change. In particular, we are assessing the role of adrenergic regulation of the heart in contributing to cardiac thermal plasticity among fish species. Using a well-established electrocardiogram (ECG) method, I am investigating the temperature tolerance and adrenergic regulation of cardiac function in lumpfish, a cold- and winter-active species that is also of aquaculture interest. My study will provide insight into the interaction between adrenaline, cardiac function and thermal limitations in cold temperate fishes.

28. Drivers of Metabolic Consequences of Freezing in the Intertidal Mussel, *Mytilus trossulus*

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The bay mussel, *Mytilus trossulus*, is an intertidal bivalve found along the west coast of North America which risks freezing during low tides in the winter. Despite being freeze tolerant, the energetic costs required to survive freezing are unknown and may affect its role as an ecosystem engineer. Using a closed respirometry system, we measured their oxygen consumption as a proxy for metabolic rate before and after single and repeated freezing events. We compared these responses to hypoxia exposures as tissues are not perfused when frozen which largely inhibits gas exchange. In general, we observed that mussels' oxygen consumption rates decreased immediately after a single freeze. This initial metabolic decrease after freezing may relate to the damage to the oxygen cascade and limit the capacity for oxygen uptake (e.g. damage to the gill). Alternatively, it may be representative of a deliberate metabolic suppression to limit oxidative damage. However, after 24 hours of recovery from a single freeze, metabolic rates were generally elevated. Additionally, repeated freeze-thaws did not cause the same decrease in oxygen consumption, and generally showed an elevation in metabolic rates. Combined, this suggests that periods of repair may be crucial to survive freezing, and that the elevated metabolism may be fueling post-freeze repair. Further work will elucidate the mechanisms underlying the main driver of this response and the broader ecological implications to the intertidal zone following cold spells.

29. Posttranslational modifications of mitochondrial proteins in hibernating thirteen-lined ground squirrels, *Ictidomys tridecemlineatus*

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Thirteen-lined ground squirrels (TLGS), *Ictidomys tridecemlineatus*, are obligate hibernators native to central North America. TLGS inhabit regions where seasonal changes result in harsh environmental conditions, and hibernation maximizes a ground squirrel's likelihood of survival. When TLGS hibernate, they experience two distinct physiological states: torpor and interbout euthermia (IBE). When entering torpor, whole-animal metabolism and mitochondrial respiration decrease dramatically over a short period. The mechanism underlying this rapid change in mitochondrial respiration between torpor and IBE is not fully understood, however past research suggests posttranslational modifications (PTMs) to mitochondrial proteins contribute. PTMs modify proteins at a similar timescale of the quick entrance into torpor. Additionally, enzymes function slower at low temperatures and faster at high temperatures, which is consistent with mitochondrial metabolism increasing slower when body temperature is cold in torpor, and decreasing faster when body temperature is high in IBE. My project compares the abundance of several PTMs to TLGS liver mitochondrial proteins in torpor, IBE, and summer euthermia. Using isolated liver mitochondria from TLGS in these three states, I performed western blotting to quantify the abundance of protein acetylation, hydroxylation, methylation, phosphorylation, S-nitrosylation, succinylation, sulfhydration, and SUMOylation. Of particular interest, acetylation, hydroxylation, S-Nitrosylation, and SUMOylation were significantly higher in torpor in comparison to IBE and summer euthermia. Conversely, phosphorylation and sulfhydration were significantly higher in IBE than in torpor, while succinylation was highest in summer euthermia. Differential PTMs between torpor and IBE correspond with changes in mitochondrial function suggesting a mechanism underlying this extreme example of mitochondrial plasticity.

30. The Influence of an Energy Deficit on Neurogenesis in Zebrafish (*Danio rerio*)

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Neurogenesis is the process through which new neurons are formed in the brain, and this process continues throughout the lifespan of zebrafish in all major brain regions. Neurogenesis is modulated by a variety of factors, but the reason for individual variation in the rate of neurogenesis remains unknown. Given that the maintenance and generation of neural tissue is energetically expensive, we tested the hypothesis that individual rates of neurogenesis are modified to maintain energy balance. Under acute energy deficits, we predicted that neurogenesis will be prioritized to sustain neural function. We exposed adult zebrafish to either increased temperature (33°C) and/or starvation for 3 days in a fully factorial experiment. Cell proliferation in the brain was quantified in individuals from each group using the mitotic marker 5-bromo-2'-deoxyuridine (BrdU) and tallying the number of BrdU+ cells in cross-sections of the telencephalon and cerebellum. Gastrointestinal tract (GIT) and body weight measurements were recorded and compared between all experimental groups. Although the acute energy deficit did not impact body weight, the GIT weights of fish in the Starved and Starved+Heated groups were significantly less than those of fish in the Control and Heated groups. If the number of BrdU+ cells are similar across treatment groups, this would support a trade-off between neurogenesis and the GIT under acute energy deficits.

31. Prevention of cold-induced ionoregulatory collapse via cryoprotectant accumulation in a freeze tolerant insect: Asian longhorn beetle (*Anoplophora glabripennis*)

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Temperature is a key environmental constraint on insect population size and distribution, and a crucial factor limiting establishment and spread of invasive species. For insects, cold exposure results in progressive loss of ion balance, which is dependent on both exposure duration and intensity. Ionoregulatory collapse is characterized by extracellular hyperkalemia, which causes tissue damage and eventually death. Preliminary data suggests mountain pine beetle larvae (*Dendroctonus ponderosae*; MPB), which are freeze-avoidant, only partially lose ion balance during chilling and maintain extracellular [K^+] just below lethal cellular limits – unlike less cold-tolerant species. Similar to MPB, freeze-tolerant, Asian longhorn beetle larvae (*Anoplophora glabripennis*; ALB), suppresses their supercooling point in winter partially by accumulating cryoprotectants (mainly glycerol). Whether cold-hardy species other than MPB can mitigate cold-disrupted ion balance is unknown, as are the mechanisms behind this phenomenon. This research will examine whether cryoprotectants, in addition to preventing freezing, increase cold tolerance by acting as osmoprotectants that mitigate cold-induced ionoregulatory collapse. Using ALB as a model system, we will characterize their cold tolerance and explore the relationship of hemolymph ion concentrations (Na^+/K^+), cryoprotectant accumulation, and their hypothesized interaction with cold. ALB larvae lab-reared on artificial diet will undergo cold exposures at temperatures approaching their lower thermal limit, hemolymph concentrations of cryoprotectants and ions will then be measured. Understanding the mechanisms underlying winter survival of cold-tolerant species will improve our ability to predict their capacity to survive in novel habitats outside their native ranges and forecast potential impact of future climate conditions on these species' distributions.

32. Investigating the relationship between ionoregulatory mechanisms and salinity tolerance in early life stages of mummichog (*Fundulus heteroclitus*)

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Maintenance of ion homeostasis via active ion transport is crucial for the survival of virtually all organisms. Euryhaline fishes can acclimate to both seawater and freshwater environments, and therefore must modify their ionoregulatory mechanisms in response to changes in environmental salinity. Freshwater-acclimated fish must actively absorb ions to mitigate passive ion diffusion to the environment, whereas seawater-acclimated fish must actively secrete ions to compensate for passive ion influx. In adult fishes, the active component of ionoregulation is coordinated by ionocytes, specialized cells localized to the gill epithelium, and in some species the opercular membrane. Mummichog (*Fundulus heteroclitus*) has served as an ecologically relevant model organism for many physiology studies, yet we lack an understanding of the ionoregulatory mechanisms used in its early life stages of this species. Furthermore, larvae of this species appear to be less euryhaline relative to adults, yet the mechanisms underlying this discrepancy have not been investigated. This study will investigate the mechanisms underlying early-life salinity tolerance of this species. We will use immunofluorescence to assess the expression of specific ionocyte transport proteins in response to environmental salinity during early-life development and further assess the expression of transporter genes. We predict that late larval developmental stages will be salinity tolerant as a function of ionocyte plasticity in response to environmental salinity, whereas salinity-susceptible individuals in early developmental stages will not exhibit plasticity nor changes in transporter expression. This research will address the long-standing question of how these organisms in early developmental stages acclimate to environmental salinities.

33. Characterizing the Structure and Post-Translational Regulation of the Alternative Oxidase (AOX) Protein of Animals, Plants, Fungi, and Protists Using a Yeast Expression System

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The alternative oxidase (AOX) in the electron transport system directly reduces oxygen to water and enables electrons to bypass complexes III and IV. AOX is important for cellular respiration and providing metabolic flexibility by modulating electron flow and reactive oxygen species (ROS) levels to mitigate oxidative stress and support redox homeostasis. AOX has a broad taxonomic distribution, but research gaps persist, such as the limited knowledge about AOX structural differences, their functional implications, and how the enzyme is post-translationally regulated across kingdoms. This study focuses on analyzing and comparing AOX protein sequences and structures in plants, animals, fungi, and protists using bioinformatics tools, and developing a yeast expression system to express AOX proteins from a variety of eukaryotic kingdoms for further analysis. In this study, initial analysis using predictive modelling software, such as AlphaFold and SwissModel, found that all AOX proteins examined exist as a monomer. In parallel to bioinformatic analysis, AOX cDNA sequences from various organisms were synthesized and inserted into the pYES2.1 protein expression vector designed for *Saccharomyces cerevisiae*. Future work will explore the post-translational modifications of AOX proteins across these kingdoms using biochemistry and respirometry. Research on AOX will continue to deepen our understanding of cellular respiration and organism adaptation strategies. Understanding AOX structure and post-translational regulation can lead to technologies for developing stress-resistant crops, treating mitochondrial diseases, and combating fungal and protistan pathogens.

34. Acid-Base Regulatory Mechanisms in the West African Lungfish, *Protopterus annectens*: An Investigation into the Role of the Sodium Proton Exchangers (NHE)

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The lungfish possesses a primitive lung and underdeveloped gills, making use of bimodal respiration. Like other freshwater fishes they must regulate ion- and acid-base equilibrium through active ion uptake (driven by pumps and ion transporters), albeit with challenges due to possessing reduced gills. This study aimed to elucidate the role of the sodium proton exchangers (NHE), encoded by the genes *slc9a1/nhe1*, *slc9a2/nhe2* and *slc9a3/nhe3*, which we hypothesized to play a role in acid-base regulation. Pharmacologically we observed that amiloride, a sodium-transport inhibitor, and 5-(N-Ethyl-N-isopropyl)-Amiloride (EIPA), an NHE specific inhibitor, affected titratable acidity and ammonia fluxes supporting a physiological function of the NHE. Next, a PCR based approach was used to identify the paralogues in the gill and kidney. The function of NHE (and associated acid-base transporters) was assessed by challenging lungfish under acidic and basic extremes (of pH 4.0 and 9.0, respectively). After pH 9 acclimation, ammonia excretion transiently decreased and recovered. Gill *nhe3* expression was upregulated , while kidney expression was unchanged. Rhesus glycoprotein C (*rhcg*), an ammonia channel, was downregulated in the gill suggesting an alternative method to nitrogenous waste excretion. Our results suggest that *nhe3* may be functioning as an Na^+/NH_4^+ exchanger under high pH conditions. The NHEs likely do not function under the low pH conditions, and changes in expression were not observed. Research into the physiological function of the NHEs in the lungfish serves as a crucial link into understanding the evolutionary transition from the aquatic to terrestrial environment and provides insight into relatively understudied processes.

35. Investigating the contribution of the gut microbiome to thermogenesis at high altitudes.

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High-altitude environments present significant challenges for endothermic animals, as they require high metabolic rates to generate heat at low temperatures (thermogenesis) but also have reduced oxygen levels (hypoxia). High-altitude natives have adapted to these conditions by enhancing thermogenesis in hypoxia. An underexplored contributor to thermogenesis in high-altitude taxa is the gut microbiome, the composition and function of which can be influenced by both host genetics and environment. The gut microbiome could contribute to heat production by communicating with the host's thermogenic tissues and by generating heat itself, primarily through anaerobic processes. Two series of experiments are examining this issue. First, deer mice (*Peromyscus maniculatus*) native to high and low altitudes are acclimated to warm (25°C) normoxia or cold (5°C) hypoxia (12 kPa O_2) for 6 weeks, and a subset of the latter are treated with broad spectrum antibiotics to disrupt the gut microbiome. Second, caecum contents from high and low altitude deer mice were transferred to domestic germ free mice via oral gavage. Thermogenic performance was then assessed in all mice by measuring cold induced VO_2max and cold endurance. Preliminary results thus far indicate that cold hypoxia acclimation enhances thermogenic performance in deer mice. Antibiotic treatment reduces cold endurance, with greatest effects seen in highland deer mice. Ongoing work will thus elucidate the role of the gut microbiome in thermogenesis at high altitude.

36. Unearthing the adaptive value of soil chambers built by an insect prior to winter dormancy

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Many insects construct chambers from soil prior to winter dormancy or metamorphosis. However, the adaptive value of these structures during dormancy is unclear. We used prepupae of the western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae) to test three potential roles for soil chambers: that chambers confer space necessary for life stage transitions, protect against natural enemies in soil, or beneficially modify the soil microclimate. Here, we experimentally manipulated the integrity of

chambers, by either manually breaching or leaving chambers intact, and measured the survival of prepupae exposed to soils that were either compacted, pathogen-inoculated, or flooded. We show that intact chambers (1) reduce mortality within soils inoculated with an entomopathogenic fungus (*Beauveria bassiana*) and (2) are necessary for prepupae to survive prolonged soil flooding. We discuss possible mechanisms by which chambers might reduce the severity of hypoxia in flooded soils.

37. A microscopic examination of the life cycle of *Tigriopus californicus*

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The marine copepod *Trigriopus californicus* has been used in science for toxicology and genetics experiments. Commercially, they are used as a nutritious food source for various animals in salt-water aquariums. As a first step towards conducting CRISPR via injection in this species, we wanted to better characterize their mating behaviors, development, and the timing of key life cycle events. Our preliminary observations suggest the following: 1) what has been previously termed an egg sac is in fact a sac containing developing embryos, not eggs; 2) once the embryo sac is evident, it detaches from the pregnant female 10-14 days later; 3) dropping of the embryo sac results in the immediate hatching of the nauplii offspring; 4) copepods prefer different locations in the water column of the cell culture container habitat depending on their life stage (e.g. nauplii prefer the bottom, pregnant females prefer the upper 25%, while mating pairs hover near the very top of the habitat. Our results suggest that injection of the embryo sac will not be effective for CRISPR as too much cell division has already taken place. A better strategy may be injection of pregnant females prior to the formation of the sac. Our current research is therefore focusing on the timing of mating pair separation and characterizing the development of the embryo sac.

38. Functional Characterization of Tyramine Receptors in the Yellow Fever Mosquito, *Aedes aegypti*

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The adult mosquito *Aedes aegypti* is an important organism to study due to its role as a chief vector of various human arboviral diseases such as yellow fever, dengue, and Zika virus. Tyramine (TA) and octopamine (OA) are two biogenic amines that, along with their corresponding receptors, are involved in regulating several physiological and behavioural processes in insects including metabolism, reproduction, homeostasis, and smell perception. TA and OA are thought to function analogously to epinephrine and norepinephrine in vertebrates. Moreover, TA and OA receptors are targets for antiparasitic agents and insecticides. However, our understanding of the specific roles of TA and OA and their receptors in *A. aegypti* mosquitoes remains limited. While there are several predicted TA and OA receptors in the *A. aegypti* genome, it is essential to confirm the functional relevance of different receptor subtypes by confirming their ligand-specific activation. In this study, three TA receptors (TAR1-3) were successfully cloned and functionally characterized using a heterologous system. The results highlight the pronounced specificity of three distinct TA receptors for TA since comparatively, OA was orders of magnitude less active while serotonin and dopamine yielded no activity. With this new evidence, three putative TA receptors can be considered bona fide functional TA receptors in the adult mosquito, *A. aegypti*. Future research will aim to unravel the functional role of TA signaling in reproductive biology, particularly involving TAR2 which is highly enriched in *A. aegypti* ovary and thus may have broader implications in overall mosquito biology.

39. The Mitochondrial Physiology of Torpor in Ruby-Throated Hummingbirds (*Archilochus colubris*)

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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°C is energetically costly. To survive cold nights, hummingbirds use torpor, a state of inactivity characterized by low Tb 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. Much less is known about mitochondrial function in avian daily torpor. The goal of my PhD is to determine how whole-animal and mitochondrial physiology change to facilitate metabolic suppression in hummingbirds experiencing daily torpor. I used flow-through respirometry to measure the metabolic rate of torpid and normothermic birds. When torpid, MR dropped by ~90%, Tb dropped to ~10°C and the RER changed from ~1 (carbohydrate metabolism) to ~0.7 (lipid metabolism). Next, I used high-resolution respirometry to measure respiration in isolated pectoralis mitochondria. The fuel-use shift I observed on the whole-animal level was not mirrored at the level of the mitochondria: oxidative phosphorylation (OXPHOS) was only suppressed by ~20% (complex I-supported respiration), but not when fueled with a lipid-based substrate. I also measured the activities of several rate-limiting enzymes and found no differences in metabolic flux between torpid and normothermic birds, although pyruvate dehydrogenase activity correlated with OXPHOS. These findings highlight interesting contrasts between birds and mammals and provide insight into their evolutionary paths to heterothermy.

40. Why do spring field crickets, *Gryllus veletis*, require three environmental cues to elicit a seasonal response?

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Gryllus veletis, becomes freeze tolerant when exposed to three simultaneous environmental cues: Decreasing and fluctuating temperature with decreasing photoperiod. However, we do not yet understand why these specific cues are required nor their roles in eliciting this response. Our goal is to untangle the molecular underpinnings responsible for freeze tolerance acclimation. We are exposing crickets to a full-factorial combination of decreasing, fluctuating temperature, and decreasing or constant photoperiod. Following acclimation, freeze tolerance will be tested by exposing crickets to -8°C for 90min. After 48-hours freeze tolerance will be determined by gently prodding the crickets and observing their response. Additionally, crickets from each treatment will be dissected to collect their fat body, Malpighian tubules, midgut, and hindgut for RNA sequencing. We hypothesize that the combination of environmental cues over a six-week period is required to induce a molecular response that does not arise from each cue individually. We also predict that if the removal of specific environmental cues results in the same transcriptomic expression, then the combination of environmental cues over a six-week period of acclimation triggers individual molecular responses that collectively express an induction of freeze tolerance.
We have a limited understanding of how multiple environmental cues interact to produce physiological shifts. By using a mechanistic approach, we will generate additional hypothesis and questions that will explore more specific molecular pathways under varying environmental cues. Ultimately, this will serve as the framework for identifying the molecular underpinnings of freeze tolerance acclimation.

41. Naked Truths: Unveiling Microglia Activation Patterns During Hypoxia and/or Immune Stimulation in Naked Mole-Rat Brain

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Mounting an immune response is an energetically expensive process. Immune activation is primarily fueled by aerobic energy production in most mammals but hypoxia throttles aerobic metabolism, which may inhibit immune activation. Hypoxia-tolerant organisms, which often minimize energy demand in hypoxia, must therefore make trade-offs between hypometabolism and immune competency when oxygen availability is limited. Microglia are macrophages located in the brain and change shape when activated (i.e., retracted processes and a more ameboid shape) by triggers such as lipopolysaccharide (LPS, a bacterial challenge) or hypoxia in hypoxia-intolerant species. We hypothesized that the microglia of hypoxia-tolerant naked mole-rats (NMRs) will not be activated by hypoxia or by LPS during hypoxia. To test this, we treated male NMRs 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 then sacrificed animals to evaluate microglia activation using immunohistochemistry. LPS and hypoxia exposures both induced microglia proliferation after 24h, but without an additive effect, whereas these changes were reversed at 48h. Interestingly, cell counts remained unchanged in breeding males across all groups. However, LPS combined with hypoxia led to more activated microglia with fewer and shorter projections, which were more pronounced by 48h. As such, morphological activation was still present, but proliferation did not increase. These results suggest that hypoxia activates an immune response in subordinate NMRs only but does not impair immune responsiveness to a bacterial challenge.

42. Is thermal plasticity in *Aedes aegypt*i sensitive to photoperiod?

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Environmental conditions serve as important cues for the regulation of seasonal plasticity in insects. Changes to photoperiod, the duration of darkness and daylight in a 24-hour cycle, can influence insect physiological responses, affecting their circadian rhythm and thermal plasticity. *Aedes aegypti*, a mosquito historically confined to tropical regions, has recently been found in more poleward climates, including southern Ontario, suggesting the thermal plasticity of this species may be more complex than previously believed. We have been working to characterize how different thermal environments affect adult performance in the laboratory and have confirmed adults are capable of cold acclimation under a standard tropical photoperiod of 12h:12h (light:dark). Cold acclimation reduces injury and enables survival at otherwise lethal low temperatures. To assess the impact of disrupting the mosquito internal clock, cold acclimation was attempted under an extreme photoperiod outside of their typical range (20h:4h). This photoperiod was introduced at three different life stages: during egg development, adult acclimation and at the parental generation, as mosquitos are known to be photosensitive at these life stages. Using a chill coma onset assay, we confirmed cold acclimation in all treatment groups except offspring receiving the light cue from the parental generation, suggesting a heritable factor influences this response. To investigate the possible molecular mechanisms underlying this response to the extreme photoperiod, we are now measuring expression of circadian clock genes using RT-qPCR data. This approach will help us gain a deeper understanding of the complex relationship between photoperiod cues and cold acclimation in *Ae. aegypti*.

43. Venlafaxine exposure alters mitochondrial respiration and mitomiR abundance in zebrafish brains

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Pharmaceuticals like venlafaxine (an antidepressant) are released into freshwater ecosystems from wastewater effluent and have many behavioral and metabolic effects on fish. Changes to metabolism can be regulated by epigenetic mechanisms like microRNA (small RNA molecules that regulate mRNA

translation) including regulating mitochondrial mRNAs. Nuclear encoded microRNA regulates mitochondrial gene expression in mammals, and we previously demonstrated that they may play a role in silencing mitochondrial gene expression in darters, a freshwater benthic fish. This study sought to identify if venlafaxine exposure changes mitochondrial respiration in zebrafish brains and determine if mitochondrial microRNA (mitomiRs) were differentially abundant with venlafaxine exposure. We found that in vitro exposure of zebrafish brain homogenate to below environmentally relevant concentrations of venlafaxine (< 1 µg/L) resulted in a decrease in mitochondrial respiration. To identify whether these effects also occur in vivo, zebrafish were exposed to 1 µg/L venlafaxine for 0, 1, 6, 12, 24, and 96 hours. Acutely in vivo, venlafaxine exposure had no significant effects to brain mitochondrial respiration, however select mitomiRs (*dre-miR-301a-5p*, *dre-miR-301b-3p*, and *dre-miR-301c-3p*) were also measured, as they were bioinformatically predicted to regulate mitochondrial cytochrome c oxidase subunit I (COI) abundance. These mitomiRs were differentially abundant based on exposure to venlafaxine, and with respect to sex and circadian rhythm. Overall, venlafaxine had demonstrated effects in vitro, which may have been attenuated during in vivo exposure due to the ability of the fish to mitigate effects through mechanisms including mitomiR regulation.

44. Investigating the importance of mtG3PDH in mitochondrial flexibility during environmental and physiological changes

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Maintaining cellular homeostasis is crucial to survival, and organisms must be able to adjust their physiology and metabolism to environmental and cellular constraints. Mitochondria are at the core of these adjustments, as they integrate multiple pathways and perform one of life's most important biological functions, the production of ATP via the oxidative phosphorylation process (OXPHOS). When confronted to stressful conditions such as reduced macronutrient availability and/or impairment of cellular signaling, these organelles can lose their ability to modulate substrate oxidation switching from NADH-bound to $FADH_2$-bound substrates, a process known as mitochondrial inflexibility. Recently, it was shown that feeding fruit flies (*Drosophila melanogaster*) with high-fat or high-sugar diets for prolonged periods induces mitochondrial inflexibility, characterized by complex I dysfunction which is however offset by an increased contribution of alternative mitochondrial complexes such as mitochondrial glycerol-3-phosphate dehydrogenase (mtG3PDH). As a result, mtG3PDH seems to be involved in metabolic adjustments and would therefore be a protein playing an important role in insect adaptation to environmental and cellular stress. The aim of this work is to generate D. melanogaster lines with reduced activity for mtG3PDH. Among a panel of modified lines obtained by Crispr-Cas9 method, we confirmed the reduction of mtG3PDH activity by spectrophotometry and selected multiple candidates. The lines selected will be used to study the importance of this enzyme in metabolic flexibility and inflexibility during environmental and physiological changes.

45. Effect of nutritional status and Zn on liver mitochondrial respiration and H_2O_2 metabolism in rainbow trout, *Oncorhynchus mykiss*

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Starvation is a common phenomenon in fishes under natural and aquaculture settings that may moderate effects of chemical contaminants such as metals. The key features of starvation include

reduced oxidative metabolism with redirection of energy resources towards maintenance and survival. Because mitochondria comprise the cellular oxidative energy conversion hubs and target for metals, we investigated the effects of zinc (Zn) on mitochondrial respiration and reactive oxygen species (hydrogen peroxide, H_2O_2) emission following starvation and refeeding in rainbow trout (*Oncorhynchus mykiss*). Fish were maintained under three levels of feed availability: satiation feeding (control), 7-day food withdrawal (starved), and one-time satiation feeding following 7-day food withdrawal (re-fed). Liver mitochondria were isolated, and the rates of respiration and H_2O_2 emission driven by glutamate-malate (complex I, CxI) and succinate (complex II, CxII) were measured without and with Zn exposure. Starvation and Zn exposure inhibited CxI state 3 respiration rate, with refeeding and low Zn concentration partially reversing the effect of starvation. While feeding regime and Zn exposure did not alter CxI state 4 respiration rate, they reduced CxI respiratory control ratio (RCR), with the low Zn concentration partially reversing the effect of starvation. CxII state 3 and 4 respiration rates were not altered by feeding regime but were inhibited by Zn. Both starvation and Zn exposure reduced CxII RCR, with refeeding reversing the effect of starvation. The rate of H_2O_2 emission was markedly increased by starvation but was not altered by Zn exposure during glutamate-malate oxidation. In contrast, starvation/refeeding did not alter the H_2O_2 emission rate, but Zn greatly stimulated it during succinate oxidation. Overall, our study indicates that effects of nutritional status and Zn exposure on mitochondrial respiration and H_2O_2 emission depend on substrate and that refeeding and exposure to low levels of Zn may reverse the effects of starvation.

46. Effects of acclimation and developmental plasticity on thermal and hypoxia tolerance in killifish

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With climate change, coastal aquatic ecosystems are experiencing dramatic increases in the duration and frequency of warming and hypoxic events. Although thermal acclimation is known to improve thermal and hypoxia tolerance, the potential for developmental plasticity has seldom been explored. Using Atlantic killifish (*Fundulus heteroclitus*), we investigated the relative roles of developmental plasticity and acclimation on these phenotypes. Killifish (*Fundulus heteroclitus*) embryos were incubated at 26°C with a diel fluctuation of + 0, 3, 5, or 7°C from fertilization to hatch, and were then reared at a common, constant, temperature of 18°C for 1.5 years. We acclimated these fish to 26, 18, or 10°C and assessed critical thermal maximum (CTMax) and time to loss of equilibrium under hypoxia (TLOE; 2% O_2 sat). 26°C-acclimated fish were more thermally tolerant (40.2+0.06°C) but had a lower thermal safety margin (13.5+0.08°C) than 18 (36.0+0.08; 16.2+0.1°C) and 10°C (32.1+0.09°C; 19.8+0.17°C) acclimated fish. Hypoxia tolerance was also affected by acclimation temperature, with TLOE's for 26, 18, and 10°C-acclimated fish of 8.2+1.0, 104.0+7.5, and 229.7+21.7 minutes respectively. By contrast, developmental temperature treatment had little effect on these traits, except in 26°C-acclimated fish. Those that experienced 26+3°C during incubation had statistically significant higher CTMax (40.3±0.1°C) than fish incubated at 26+0 or 7°C (40.0±0.1°C), although this difference in contrast to differences between acclimation temperatures is extremely small. Similarly, there were few significant differences in hypoxia tolerance between developmental groups. These data suggest that acclimation temperature has a stronger effect on thermal and hypoxia tolerance than does developmental temperature.

47. Exploring Sex-Specific Molecular Adaptations in Gill Tight Junctions of *Limia nigrofasciata* Across a Salinities: From Freshwater to Hypersalinity

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Poecilid teleost fishses distinguished by sexual dimorphism and physiological adaptability, serves as the focal species for this investigation into gill epithelium adaptation under varying salinity. Using Limia nigrofasciata we examined tight junction (TJ) proteins, particularly Claudin (Cldn) proteins, for sex-specific molecular responses to different saline environments. By acclimating fish to fresh (FW), salt (SW, 35‰), and hypersaline water (HSW, 58‰) for two weeks, and separating males from females with a clear partition, we assessed changes in *cldn* mRNA via quantitative PCR. The analysis was two-pronged: (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). The findings revealed several cldns responded to salinity shifts (e.g. upregulation of *cldn-8a*, *-10d*, *-30c*, and downregulation of *cldn-3b*). Notably, some *cldn* expressions were predominantly sex-specific, such as the *cldn-8a* increase in females. We also explored heat shock proteins (HSP) 70 and 90 to gauge stress responses to salinity. Conclusively, this research presents evidence of sex-specific differences in the molecular physiology of fish gill TJ complexes. These variations are inherent regardless of salinity levels but can be accentuated under environmental stress, underscoring the complexity of physiological adaptation in sexually dimorphic species.

48. Plasticity and Evolved Changes in Mitochondrial Physiology Across Skeletal Muscles in Deer Mice Native to High Altitude

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High-altitude is amongst the harshest terrestrial environments inhabited by animals, 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 mitochondrial respiration. While skeletal muscles play an essential role in both shivering and non-shivering thermogenesis, the pervasiveness of mitochondrial adjustments in high-altitude mammals remains unclear, with studies limited to a small number of muscles. 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 8-10 weeks) in a full-factorial design. Mitochondrial function was investigated in permeabilized fibers extracted from diaphragm, gluteus maximus, vastus medialis, and vastus lateralis using a comprehensive substrate titration protocol by high-resolution respirometry and fluorometry. Preliminary results suggest that acclimation to cold hypoxia increases leak respiration across muscles, which could increase mitochondrial heat generation. Mitochondrial ROS emission was lower in highlanders than in lowlanders for some muscles. Overall, this study will provide important insight into the roles of plastic and evolved changes in the function of muscle mitochondria in small mammals at high altitude.

49. Characterization of regional current densities on cardiomyocyte electrophysiological properties in Lake Sturgeon

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Understanding the coordinated processes contributing to an integrated cellular response - like an action potential - is essential for understanding the basic and applied electrophysiology of a cell in an electrically excitable tissue/organ. Cardiomyocyte electrophysiological properties in fish have been extensively studied, yet unlike in non-fish model species electrogenic cells, their spatial distribution of channels and corresponding currents have not yet been investigated. Further, general cardiomyocyte electrophysiological properties, including channel activity and current dynamics tied to cellular electrogenic events, in more classically considered 'basal' vertebrates are understudied. To address these disparities, we aim to investigate the regional distribution of numerous ionic currents along the

bodies of atrial and ventricular cardiomyocytes isolated from Lake Sturgeon, *Acipenser fulvescens*. Using whole-cell patch clamp techniques, channel-specific current type, ionic current densities, and current-voltage relationships will be characterized along the lengths of cells split into three distinct regions: 0 - 25 µm, between 25 – 50 µm, and between 50 – 75+ µm of cell ends. The main ion currents (and channel-specific currents of each ion type) recorded within each cardiomyocyte zone will be Na^+ (INa), Ca^{2+} (ICa; L-type and T-type), and K^+ (IK; both delayed-rectifying and inwardly rectifying). These analyses will crucially address the question of equal versus unequal spatial distribution of cardiomyocyte channel activity (a novel and previously never determined metric in fish) and provide key comparative information on INa, ICa, and IK properties. Our analyses will additionally contribute cardiac electrophysiological information on an ancient species with clear evolutionary importance.

50. A comparison of macromolecule extraction and quantification methods in the house cricket, Gryllodes sigillatus

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Bioenergetics is an essential component linking the physiology of any organism to its ecology. Understanding the metabolism of stored energy in organisms is a fundamental aspect of bioenergetics. Organisms store energy in the form of macromolecules (carbohydrate, lipid, and protein). The metabolism of these macromolecules is crucial for growth, reproduction, and survival. Studies on fruit flies and mosquitoes show that macromolecule reserves are a key regulating factor in life-history decisions (e.g., growth, reproduction, and metamorphosis). Linking changes in macromolecule content with changes in critical life stages can provide ecologically relevant information about the impact of biotic and abiotic environmental stressors. Accurate quantification of macromolecules is a key first step in this process, and several different methods are used for macromolecule extraction and quantification. In this study, we have extracted macromolecules from the common house cricket, *Gryllodes sigillatus* using two different extraction methods and quantified each macromolecule using different methods. Each cricket was bisected longitudinally; extraction of one-half was done with Na_2SO_4 and 75% methanol and the other half was extracted in 0.1M Tris buffer. Protein was quantified using Bradford reagent and Bicinchoninic acid (BCA) assay. For glycogen, we compared the sulfuric acid-anthrone method and the sulfuric acid-phenol method. Total lipids for both extraction methods were measured using the sulpho-phospho-vanillin method. The goal of this study is to identify a reliable, time- and cost-effective quantification method of the macromolecules in small organisms to use bioenergetics as a marker to understand the effects of environmental stressors.

51. Can aquatic acidification impair olfactory imprinting in Pacific salmonids?

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Pacific salmon (*Oncorhynchus spp.*) undergo fantastical life history events, culminating in a return from the sea to natal freshwater habitat where salmon spawn and then die. The remarkable fidelity Pacific salmon have for their natal habitat is partially driven by their ability to remember chemical cues that they detect through their sense of smell. This process is called olfactory imprinting and is hypothesized to occur during early development before juveniles migrate to the sea. Improper, incomplete, or impaired imprinting is thought to lead to salmon straying from their natal grounds to new habitats and is a phenomenon of conservation concern. While Pacific salmon stocks have been enhanced by the activities of conservation hatcheries for decades, the chemical cues in waters that salmon imprint on in hatcheries can exacerbate straying. Here, we propose that another phenomenon, aquatic acidification

by carbon dioxide (CO_2), might contribute to straying by impairing olfaction during critical developmental windows when imprinting occurs. Pink salmon (*Oncorhynchus gorbuscha*) have been the subject of separate studies on imprinting and aquatic acidification, demonstrating that olfactory imprinting occurs at the yolk-sac absorption (YSA) stage, and that olfaction is also impaired by CO_2 acidification at YSA. However, there is an overall lack of understanding of molecular mechanisms underlying imprinting and whether those mechanisms are plastic to acidification. Thus, we present a preliminary investigation into a putative molecular marker of imprinting, salmon olfactory imprinting gene, across five ecologically and economically important Pacific salmon species in preparation for larger-scale studies on CO_2 acidification and olfaction.

52. The dynamic transcriptomic response of the goldfish brain under chronic hypoxia

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Oxygen is essential to fuel aerobic metabolism. Some species evolved mechanisms to tolerate periods of severe hypoxia and even anoxia in their environment. Among them, goldfish (*Carassius auratus*) are unique, in that they do not enter a comatose state under severely hypoxic conditions. There is thus significant interest in the field of comparative physiology to uncover the mechanistic basis underlying hypoxia tolerance in goldfish, with a particular focus on the brain. Taking advantage of the recently published and annotated goldfish genome, we profile the transcriptomic response of the goldfish brain under normoxic (21 kPa oxygen saturation) and, following gradual reduction, constant hypoxic conditions after 1 and 4 weeks (2.1 kPa oxygen saturation). In addition to analyzing differentially expressed protein coding genes and enriched pathways, we also profile differentially expressed microRNAs (miRs). Using in silico approaches, we identify possible miR-mRNA relationships. Differentially expressed transcripts compared to normoxia were either common to both timepoints of hypoxia exposure (n=174 mRNAs; n=6 miRs), or exclusive to 1-week (n=441 mRNAs; n=23 miRs) or 4-week hypoxia exposure (n=491 mRNAs; n=34 miRs). Under chronic hypoxia, an increasing number of transcripts, including those of paralogous genes, was downregulated over time, suggesting a decrease in transcription. GO-terms related to the vascular system, oxidative stress, stress signaling, oxidoreductase activity, nucleotide- and intermediary metabolism, and mRNA posttranscriptional regulation were found to be enriched under chronic hypoxia. Known 'hypoxamiRs', such as *miR-210-3p/5p*, and miRs such as *miR-29b-3p* likely contribute to posttranscriptional regulation of these pathways under chronic hypoxia in the goldfish brain.

53. Unlock in progress: Physiology tools in an ecological context

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To disentangle molecular and cellular mechanisms, physiologists can rely on specific tools, designed to be mainly used on few model organisms and in very controlled conditions. However, studying how organisms can respond physiologically to environmental stressors in an ecological context (i.e. Ecophysiology), could represent a great challenge. Indeed, wild animals exhibit a huge inter-individual variability due to specific life-history trajectories, ages, sex… that could not be avoid in natural

population sampling. It is also noteworthy to add that counteracting this issue by working with very large sample size is impossible, because of the invasiveness of physiological technics. This variability has therefore to be considered within data analysis, to eventually give trustful conclusion.
To answer this methodological weakness, it is now necessary to think of different manners to fill this gap by first a better use statistical tools (i.e PGLS analysis, Bayesian approach), and by the fine-tuning development of different physiological tools that could be adapted for ecological studies. For example, muscle micro biopsy technic was recently validated on small fish, allowing to study longitudinally mitochondrial function without killing animals. Moreover, a series of DIY-low-tech systems to precisely control temperature in several aquaria is currently developed in the lab to better mimic stochastic small thermal variations that could be met by fish in natural environment. This growing interest in improving techniques to perform physiological measurements in ecologically relevant conditions should allow a better understanding of how animals respond to climate changes in the wild.

54. Effects of experimental parasite infection on aerobic scope and hypoxia tolerance in freshwater sunfish

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Water temperatures in freshwater ecosystems are rising concurrent with rising atmospheric temperatures, elevating the oxygen demands of resident fishes while simultaneously reducing dissolved oxygen levels in the water. These phenomena constrain the fishes' aerobic scopes (AS) for activity and render them more susceptible to hypoxia. Compounding this challenge is the risk of parasite infection, which, although common in fishes, is projected to increase as rising temperatures enhance the metabolic and reproductive rates of many parasites and their intermediate hosts. Because parasite infection can trigger an energetically costly and time-dependent immune response in the host, this could further impact AS and hypoxia tolerance. We hypothesized that parasite infection reduces the AS and hypoxia tolerance of fishes in a time-dependent manner. To test this, we experimentally infected pumpkinseed sunfish (*Lepomis gibbosus*) with a trematode parasite (*Uvulifer sp.*) causing blackspot disease, a common infection among centrarchid fishes. Then, at five time points over 56 post-infection days, we made respirometry-based measurements of metabolism and hypoxia tolerance, namely standard and maximal metabolic rates, AS, critical oxygen tension and PO_2 of loss-of-equilibrium, as well as underlying hypoxia-related variables including plasma lactate and hematocrit. This poster will detail the results of this project and speculate on its implications.

55. The Madagascar Hissing Cockroach: A New Neuroethological Model Species

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Due to the relatively large brains of cockroaches, American (*Periplaneta americana*) and German (*Blatella germanica*) cockroaches are common invertebrate species in neuroethology research. While valuable models, these species have drawbacks: they are fast and difficult to handle, and they are major infestation risks. Madagascar hissing cockroaches (MHCs) (*Gromphadorhina portentosa*) provide many benefits over traditional cockroach models: they are large, slow, and easy to handle, they cannot fly, they live up to five years in captivity, and they are not an infestation risk. Additionally, MHCs exhibit cognitive behaviours not seen in many insects: they are semi-social and maintain a dominance hierarchy, and they communicate by their hiss. Yet, despite MHCs being charismatic insects commonly kept as pets, relatively little is known of their biology. Copious anecdotal information is available from MHC enthusiasts, but few controlled studies have been performed. To start, we explored gustatory preference using common foods of varying nutrients, separately for adult males, adult females, and

late-instar (unsexed) nymphs. Considering reproductive investment needs, we hypothesized that females prefer more protein-rich foods than do males. Similarly, due to growth needs, we hypothesized that nymphs prefer more protein-rich foods than do adults. We performed two experiments. First, foods were individually placed in the colony enclosure for 24 hours. Consumption was measured (by weight), and time-lapse recordings were used to compare food interactions between adults and nymphs. Second, 24-hour consumption was measured in separate cohort cages for each food. Preliminary results will guide future studies involving carefully controlled nutritional choice-based experiments.

56. Developmental and behavioural responses of the obliquebanded leafroller (*Choristoneura rosaceana*) to heatwaves and insecticide

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Climate change-fueled heatwaves are expected to become longer, hotter, and more frequent over the next 20 years. Temperature plays a significant role in both the development and growth of insects, and could alter their susceptibility to pest control agents. We investigated how heatwaves and insecticide may interact to affect the growth, development, and foraging behaviour of the caterpillar *Choristoneura rosaceana* (Obliquebanded Leafroller, OBLR). OBLR are a common apple pest across North America (including Nova Scotia), and their impact on orchards will likely increase with climate change. Using third instar OBLR, we simulated a 5 day heatwave using temperatures expected in Kentville, NS within the next 20 years. During, or after, the heatwave, each caterpillar was placed on artificial OBLR diet. Half the food dish was covered with the insecticide DiPel, which is derived from Bacillus thuringiensis and damages the caterpillar's midgut wall. There were 4 groups per timepoint: heatwave exposure only, DiPel exposure only, exposure to both, and exposure to neither. Caterpillar location (on the food), weight, instar, and mortality were measured daily. Heatwave exposure accelerated growth and development, but this trend was absent when DiPel was applied early. Mortality was highest when DiPel exposure occurred early in the heatwave, suggesting a cost to accelerated development in younger OBLR. Regardless of treatment, there were no differences in foraging behaviour, suggesting OBLR do not actively avoid ingesting DiPel. These data could help guide the timing of pesticide use for greatest efficiency in the future.

57. A Study in Fur: Pine martens, fur trappers, and their joint future under rising temperatures in Maine

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Increasing temperatures from climate change are predicted to alter and constrain species distribution in unpredictable ways over time. For cold-adapted species, such as American martens (*Martes americana*), we have a limited understanding of what temperature ranges might have physiological impacts significant enough to affect their distribution. This project will develop a mechanistic model of martens' heat balance and exchange with their environment, integrating functional trait and microclimate data to develop a model specific to conditions in Maine. This project will also incorporate a social analysis of fur trapper perceptions and practices related to our species of interest. We will conduct semi-structured interviews and analyze the collected data through a phenomenological theoretical lens. By combining biophysical and social research, we hope to produce a more complete

understanding of the ways in which increased temperatures affect American martens' physiology and distribution at the southern end of their geographic range.

58. A New Approach to Evaluating Honey Bee Learning in Free Moving Associative Learning Tasks

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Learning is an adaptive process that allows for experiential modification of behaviour. An organism's ability to learn is partially dependent on its sensory systems, neural architecture, and its evolutionary and ecological context. Studying learning capacities in a species allows for better understanding of the environmental demands placed on that species, and its potential resiliency to environmental changes. However, learning is a complex process that can be difficult to quantify. Traditionally, honey bee (*Apis mellifera*) studies quantified learning by examining cumulative performance for an entire cohort rather than individuals. This method is flawed because it assumes learning is a homogeneous process among individuals within a population. Thus, it can hide variability in learning capacity, diminishing our understanding of behavioural adaptability of a population. Psychophysics is a field dedicated to developing mathematical models for evaluating cognitive processes in humans and other species, but psychophysical methods have seldom been used with bees. Binary data present a special challenge for evaluating performance over time at the individual level. Here, we conducted colour-learning experiments to develop models of binary-choice associative learning in honey bees. Bees were trained to associate escape from a y-maze with going towards either a blue or green LED. Performance for each bee was found to follow one of four patterns: learning the correct colour, bias towards the correct colour, bias towards the incorrect colour, or no learning. For bees that learned the association, the model allowed behaviour to be quantified in terms of learning strength, learning time, and learning rate.

59. Decoding the Dance: Exploring Honey Bee Responses to Modulated Electric Fields in the Waggle Dance Recruitment Language

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Although honey bees (*Apis mellifera*) they do not possess an electroreceptive organ, per se, they are capable of detecting small antennal movements induced by changing electric fields in the immediate environment via the Johnston's organ. To investigate the role of electric fields emanating from foragers performing the waggle dance on the behaviour of nearby recruits, we developed an artificial waggle dance generator that produces electric fields mimicking those of a waggle dancing bee, including both the high-frequency (~200 Hz) modulation associated with wing beats and the low-frequency (~15 Hz) modulation associated with the waggling abdomen. Honey bee foragers were then captured for two series of experiments. First, harnessed bees were placed in front of the generator electrode, and a camera recorded antennal movement. We then compared antennal velocity between signal-on and signal-off conditions. Second, harnessed bees were placed on an air-suspended ball treadmill in front of the generator electrode to record locomotor activity. We then compared locomotion between signal-on and signal-off conditions. Preliminary results suggest activity decreases in the presence of the artificial waggle dance signal. Future directions involve installing the generator electrode on the dance floor of an observation beehive to determine if modulated electric fields affect observers' behaviour in a functioning hive. These findings contribute to our understanding of the complex interplay between electric fields and honey bee behaviour, shedding light on the mechanisms underlying communication and navigation in these important pollinators.

60. The Metropolitan Wasp: Understanding Behavioural Responses to Urbanization in Yellowjackets

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Urban environments provide unique challenges for many animals, often leading to population reduction and diversity loss. Vespid wasps (commonly known as yellowjackets), however, display a high degree of behavioural plasticity and thrive in urban environments. For this reason, they are a good system for studying behavioural adaptation to urbanization and human-wildlife conflict. Despite this, yellowjackets are usually studied only as pests and invasive species; relatively little is known of their behavioural ecology or neurobiology. In the present study, we examined changes in defensive behaviour between urban and rural yellowjacket colonies, along with the neural correlates of these changes. Colony-level aggression was measured using an automated decoy that recorded strikes after a disturbance was provoked at the nest entrance. Response duration and total number of strikes per response were compared between urban and rural colonies. Additionally, because serotonin is implicated in aggression in insects, we measured serotonin levels in the brains of individual yellowjacket specimens from each nest. Brains were sliced, immunolabelled for serotonin, and imaged on a confocal microscope. Serotonin levels in sensory and cognitive brain centres were then compared between urban and rural colonies. Here we present validation of these methods and the results of a small pilot study.

61. Examining Bitter Detection and Gustatory Preference in Honey Bees

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Honey bees (*Apis mellifera*) generally need not discern the safety of foods they collect, as their foods are “gifts” from plants. This is likely why bees have far fewer gustatory receptors compared to other insects. However, exactly which receptors bees possess and what tastants they can detect is unclear. Their genome appears to contain many gustatory pseudogenes. Specifically, it is unclear if bees possess bitter receptors, despite quinine (typically a bitter tastant) frequently being used as a gustatory punishment in bee training experiments. While bees clearly prefer sucrose over sucrose+quinine, the preference is possibly due to quinine acting as a sweet receptor antagonist rather than a bitter receptor agonist. We performed two series of gustatory preference experiments. First, we placed individual fasted bees in cages with pairs of coloured wells containing two different solutions. Bees learned to associate well colour with the preferred solution, and we measured the quantities of the solutions consumed. Second, we placed individual fasted bees in a y-maze, where they learned to associate the colour in one arm of the maze with the preferred solution. We then calculated preference scores for each bee. In both series, we tested sucrose vs sucrose+quinine, sucrose vs water, water vs quinine, and as a control, sucrose vs sucrose+NaCl. Bees demonstrated a preference for sucrose over water, quinine, and NaCl, but no preference between quinine and water. This work is part of a larger study investigating sensory systems and phytochemicals, in particular allyl isothiocyanate (the “wasabi compound”) in canola nectar.

62. Naphthenic acids contribute to oxidative stress by eliciting the emission of reactive oxygen species in mitochondria

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Oxidative stress is a major cellular mechanism of toxicity elicited by a plethora of toxicants. The mitochondrial oxidative phosphorylation pathway is a major cellular source of reactive oxygen species contributing to oxidative stress. Recent research shows that 3,5-dimethyladamantane-1-carboxylic acid, a model quaternary naphthenic acid, increased ROS emissions in isolated mitochondria. The purpose of this study was to determine if a primary carboxylic acid, the moderately hydrophobic naphthenic acid, 3,5-dimethyladamantane-1-acetic acid, would cause the hydrogen peroxide (H_2O_2) production by mitochondria in multiple bioenergetic states. It was hypothesized specific mitochondrial electron transport complexes are the site of ROS emission. Mitochondria isolated from rainbow trout (*Oncorhynchus mykiss*) liver were exposed to commercially available 3,5-dimethyladamantane-1-acetic acid. The emission of ROS during States 3 and 4 respirations was quantified using a respirometer. Subsequently, each ROS emission site in the mitochondrial complexes was isolated using inhibitors, and the ROS emission of each site was measured in multiwell plates using the AUR-HRP system. The compound 3,5-dimethyladamantane-1-acetic acid caused ROS emission in state 3 and state 4 with EC_{50} of 0.77 mM. The test compound increase ROS emission in a dose-dependent manner at all ROS production sites, OF, IF, IQ, IIF, IIIQo. However, some sites were more responsive to the production of ROS than others with IQ > IIIQo > IF > IIF > OF. Overall, the research shows that the mitochondrial H_2O_2 metabolism in rainbow trout liver is a possible source of oxidative stress in response to naphthenic acids.

63. Towards a Baseline Behavioural Survey of Juvenile Lobsters (*Homarus americanus*) in the Southern Gulf of St. Lawrence Scallop Buffer Zone

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Scallop Buffer Zones (SBZ) were created in the southern Gulf of St. Lawrence by the Department of Fisheries and Oceans to protect juvenile lobster habitat by restricting scallop dragging. As the SBZ are newly established and encompass almost all of the available juvenile habitat in the region, gathering baseline and monitoring data will be important for evidence-based management decisions for lobster populations. We conducted a pilot survey of juvenile lobster abundances and behaviours at one site in the SBZ to collect baseline behavioural data of juvenile lobsters across environmental factors of food availability, depth, and substrate. We hypothesized that juvenile lobster behaviour will change across all three factors. We recorded continuous top-down underwater video from the shallow subtidal habitat of juvenile lobster. For analysis, we determined abundances of lobsters in the videos and constructed a time budget for six broad behavioural categories. Variations in sheltering and digging behaviour were observed, corresponding with either food availability or depth (or both), as well as across substrate types. Increased sheltering on cobble is expected because lobster shelters are typically available amongst the cobble, while burrows cannot be typically constructed in unconsolidated sand. Despite this, we found increased digging behaviour on sand, either as a futile effort at shelter construction, or more likely a foraging strategy. This pilot study will contribute to the development of the full baseline survey methods as well as later monitoring efforts to document the status of juvenile lobsters in the southern Gulf of St. Lawrence.

64. Hybridization along a salinity gradient between two species of Killifish

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A species can be defined as a group of individuals capable of reproducing with each other and producing viable, fertile offspring. Nevertheless, some species can reproduce with each other and produce viable, fertile offspring. This is the case for *Fundulus diaphanus* and *Fundulus heteroclitus*, two fish species found in New Brunswick. In 2022, a hybridization zone was identified in the Richibouctou River. Mitochondrial DNA (mtDNA) analyses revealed that all individuals sampled were of the *F.*

heteroclitus type, although their phenotype was not always concordant. The 2023 results seem to confirm this finding, suggesting the presence of *F. heteroclitus* mtDNA in *F. diaphanus* populations. This suggests that New Brunswick hybrids have the ability to reproduce sexually with individuals of the parental species, and that the maternal species of the individuals is *F. heteroclitus*.

65. Preliminary Exploration of Fjord Wall and Sea Floor Epibenthic Invertebrate Communities in the Saguenay-St. Lawrence Marine Park

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The Saguenay-St. Lawrence Marine Park of Tadoussac, Quebec was the first park established in Canada for the protection of solely marine habitat. Its boundary contains highly productive waters in both fjord and estuarine environments, with well known and abundant populations of marine mammals such as the southernmost beluga whales on Earth. Invertebrate communities play an integral role in the local food web supporting these populations. The diversity, abundance, and distribution of epibenthic invertebrate communities along the 80km long Saguenay Fjord was largely unknown. In Fall 2023 as part of a wider research initiative and with the support of the marine park and the Trebek Initiative, a combination of underwater ROV and drop camera quadrats were used to begin to address this research gap. Seven randomized vertical fjord wall surveys were conducted with an ROV to a depth of 100 metres. Survey video and photographs were collected at pre-identified depth intervals along with accomanying CTD data. A 50 cm^2 drop camera quadrat was dropped at 45 randomized locations along the fjord floor. Extensive and diverse populations of invertebrates were observed. Preliminary results indicate that the walls of the fjord are critical habitats for local epibenthic invertebrate populations when compared against the fjord floor, and observed species diversity and abundance decreased further upstream in the fjord. Further data analysis and collection is needed to understand the state and composition of invertebrate communities within the Saguenay-St. Lawrence Marine Park.

66. Quantifying post-larval American lobster (*Homarus americanus*) benthic recruitment in relation to depth and temperature in southwest Bay of Fundy

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Benthic recruitment of American lobster, which provides an index of future fisheries recruitment, has historically been monitored in shallow waters (5-15m) due to an assumed avoidance of colder (i.e. deeper) waters by settling post-larvae. However, climate-driven warming and strong tidal mixing could be expanding the depth and total area of seafloor over which lobster post-larvae are recruiting in the Bay of Fundy. The main objective of this study is to test this hypothesis. Bio-collectors will be deployed in 2024 to quantify temperature and benthic recruitment of lobster between 5-95m within three study regions in southwest Bay of Fundy. Although we expect greater recruitment in shallow waters, we predict benthic recruitment to occur at greater depths than historically monitored. Furthermore, using bathymetric maps and our recruitment at depth data, we will estimate the proportion of benthic recruitment that occurred at different depths in our study domain. We will also explore the relationship between recruitment density and temperature over different time periods in our study year and will create a model that can hopefully be used to hindcast changes in the extent of suitable habitat for lobster based on historical records of water temperature. Quantifying American lobster post-larval benthic recruitment at depths greater than historically sampled in a region where climate change is changing habitat suitability is crucial in understanding how fisheries recruitment may be affected down the line.

67. Fish master: Foraging behaviour of northern gannets (*Morus bassanus*) feeding on five main prey species in the Gulf of St. Lawrence

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Northern gannets (*Morus bassanus*) are piscivorous predators that feed on a wide range of prey species and sizes. Notably, gannets from the colony at Parc national de l'Île-Bonaventure-et-du-Rocher-Percé (Québec, Canada) typically consume five pelagic fish species during the breeding season: Atlantic mackerel (*Scomber scombrus*), capelin (*Mallotus villosus*), sand lance (*Ammodytes sp.*), Atlantic herring (*Clupea harengus*) and rockfish (*Sebastes sp.*). Their broad diet implicates gannets' flexible foraging behaviour regarding search and capture techniques for prey. Indeed, studies have shown that gannets utilize a variety of diving strategies to capture prey of different sizes, including variation in terms of dive shape, depth reached and duration. Another peculiar feature of their foraging habits is that gannets regurgitate food to their chicks or when they are slightly disturbed by researchers. In this study, we used the fact that birds handled upon their return from a foraging trip were regurgitating the last prey captured. Our primary goal is to characterise the foraging behavior of adult northern gannets in the Gulf of St. Lawrence during the breeding season, focusing on the five main pelagic fish species they consume. Using GPS and diving loggers deployed from 2020 to 2023, we aim to identify key elements of travel, diving, and resting throughout foraging trips where the bird regurgitated a single prey species upon returning to the colony. Furthermore, we aim to quantify and compare the profitability, in terms of energy gained per unit of foraging time, of the five main prey species. Here, we present our preliminary findings.

68. A New Invasive Exotic Slug in Québec: *Arion vulgaris*
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Species monitoring is required to quickly identify new introductions. Early detection of invasive exotic species generally increases the success of control methods, thus limiting the species impacts. However, cryptic species, which are by definition hard to identify, require additional identification work and can remain undetected for years. This is the case of the exotic slugs of the genus Arion, known for their significant ecological and economic impacts worldwide. Despite the challenges associated with their identification (e.g. externally similar species or hybridizations), it is essential to know their identity as different slug species have their own invasion specificities. Here we present the first record of the European slug *Arion vulgaris* in Québec, a slug classified among the 100 most invasive species in Europe. A combination of genital apparatus morphological analyses and 16SrDNA gene sequencing were used to confirm the identity of the species. As this species has a high invasive potential, it should be carefully monitored in Québec to limit its spread and evaluate its impacts.

69. The effect of turbidity on nasal and olfactory epithelia in the Redside Dace
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Anthropogenic activities and natural disasters increased water turbidity and endanger aquatic species. In fish, turbidity damages gills impairs vision, alters ventilation, disrupts reproduction, impacts growth, hampers movement and elevates mortality. Turbidity may also impact olfactory sensory neurons and other nasal cells, including crypt cells which are associated with chemosensation for predator/prey interactions, reproduction, and migration. This study investigated the effect of water turbidity on the olfactory rosette of Redside dace, a species challenged due to urbanization-induced effects. Bentonite clay was added to increase water turbidity and olfactory sensory neurons in the nasal cavity were examined. Immunohistochemistry was utilized to label olfactory crypt cells with anti-S100 protein, microvillous olfactory sensory neurons with anti-calretinin and anti-acetylated tubulin to label ciliated

olfactory sensory neurons. Exposure to bentonite was followed by an increased crypt cell abundance in the nasal epithelium, but not in the olfactory epithelium. Variations were observed in calretinin-immuno-labeled microvillous cells, with some rosette regions not labelling in bentonite-treated samples. This study highlights the sensitivity of Redside dace to environmental changes, particularly to water turbidity. These implications for the olfactory system may impact the survival and reproduction of this species. Understanding these impacts is crucial for conserving and managing Redside dace populations in their natural habitat and emphasizes the broader importance of environmental factors in shaping fish physiology and ecology

70. HemaP injection may rescue feeding motivation in the parasitized caterpillar *Manduca sexta*

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The parasitic wasp *Cotesia congregata* employs a multifaceted approach to manipulate the brain and behavior of its caterpillar host, *Manduca sexta*. The wasp injects her eggs, along with venom and a virus, into the caterpillar. During wasp larval development there are no changes in host behaviour. However, once the wasps emerge from their hosts, the hosts lose self-generated behaviors such as feeding and locomotion, while retaining defensive responses, effectively acting as bodyguards for the developing wasp cocoons. Proteomic and peptidomic analysis of host hemolymph has revealed profound changes, including a reduction in the peptide HemaP. The elevation of HemaP increases feeding motivation and foraging behaviour in healthy caterpillars of other species. We attempted to rescue feeding behaviour by injecting HemaP into parasitized caterpillars. Preliminary data suggests that both movement and feeding increase following HemaP injection. This study suggests that one method wasps may use to suppress host feeding is by preventing the release of a key feeding neuropeptide – HemaP.

71. Are Lungworms Emerging in Prince Edward Island Coyotes?

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Crenosoma vulpis, *Capillaria aerophila*, and *Angiostrongylus vasorum* are metastrongyloid nematodes of domestic and wild canids. *Crenosoma vulpis* and *C. aerophila* reside in the trachea and bronchi, and *A. vasorum* is found within the pulmonary arteries and right ventricle. All three lungworms can cause dyspnea and respiratory issues in infected hosts. *Crenosoma vulpis* and *C. aerophila* are common in canids worldwide, while *A. vasorum* is prevalent throughout Europe, Newfoundland, and recently was detected in PEI foxes for the first time. The aim of this study is to determine the prevalence of *A. vasorum*, *C. vulpis*, and *C. aerophila* in the coyote population of PEI. Coyotes were hunted or trapped for other purposes and their carcasses made available for this study. So far, 16 coyote lungs and hearts collected between 2020 and 2024 were dissected to recover lungworms. Parasites were identified, sexed, and counted. For coyotes collected in winter 2020-2021, the *A. vasorum* prevalence was 12.5% (1/8), the *C. vulpis* prevalence 62.5% (5/8), and the *C. aerophila* prevalence 12.5% (1/8). Comparatively, in coyotes collected in winter 2023-2024, the prevalence was 62.5% for *A. vasorum* (5/8), 25% for *C. vulpis* (2/8), and 12.5% for *C. aerophila* (1/8). This is the first study to report the presence of *A. vasorum* in PEI coyotes. Our preliminary results suggest an increasing *A. vasorum* prevalence and a decreasing *C. vulpis* prevalence over a three-year period, while the *C. aerophila* prevalence remained stable. Research is ongoing and further samples will be analyzed.

72. Isolation and identification of Clonostachys rosea (*Ascomycota: Hypocreales*) from deceased ticks, revealing a novel natural tick pathogen

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The American dog tick (*Dermacentor variabilis*) and the black-legged tick (*Ixodes scapularis*) are vectors of pathogens that cause harmful diseases to humans and animals. Entomopathogenic fungi can replace the use of chemical acaricides for tick management, reducing risks to the environment, and human and animal health. We identified a fungus in wild ticks collected in Coldbrook, NS, Canada that could be used as a biocontrol agent against them. Carcasses of *D. variabilis* ticks with visual evidence of infection were suspended in sterile water containing 0.05% (v/v) Tween 80 to collect spores and mycelium fragments. The suspension was plated on PDA and incubated at 25±1 °C and 12 h:12 h [L:D] photoperiod for 10 days. For molecular identification, the ITS region of ribosomal DNA was targeted for PCR amplification. Lab-reared *D. variabilis* and I. scapularis unfed females were sprayed with 1 x 108 conidia/mL and monitored for mortality over 15 days. The fungus species *Clonostachys rosea* (Ascomycota: Hypocreales) was identified through molecular characterization, with a 100% GenBank match. After 72h of treatment with the conidial suspension, fungal growth was observed on the tick cuticle, mainly on the anal plate, joints, and hypostome. *D. variabilis* showed more fungal colonization than *I. scapularis*. The concentration of spores applied on ticks exerted acaricidal activity on *D. variabilis*, causing 80% mortality after 10 days, while 20% of infected I. scapularis died after being exposed to the fungus. These preliminary results indicate that *C. rosea* has potential entomopathogenic activity against ticks, particularly towards American dog ticks.

73. Modulation of Immune-inflammatory Responses in the Intestine of Atlantic Salmon Upon Sea Lice Infestation

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The salmon louse, *Lepeophtheirus salmonis*, is an ectoparasitic crustacean that has threatened the sustainability of salmonid mariculture. In this study, the effects of sea lice infestation on gene expression responses in the intestine of Atlantic salmon were studied using real-time quantitative PCR. Atlantic salmon (120 g) were distributed into two tanks (control and lice) prior to exposure to sea lice. Fish in the first tank (control group) were not exposed to sea lice, whereas fish in the second tank (lice-infected group) were infected with copepodids of L. salmonis (50-100 infectious copepodids/fish). The water temperature was maintained at 10 (±2) °C in both tanks before and after the challenge. Sea lice counting occurred 240-degree days postinfestation at which point the lice had reached the pre-adult stage. The posterior intestine samples were collected from 25 fish per group for further processing and gene expression analyses. Based on the results, the expression of several genes involved in immune responses/inflammation (*CD8*, *IFN-α*, *IFN-γ*, *IL-4/13a*, *IL-6*, *IL-8*, *IL-12*, *IL-17*, *SAA*, *TLR-9*) and cell growth (*TGF-β*) were significantly down-regulated in the intestine of fish infected with sea lice. In contrast, the expression of matrix metalloproteinase-9 (*mmp9*), which plays a key role in the remodeling and destruction of extracellular matrix (ECM), significantly increased in lice-infected fish. The transcription levels of other genes examined (*CATH1b*, *CATH2*, *COX-2*, *GAL9*, *IL-1β*, *IL-10*, *MHC-II*, *SOD2*, *TRIM25* and *TRX*) were not remarkably altered postinfection with sea lice. Our findings are similar to those found in the other mucosal surfaces affected by lice infection such as the skin and suggest that infection with sea lice results in reduced inflammation and disturbed anti-viral responses both systemically and in

multiple mucosal tissues, which may impact the integrity and immune protection of these mucosal surfaces to other infections. Further studies are required to investigate whether infection with lice may lead to the imbalance of microbial community in the intestine of Atlantic salmon.