CEW 2024 Abstract Book

50th Annual Canadian Ecotoxicity Workshop: October 6 – 9, 2024, Waterloo, Ontario
Celebrating 50 Years of Ecotoxicity Research: Reflecting on the Past, Charting the Future

NOTE: For the regular sessions (e.g., non plenary or award talks) presenting authors are either the first author or identified by an underline; platform presentations are denoted with (PL) and poster presentation by (PO).

Special 50th ATW/CEW Anniversary Celebration Plenary Session

50 Years of CEW – How We Got Here – Where We Go

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We will review the stages of environmental protection from the beginnings of European settlement, immigration, the growth of cities, public health crises to the demand for clean water, air and soil. Technological advances in chemistry and microbiology provided the capabilities of analysis and provided the answer to "how clean is clean?". Biological quality became quantifiable after World War II and environmental quality became measurable. Industry, government and the public needed a number and environmental toxicology delivered. Over the last 50 years CEW has provided a forum for the rapidly evolving field of environmental toxicology in Canada. As we look towards the future, CEW has a pivotal role to play in providing a platform for face-to-face interaction among ecotoxicologists across academia, government, and industry, facilitating the exchange of cutting-edge research and fostering collaboration and innovation. In an era of rapid scientific advancement and shifting communication landscapes, CEW's role is more important than ever to ensure that scientific integrity and accessibility is maintained. We must continue to evolve in our practices, acknowledging equity, diversity and inclusion, supporting the incorporation of Indigenous knowledge into Western science, and navigating our role as ecotoxicologists in the face of AI and technological advancements.

Standardized Toxicity Methods for Regulatory Application: A Brief History and the Future

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Over the past 35 years, the Biological Assessment & Standardization Section has produced 28 biological method documents for aquatic, sediment and soil testing applications. Each of these test methodologies has a federal and/or provincial regulatory application, whether for risk assessment, pollution prevention or compliance limit application. Based on solid research procedures for organism culture/holding/testing, these methods can require up to 5 to 10 years for the refinement, validation and standardization process. However, to ensure the generation of high-quality test data by Canadian toxicological testing laboratories, the development and validation of standardized testing methodologies

is only half of the equation. The other half requires on-going confirmation that the test is performing as expected in laboratories across Canada. To support that goal, laboratories' policies, procedures and practices can be assessed using international accreditation standards. For tests which are used routinely in regulations, many laboratories are accredited through organizations such as Canadian Association for Laboratory Accreditation (CALA). Examples of standard biological test methods needed for regulatory applications will be presented and future ECCC method development effort will be covered in this presentation.

Evolution of environmental monitoring studies presented at the Canadian Ecotoxicity Workshop: past trends and future tools

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During the 1970s, field studies made up about 16% of studies presented at ATW, and doubled to more than 25% of studies in the 1980s. In the early 1990s Environmental Effects Monitoring requirements came into focus as requirements were being developed for monitoring pulp and paper mill effluents. EEM development changed the monitoring studies presented at ATW/CEW from a biomarker and chemical residue perspective, to include population and community-level indicators. As time progressed, monitoring studies shifted to include a new generation of tools including proteomics, transcriptomics and metabolomics, as well as eDNA. The focus of concerns evolved from metals, industrial chemicals, and PBTs to concerns about natural and synthetic hormones, pharmaceuticals and personal care products, flame retardants, and more recently to microplastics, nanoplastics and microfibres. In recent years, development of cutting-edge technologies and tools has been complemented by a shift to more holistic and inclusive monitoring approaches that increasingly involve citizen science and community-based monitoring. Progress in achieving meaningful engagement with Indigenous communities and inclusion of Indigenous knowledge has been slow but measurable, and is slowly evolving into knowledge co-production in some areas, although much effort and progress is still required. The application of monitoring data is evolving from single to multi-purpose as Open Data initiatives prompt researchers to make their data more findable, accessibly, interoperable, and reusable. While difficult decisions and trade-offs abound, we are at an exciting cross-roads in environmental monitoring in Canada.

A retrospective on the evolution of microbiotests and future perspectives on molecular and “Omics” approaches in ecotoxicology

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Microbiotests, standardized bioassays utilizing microorganisms, have been instrumental in the field of ecotoxicology for several decades. This retrospective traces the historical development and application of microbiotests in ecotoxicology, highlighting key milestones and advancements. From their inception, microbiotests have provided rapid, cost-effective, and reliable tools for detecting effects of contaminants in various environmental matrices, including water, soil, and sediment. Pioneering studies utilized bacterial, algal, and protozoan assays to evaluate acute and chronic toxicity, contributing to regulatory monitoring programs worldwide. More recently, these tests have been developed as “kits” that can be

used directly with samples from field collections. We examine the evolution of microbiotests, method refinements, technological advancements, and integration of high-throughput screening techniques. "Omics" approaches involve simultaneous, large-scale study of gene, proteins, metabolites, and other biomolecules. These techniques are more observatory in nature than traditional, targeted, hypothesis testing experimental designs, and can help identify unknown/unexpected toxicological responses and mechanisms. Current technology supporting these techniques has driven innovation in 'Omics, and the tools have evolved at an ever-increasing pace, making it challenging for newcomers to identify and learn current and state-of-the-art methods. 'Omics methods are often considered new approach methods (NAMs), because they can help reduce the use of animals in research by providing a greater amount of information from a single study, and because molecules can be detected in small samples of tissue and biofluids, allowing for the use of non-lethal sampling for biomonitoring and laboratory studies. Policy makers and regulators are only beginning to discuss how to incorporate these NAMs into environmental and resource protection through ecotoxicity assessments and environmental monitoring to support decision making.

Reflecting on the last fifty years of society and environmental contaminants: evolution of science, technology and public policy

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It is not possible to think about the history of environmental contamination without placing it in the context of societal and technological change. The development of synthetic chemicals transformed society and provided a promise for a better future. However, Silent Spring soon focused our attention on the adverse effects occurring in the environment. Early pesticides and industrial chemicals were transported globally and were found to bioaccumulate in food chains, threatening wildlife and human health. New regulations and risk management programs eventually were adopted to control or even virtually eliminate many of these substances (e.g. POPs). However, these sources of these chemical are diverse, and the global distribution means they will remain with us for generations. Unfortunately, almost every human activity results in some form of pollution or environmental change. Although remarkable progress has been made on controlling legacy pollutants, other chemicals continue to emerge and/or replace them. As we learn more about their properties, environmental pathways, and toxicology we are better able to predict adverse outcomes and take remedial action. However, the regulatory response can be slow, and humans continue to develop novel chemicals at a startling pace, and these will continue to enter and potentially harm our waterways. Things we once saw as environmentally benign are now creating public concern (e.g. drugs, tires, plastics). The environment is now a complex mixture of contaminants and other stressor that interact and cause cumulative effects that are difficult to define or manage. CEW (ATW) has played a key role in building and communicating the foundations of the science used to assess, regulate and remediate environmental contaminants and the need for this role has not diminished

Population and community responses in ecotoxicology: how have field assessments changed and where are they going?

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A key goal in ecotoxicology is to protect populations, communities and biodiversity given their inherent value and provision of ecosystem services. However, linking specific chemical stressors to higher-level responses has been a challenge since the field of ecotoxicology began. In this presentation we discuss how our approaches to link changes in aquatic populations and communities to contaminants have evolved from early field studies to current day and the promising new technologies available for future assessments. In addition, we will profile some of the successes and major contributions that Canadian ecotoxicologists have made in this area, from assessments at contaminated sites to whole-ecosystem experiments, and discuss the contributions that early career researchers can make to the field.

Health of the ocean – the intersection of marine ecotoxicology and human health research in an era of climate change

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The ocean is suffering from many threats to its natural biodiversity putting at risk coastal communities who benefit from its many resources. Amongst those threats are pollution (chemicals, plastics and pathogenic microbes), over-fishing, invasive species, natural toxins, and above all, climate change with its effects such as rising sea temperatures and acidification. Advances in marine ecotoxicology have helped to identify the significant threats, reveal effect mechanisms and encourage appropriate regulation and control. This helps protect people from well-recognised dangers and newly emerging threats.
This talk discusses where we are currently in the field of marine ecotoxicology and how it intersects with the wider meta-discipline of ocean and human health (OHH). At their intersection critical new approaches and ways of understanding can be developed to ensure a healthier ocean for all of its inhabitants and users. The importance of the two fields in fostering cooperation among its practitioners cannot be underestimated, given climate change, growing numbers of people living along the coasts, and the increasingly high demand for marine protein. Shortening the time delay between understanding a marine threat and its resolution by policy and decision makers, and ocean managers is a key challenge to overcome in making further progress. Marine ecotoxicology is making a vital contribution within the context of protecting both ecosystem health and human health. Clean seas protect people.

Dr. Richard C. Playle Awards for Outstanding Theses in Ecotoxicology

M.Sc. Winner: Towards high throughput determination of biotransformation rates of chemical mixtures using isolated perfused trout livers | Matthew Schultz, University of Saskatchewan

Chemical risk assessment focuses on screening substances for criteria of persistence, bioaccumulation potential, and toxicity (PBT). Of these criteria, bioaccumulation presents unique challenges due to the difficulty of testing compounds that undergo biotransformation, compounded by the diversity of chemical pollutants. Current assessment frameworks rely largely upon in vivo assays (OECD 305). The adoption of new approach methodologies (NAMs) such as in vitro-in vivo extrapolation assays (OECD 319) has been limited due to concerns of overprediction, uncertainty, and limited domain of applicability. These concerns can be addressed using the isolated perfused liver model, representing an intermediate level of biological organization. This model delivers a physiologically relevant measure of hepatic clearance and thus can estimate bioaccumulation potential. The present study uses this model to

validate prior IVIVE methods by comparing direct clearance measurements with those of IVIVE prediction in a range of reference chemicals. To demonstrate an expanded domain of applicability, these assays were also performed using a mixture of chemicals found within the US EPA's Non-Targeted Analysis Collaborative Trial (ENTACT) trial. Samples were analyzed using liquid-chromatography-high-resolution-mass-spectrometry (LC-HRMS) to semi-quantitatively measure individual chemicals within mixture and calculate hepatic extraction fraction. Our results serve to both validate prior in vitro methods, as well as demonstrate the potential use of the isolated perfused liver model as a tool for high-throughput bioaccumulation screening within a tiered risk assessment approach.

B.Sc. Winner: Impact of Freshwater Salinization on Zooplankton Communities in Shallow Wetland Mesocosms | Kailey Carriere, University of Manitoba

Kailey Carrière[1], Braedon Humeniuk[1], José Luis Rodriguez Gil[2], Mark Hanson[1]
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While chloride salts occur naturally in the environment, the salinization of freshwater systems can pose ecological risks when found in excess, typically via anthropogenic activity. To better understand these risks, we sought to characterize chronic zooplankton community responses to freshwater salinization using wetland mesocosms that were monitored over the course of 16 weeks. A total of twelve mesocosms were established with aquatic macrophytes, invertebrates (e.g., clams, zooplankton, aquatic insects), phytoplankton, and other organisms. We treated systems along a gradient of nine chloride concentrations in a regression design (linear on a logarithmic scale, n=1), ranging from 22 to 2000 mg Cl^-/L (derived from NaCl), with controls (no salt added, n=3). Zooplankton communities did not experience statistically significant ($p<0.05$) changes in composition due to salinization. However, strong correlations ($|r| > 0.5$) between treatment and abundance were observed, as well as a strong negative correlation between treatment and diversity metrics (species richness, evenness, Shannon's index, and Inverse Simpson's). Zooplankton exposed to 2000 mg Cl^-/L experienced a shift from a cladoceran and copepod dominant community to a rotifer dominant community. Higher treatments, including more environmentally relevant treatments, saw an increase in salt tolerant species over time. These data will aid in understanding the impact of salinization on lower trophic levels, inform risk assessments, and the development of water quality guidelines for chloride salts in freshwater.

6PPD-Quinone & Other TRWP-Derived Contaminants: Analytical Methods, Environmental Occurrence, & Toxicology

A Rapid Analytical Method for Measuring PPDs and PPD-Qs in Water Samples (PL)

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Recently, Phenyl-*p*-phenylenediamines (PPDs) and Phenyl-*p*-phenylenediamine-quinones (PPD-Qs) have drawn wide attention since 6PPD-Q was identified as the culprit of the lethality of Coho Salmon. This

leads to the critical needs to analyze their concentrations in surface waters. Reliable measurement of PPDs in surface is challenged by their instability during sample storage and extraction. In this study, we tested the stability of PPDs and PPD-Qs in water, with different sampling tubes (polypropylene, amber glass), percentages of methanol (0%, 10%, 20%), pH (5, 7, 9), and storage temperature (4 and -20°C). We found that PPDs in amber glass vials with pH = 5 and 20% methanol, are stable at -20°C for at least 42 days. Grab and autosamplers were compared and consistent results were observed. To reduce the degradation of PPDs during sample extraction, a large injection volume - based direct water analysis method was employed. Fortunately, the detection limits of all chemicals were lower than 10 ng/L by increasing the injection volume to 50 µL, which is sufficiently sensitive to measure PPDs in environmental waters. In summary, we herein developed an analytical method for measuring PPDs and PPD-Qs in runoff water with sufficient sensitivity and reproducibility

Investigating alternative environmental sources of the toxic tire-derived chemical 6PPD-Quinone (PL)

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In 2021, mass mortalities of coho salmon were linked to 6PPD-Quinone (6PPD-Q), a transformation product of the antioxidant 6PPD that is used in rubber tires. Since then, the major focus of scientific efforts concerning 6PPD-Q contamination pathways has been on paved surfaces. However, there remain other areas through which 6PPD-Q can be formed and released into the environment. For example, tire recycling has resulted in the diversion of 6PPD-containing material from landfills into various industries, such as infill for artificial turf. As such, the recycled tire rubber is exposed to environmental conditions that have the potential to create 6PPD-Q and subsequently leach it into the storm drain network. Despite this, few studies have examined the 6PPD-Q leaching potential of recycled rubber tire materials under environmental conditions. To fill this knowledge gap, we performed a long-term leaching study to detect 6PPD-Q and other tire additives in rain and meltwater captured after it had filtered through a layer of the recycled tire material. The 6PPD-Q concentrations in the captured leachate were quantified using liquid chromatography high-resolution mass spectrometry, and ranged from 9 µg/L to 172 µg/L, with a median concentration of 39.48 µg/L. The results of this study demonstrate that using recycled tire material in a landscaping context has the potential to contribute 6PPD-Q into the environment. Furthermore, the leachate concentrations found in the leachate greatly exceed the median lethal concentration for various salmonids. The results of this study raise further questions about the use of recycled tire materials in outdoor contexts.

Unraveling the Mechanism and Species Sensitivity Differences in 6PPD-Quinone Toxicity by Using Whole Transcriptome Analysis in Four Fish Species (PO)

Junyi Lin[1], Catherine Roberts[1], Evan Kohlman[1], Mawuli Amekor[1], Niteesh Jain[1], Alper James Alcaraz[1], Natacha Hogan[1,2], Markus Brinkmann[1,3,4], Markus Hecker[1,4]
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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone(6PPD-quinone), has recently been identified as a cause of acute mortality in coho salmon exposed to urban runoff. 6PPD-quinone poses significant concern in aquatic environment as it exhibits a wide distribution of species sensitivities among fishes and the underlying mechanism of toxicity remains unknown. Therefore, there is urgent need for assessment of hazards and risk associated with 6PPD-quione; however, current methods in chemical hazard assessment heavily rely on costly, time-consuming, and ethically questionable live animal testing. This study applied an alternative approach that employs next-generation whole transcriptome analysis to investigate the toxicity of 6PPD-quinone in early-life stages of rainbow trout (*Oncorhynchus mykiss*), lake trout (*Salvelinus namaycush*), Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). An advanced dose-response modeling approach to derive transcriptomics points of departure (tPODs) was successfully applied to both rainbow trout (Acute LC50: 1 µg/L) and lake trout (Acute LC50: 0.50 µg/L). Data for Atlantic salmon and brown trout are still in the process of analysis. We found that tPODs effectively estimated previously reported apical benchmark concentrations (BMC) from acute and sub-chronic tests with adult and early-life stages. Lists of differential express genes were used for pathway analysis, which indicated that rainbow trout and lake trout shared several pathways that were indicative of certain apical outcomes. In contrast, Atlantic salmon exhibited a greater number of unique pathways when compared to both rainbow trout and lake trout, which suggests that potential compensatory mechanism may dominate responses in insensitive species. Our results highlight the potential of using transcriptomics for chemical hazard assessment and provide a deeper understanding of species-specific responses to 6PPD-quinone.

Metabolomic changes in juvenile coho and Chinook salmon exposed to the vehicle tire associated chemical 6PPD-Quinone (PL)

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N-(1,3-Dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone, 6PPD-Q), an ozonation product of the tire rubber antioxidant 6PPD, has been identified as driver of urban stormwater runoff mortality syndrome, a phenomenon where coho salmon (*Oncorhynchus kisutch*) suffer pre-spawn mortality upon entry into urban streams. Juvenile Pacific salmon spend a minimum of several months in freshwater systems where if near urban centers are likely to result in exposure to lethal and sublethal concentrations of 6PPD-Q. Here we investigated the sublethal effects of 6PPD-Q in newly feeding juvenile coho and Chinook salmon (*Oncorhynchus tshawytscha)* using a targeted metabolomic platform (633 metabolites; performed by the Metabolomics Innovation Center, AB) in a subset of samples collected during an acute 24h exposure. Sample exposure concentrations ranged from 11.9-38.5 ng/L for coho, and 3,112-25,808 ng/L for Chinook, with both ranges inducing mortality in a minimum of one treatment. Metabolite classes included biogenic amines, acyl carnitines, organic acids, ketones, lipids, amino acids, nucleotides. Up to 530 and 556 metabolites of the 633 targeted panel were detected in juvenile coho and Chinook, respectively. Acute exposure to 6PPD-Q resulted in markedly different profiles of altered metabolites between species. Lipids accounted for a large portion of metabolites altered in both species, however, the number of lipids altered was nearly double in coho compared to Chinook. These findings suggest that a toxic mode of action for 6PPD-Q involves altered lipid metabolism. Ongoing metabolomics data analysis in the two Pacific salmonid species will elucidate metabolic pathways involved in the sub-lethal responses to 6PPD-Q exposure.

Sub-chronic and acute toxicity of 6PPD-quinone in early life stages of lake trout (*Salvelinus namaycush*) (PL)

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The rubber-tire derivative N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-q) has been identified as a possible driver of Urban Runoff Mortality Syndrome, a phenomenon in which mass die-offs of pre-spawn salmon occur in association with urban settings. Tire-wear particles left on the road's surface are swept into roadway runoff during precipitation, resulting in pulses of 6PPD-q in aquatic systems. Sensitivity to this toxicant is highly variable across fish species, and with environmental concentrations meeting or exceeding sensitivity thresholds, the potential for toxicity in sensitive aquatic species is significant. In addition, there is little known regarding chronic effects, nor effects on early life stages of fishes, which are typically more sensitive to toxicants than adults. Lake trout (*Salvelinus namaycush*; LT) are a native species of concern for which the sensitivity is unknown. Sub-chronic exposures to 6PPD-q were conducted starting at hatch, with LT exhibiting a 45-day LC50 of 0.39 µg/L. This experiment also found sub-lethal effects which occur at this sensitive life stage, including yolk sac edema, spinal curvature, and pooling of blood in the eye and caudal fin. Follow-up juvenile studies were conducted with older fry, finding 96-hr LC50s of 0.50 µg/L. Samples were also taken for transcriptomic analysis, highlighting pathway-level disruption. These studies demonstrate the potential risk of 6PPD-q to early-life stage inland salmonid species, which may have significant effects on already vulnerable populations.

First assessment of the ecotoxicological effects of the tire antioxidant 6PPD on early life stages of largemouth bass (*Micropterus salmoides*) (PL)

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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is a widely used rubber antioxidant, entering aquatic ecosystems during tire wear and through roadway runoff. A transformation product of 6PPD, 6PPD-quinone, has been detected in watersheds at concentrations acutely toxic to select fish species. We evaluated the toxicity of 6PPD to embryonic and larval largemouth bass (*Micropterus salmoides*) in a series of outdoor microcosm experiments. Wild-collected bass embryos were chronically exposed to 6PPD and its potential environmental transformation products in lake water under ambient conditions through daily chemical additions. In a preliminary 96-h experiment, 6PPD was toxic to largemouth bass embryos with a 96-h LC50 of 553 µg/L. Interestingly, we found that exposure to 6PPD-quinone did not cause mortality in largemouth bass embryos up to nominal concentrations of 1,000

µg/L. To further investigate the developmental toxicity of 6PPD, largemouth bass were exposed to nominal concentrations ranging from 1.6 - 62.5 µg/L for 10 days, during embryonic to early larval life stages. We found moderate evidence suggesting 6PPD treatment reduced developmental success in a concentration-dependent manner, with an extrapolated 10-d EC50 of 76.9 µg/L 6PPD for reaching swim-up. Moreover, 6PPD exposure significantly increased time to swim up, yolk sac retention, and occurrence and severity of malformations compared to the solvent control. This study is the first toxicological assessment of 6PPD with largemouth bass, an ecologically and economically significant native fish species. Considering the proximity of roadways to inland waters in Canada, it is important to understand how tire wear particles and additives like 6PPD affect the health of aquatic ecosystems.

6PPD-quinone induces developmental cardiotoxicity in fathead minnows (*Pimephales promelas*) following microinjection (PL)

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N-(1,3-dimethylbutyl)-N'-phenyl-*p*-phenylenediamine-quinone (6PPD-quinone), an oxidation product of the rubber tire antioxidant, 6PPD, is an emerging contaminant of concern that was first identified as the cause of Urban Runoff Mortality Syndrome – a mass lethality event observed in coho salmon (*Oncorhynchus kisutch*). Acute lethality of 6PPD-quinone is highly species-specific, with the only sensitive species identified to date being some salmonids. Using waterborne exposures, we previously found that neither sexually mature fathead minnows (*Pimephales promelas*) exposed to 6PPD-quinone at 9.4µg/L, or embryos exposed to 6PPD-quinone at 40 µg/L suffered acute lethality. Further, embryos did not show any developmental malformations. Using metabolomics and transcriptomics, we showed evidence of sublethal oxidative stress in adults. Based on these findings, we selected fathead minnows as a model species to further investigate potential sublethal effects of 6PPD-quinone. To ensure consistent and precise dosing, newly fertilized embryos were microinjected with 6PPD-quinone at doses of 530, 1050, 2570, 5260, and 9550 ng/g of egg. Consistent with our previous findings, exposure to 6PPD-quinone did not induce mortality up to 12 days post-fertilization. However, a suite of developmental malformations was observed, including abnormal heart morphology, lack of common cardinal vein development, and hemorrhaging at sporadic anatomical areas. To understand the mechanisms of these results, RNAseq and metabolite analysis will be performed. These studies will provide novel insight into mechanisms of toxicity of 6PPD-quinone to fishes.

Time-course of physiological and histological changes in Brook trout (*Salvelinus fontinalis*) during exposure to lethal concentrations of 6PPD-quinone (PL)

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Recent studies have implicated a tire wear leachate, *N*-(1,3-dimethylbutyl)-*N*'-phenyl-p-phenylenediamine-quinone (6PPD-quinone), to be responsible for the mass mortality of Coho salmon (*Oncorhynchus kisutch*) exposed to urban stormwater runoff. The mechanism of action of 6PPD-quinone is not well understood, studies suggest that metabolic, respiratory, cardiovascular, and/or neurological toxicity all playing a role in the observed effects. To address this data gap we conducted 3.5 hr exposures to the 6hr LC95 concentration of 6PPD-quinone with juvenile brook trout and sampled individuals after 1, 2, 3, and 3.5 hrs of exposure to measure changes in the blood chemistry and gill tissue prior to

mortality. Blood parameters were measured using an i-STAT blood analyzer CHEM8+ and CG4+ cartridges. Exposure concentrations were measured at the start and end of each sampling duration. Changes in blood chemistry were evident in asymptomatic fish within 1 hr of exposure, with significant increases in hematocrit, calcium, potassium, and decreases in glucose, chloride, sodium, bicarbonate, total carbon dioxide concentrations, blood pH, and oxygen saturation. There were also additional increases in blood lactate and an even more pronounced increase in blood potassium concentrations. By 3 hrs of exposure morbidity and mortality was beginning to be observed in the exposed fish and the blood parameters measured were similar to the effects observed at 1 and 2 hrs. Histological examination of the gill filaments revealed morphological changes consistent with mounting a response to osmotic dysregulation. Ion dysregulation and gill inflammation appear to be contributing factors in 6PPD-quinone toxicity to juvenile Brook trout. The changes in blood chemistry we observed give insight into the possible mechanism of action of 6PPD-quinone in sensitive salmonid species.

Synergistic Toxicity of Zinc and 6PPD-Quinone Towards Rainbow Trout (*Oncorhynchus mykiss*) (PL)

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Aquatic ecosystems are experiencing increased contamination by complex mixtures of pollutants, raising concerns about the potential synergistic effects on the aquatic environment. Stormwater runoff often carries multiple contaminants, such as metals, hydrocarbons, and organic compounds, from roads, industrial sites, and residences into nearby waters into nearby water bodies. Tire-wear particles are a significant source of environmental contamination, and can release chemicals which have been shown to be toxic to fish and other aquatic life. This study investigates the individual and combined toxicity of zinc (Zn) and 6PPD-quinone, a transformation product of a widely used tire additive (6PPD), on rainbow trout (O*ncorhynchus mykiss*). While both Zn and 6PPD-q are individually known to pose significant risks to aquatic life, their interactive effects remain poorly understood. Our study revealed an LC50 of 1.5 µg/L (nominal) to 6PPD-q alone, with adverse effects within 6 hours, whereas zinc alone showed no adverse effects on survival until 24 hours, with an LC50 of 1500 µg/L. Further testing involved low, mid, and high range concentration combinations, where only the mid range concentrations of zinc (1000 µg/L) in combination with low concentrations 6PPD-q (0.5 µg/L) had significant effects on mortality, suggesting a synergistic effect. At the highest zinc concentration of 1500 µg/L, the previously observed almost additive effect diminished, suggesting antagonist interactions between the two contaminants. Overall, this study offers insights into the combined effects of two anthropogenic contaminants on a sensitive aquatic species, which is essential for developing strategies to mitigate the impact of urban runoff on freshwater fish populations.

Investigating age-specific toxicities of 6PPD to largemouth bass (*Micropterus nigricans*) in an outdoor microcosm experiment (PO)

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N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is a commonly used antioxidant in tire rubber. Its transformation product, 6PPD-quinone, has been detected in the environment at concentrations acutely toxic to select organisms. Differences in sensitivity to 6PPD-quinone have been

demonstrated in juvenile and adult coho salmon, but this age-specific sensitivity remains unexplored in other fish species. Largemouth bass (*Micropterus nigricans)* is an ecologically and economically important fish species that spawn in littoral zones of temperate lakes and rivers, where exposure to contaminants such as 6PPD and its transformation products may occur during critical stages of development. Our study aims to assess if toxicity responses to 6PPD exposure differ between embryo and larval life stages of largemouth bass. We hypothesize that larvae will be more sensitive than embryos due to the absence of the chorion, which can act as a physical barrier to xenobiotic insults. To test this, we will conduct 48-h toxicity tests on wild-caught largemouth bass using a 2-factorial ANOVA design. Fish will be randomly separated into two groups and exposed to 62.5 ug/L 6PPD or solvent control (0.02% v/v dimethyl sulfoxide) at either the embryonic or larval life-stage. Each treatment will consist of 5 replicates of 20 fish. Toxicity testing will occur in lake water under ambient conditions in outdoor microcosms, with 70% exposure water renewals every 24-h. We will assess mortality, heart rate, spontaneous movement, and incidence of malformations in 6PPD-exposed and control fish. Our study will provide insights into potential changes during development and critical windows of vulnerability for largemouth bass.

Assessing transepithelial electrical resistance (TEER) in rainbow trout RTgill-W1 and Atlantic salmon ASG-10 gill cells exposed to 6PPD-quinone (PO)

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The compound N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has been linked to significant mass mortalities of coho salmon along the western coast of North America. This toxicant forms from the antioxidant 6PPD through the environmental oxidation of tire wear particles, which enter aquatic systems via urban stormwater runoff. Although the acute lethality of 6PPD-quinone at concentrations ≤ 1µg/L has been established in various salmonid species, sensitivity to this toxicant varies greatly among fishes. The mechanism behind its species-specific acute toxicity remains unknown. Evidence suggests that 6PPD-quinone may cause molecular dysregulation of genomic pathways related to cell adhesion and endothelial permeability. Therefore, this study aims to evaluate the impact of 6PPD-quinone on the integrity and permeability of a modeled gill epithelium using two salmonid cell lines: RTgill-W1 (rainbow trout, sensitive species) and ASG-10 (Atlantic salmon, tolerant species). Gill cells will be cultured on a semipermeable insert that separates apical and basolateral compartments. Once they reach confluence, cells will be exposed to incremental concentrations of 6PPD-quinone for specified durations in 24-well plates. Transepithelial electrical resistance (TEER, in ohms) will be measured with chopstick electrodes to quantify the barrier integrity. Given that the gill epithelium is a primary target site for water-borne pollutants, this research aims to reveal the effects of 6PPD-quinone on gill permeability in both sensitive and tolerant species as a potential mechanism of toxicity.

Tire-additive transformation product 6PPD-quinone in Lake Ontario urban watersheds and receiving waters – hazard considerations (PO)

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Urban stormwater is a conduit for nutrients, pathogens, and pollutants such as tire-wear compounds to nearshore Great Lakes receiving waters. A transformation product of the anti-ozone compound 6PPD in tires, 6PPD-quinone (6PPD-Q), has been implicated in causing fish mortality. In this study, surface waters from Lake Ontario urban tributaries and nearshore waters of Toronto and Hamilton, Ontario, were sampled to assess whether 6PPD-Q was present at levels that may impact aquatic health. Stream samples were collected during wet-weather flows and dry conditions during fall and spring seasons in 2021-2022, overlapping with fish spawning times, and analyzed for 6PPD-Q and water chemistry parameters. Concentrations of 6PPD-Q were elevated during wet-weather, reaching a maximum concentration of 82 nanograms per liter (ng/L), and were highest in streams with the greatest degree of urbanization. Concentrations were low (<2.0 to 15 ng/L) in nearshore waters, including near river mouths, suggesting rapid dilution. Precipitation and road density within watersheds were positively correlated to 6PPD-Q concentrations and other road contaminants (e.g. chloride from road salts). A screening level hazard assessment using safety factors (Hazard Quotient of 0.1) and available toxicity endpoints for fish (rainbow trout, brook trout, coho salmon), shows that 6PPD-Q concentrations in urban streams exceed protective levels for fish in wet-weather, and dry weather flows may be at or above the recent USEPA acute toxicity guidance value (11 ng/L). These results support the need for further assessment of Ontario streams, particularly where trout habitat is influenced by road run-off.

State of knowledge of 6PPD-quinone in Canada: A comprehensive review (PO)

Jason Durante[1], Benjamin de Jourdan[2], Rebecca Eldridge[1], Jamie Mario[1], Emily Davis[1]
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The significance of 6PPD-quinone as a contaminant of interest has grown in recent years following observations of acute toxicity to coho salmon (*Oncorhynchus kisutch*) in the natural environment. This state-of-knowledge paper seeks to consolidate existing insights around 6PPD-quinone in Canada to enhance our technical comprehension and determine knowledge gaps for future work. The analysis encompasses a comprehensive exploration of various aspects including 6PPD-quinone interactions with aquatic environments (e.g., pathways, fate, transport, and behaviour), impacts and risks to aquatic and terrestrial ecosystems as well as human health, environmental monitoring, and analytical methods (including current challenges and limitations). This paper also discusses how 6PPD-quinone fits into the current regulatory regime in Canada and explores emerging trends and future directions. Emphasizing a holistic approach, this paper aims to construct a robust foundation that amalgamates current knowledge on 6PPD-quinone in the Canadian context, paving the way for informed decision-making and potential policy advancements.

Xenometabolome of Early-Life Stage Salmonids Exposed to 6PPD-Quinone (PO)

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N-(1,3-Dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine-quinone (6PPD-Q) is a ubiquitous and acutely toxic transformation product (TP) derived from the rubber tire antioxidant 6PPD. While not all salmonids are sensitive to acute lethality, several species are several orders of magnitude more sensitive to 6PPD-Q compared to other fish species. The main driver of species differences in sensitivity is a pressing question, with one area examining whether differences in their ability to biotransform and detoxify 6PPD-Q might be a driving factor. This study utilized high-resolution mass spectrometry (HRMS) to assess biotransformation and metabolome-wide effects of 6PPD-Q on early-life staged salmonids, including two sensitive species, rainbow trout (*Oncorhynchus mykiss*) and lake trout (*Salvelinus namaycush*), and one tolerant species, brown trout (*Salmo trutta*). We detected three Phase I TPs and seven Phase II TPs and revealed that brown trout had the greatest ability to detoxify 6PPD-Q. TP-OH1, a phase I TP, was detected in both rainbow and lake trout, but not brown trout, with more research needed to understand potential toxicity of TP-OH1. Several metabolites were found to be dysregulated in rainbow and lake trout, indicative of mitochondrial dysfunction and alterations in metabolism. Our results indicate a difference in biotransformation capability between salmonid fish species and subsequent metabolome response. A time-course metabolome response to 6PPD-Q would provide information on initial and compensatory responses to toxicity.

Interlaboratory study on analytical methods for aqueous 6PPD-quinone: on the path towards comparability – an update (PO)

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To respond to the discovery of 6PPD-quinone, the toxic agent responsible for road runoff mortality syndrome in Coho salmon, research to determine aquatic concentrations and to characterize its' mechanisms of action has accelerated around the world. Many distinct projects are underway and as multiple laboratories respond to the need for data, analytical methods are simultaneously being developed to measure this compound in water. Determining how these different methods compare is important for the interpretation of currently available evidence, but is also a critical step towards the development of comparable methods that will ultimately ensure that different studies have compatible results. The objective of this project is to evaluate the initial assessment of capability of multiple laboratories around the world for the analysis of 6PPD-quinone in natural water through standard proficiency testing methodology employed by IQM (ECCC). This project was announced at CEW in 2023 and is now underway. Natural water has been collected from Vancouver and Toronto, timed to follow rain events and capture the highest possible environmentally relevant concentrations. Background 6PPD-quinone screening has been conducted by a reference laboratory on samples from these areas as well as

stored bulk water and spiked test solutions. A series of test solutions will be generated over a range of concentrations and shipped to voluntary participants globally. Data on the concentrations measured will be analyzed for comparability of the methods used and results will be shared with the scientific community.

Ecotoxicological Impact of Critical and Strategic Minerals on Aquatic and Terrestrial Ecosystems

Evaluation of the toxicity of two platinum group metals (palladium, platinum) individually and in mixture on *Chironomus riparius* and *Hyalella azteca* (PL)

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Platinum group metals (PGMs), such as platinum (Pt) and palladium (Pd), are essential in various sectors including automotive, electronics, and medical fields. However, significant ecotoxicological data gaps hinder their environmental impact assessment. To address these environmental issues, this project aimed to i) assess the acute and chronic toxicity of Pd and Pt individually in *Hyalella azteca* and *Chironomus riparius*, ii) determine the effects in *C. riparius* when both metals are combined. *C. riparius* and *H. azteca* were exposed to Pd and Pt individually for 10 or 14 days respectively, and survival, growth, and bioaccumulation were measured. Subsequently, *C. riparius* underwent 28 days exposure to both metals individually to assess survival, sex ratio, and emergence time. Further experiments exposing *C. riparius* to sediments containing a mixture of Pd and Pt were performed. Survival results (LC_{50} in $\mu g.g^{1}$ dw) indicated that Pt (*C. riparius*: 192 ± 14 ; *H. azteca*: 520 ± 57) was found to be more toxic than Pd (*C. riparius*: 283 ± 90 ; *H. azteca*: 2627 ± 1024) for both organisms. However, when examining growth rate (LOEC in $\mu g.g^{1}$ dw), Pd (*C. riparius*: 1600 ; *H. azteca*: 200) was more effective than Pt (*C. riparius*: NA ; *H. azteca*: 800). For chronic exposures in *C. riparius*, Pt demonstrated higher toxicity (LC_{50} in $\mu g.g^{1}$ dw) compared to Pd (Pt: 75 ± 7 ; Pd: 164 ± 151). In contrast, Pd exhibited greater toxicity than Pt when considering the emergence time (LOEC in $\mu g.g^{1}$ dw) of the adults (Pd: 100 ; Pt : NA). These findings indicate that Pt and Pd toxicological effects differ depending on the endpoint. Ongoing mixture experiments will provide further insights into the interaction of both metals. This study provides innovative toxicological data on PGMs in sediment habitats. Subsequent research will concentrate on elucidating the mode of action of Pd and Pt and the handling strategy of *C. riparius*.

Lexicon:
PGMs: Platinum group metals, Pt: platinum, Pd: palladium, EC5: median effective concentration, LC50: lethal effective concentration, LOEC: lowest observed effective concentration

Fate, distribution, bioaccumulation potential, and risk assessment of rare earth elements (REEs) in aquatic ecosystems post-exposure to REE-coagulants (PL)

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Rare earth elements (REEs) are used in various high-tech and environmental applications including renewable energy, electronics, and coagulants for aquatic remediation. As the demand for rare earth

elements in high-tech products and decarbonisation technologies soars and the need to build an independent REE supply chain to protect national security increases, the Canadian government has identified these elements as critical minerals of high priority. In this context, the perspective of increasing REE exposure from mining, manufacturing, and wastewater effluent discharge can negatively affect the water resources and human health where many mineral projects are located. Thus, there is an urgency to investigate the potential environmental impacts of REEs post-exposure to pollutants, especially when there are no environmental guidelines in many countries worldwide. Therefore, this study investigated the fate, distribution, and bioaccumulation potential of REEs in both abiotic and biotic fractions in Swan Lake, Markham, Ontario, after exposure to a lanthanum-based coagulant in 2013, used to reduce eutrophication. Elemental analysis using ICP-MS on various abiotic and biotic components has allowed us to evaluate post-exposure risks. Organisms were exposed to La levels in sediments 30 times higher than the upper continental crust. Within the water, particulate La is found highest in the intermediate (26-70%) and deep levels (85-97%). Further, the uptake mechanisms of REEs into organisms were determined using REE fractionations. Organisms are highly susceptible to uptake particulate and diet REEs. For instance, the high REE concentrations in aquatic plants and plankton which transfer to vertebrates, also suggest trophic transfer via the REE diet exposure.

Aquatic Geochemistry of Critical Elements in Ontario Rivers (PL)

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Mining projects are expected to expand globally to address concerns regarding the supply of critical minerals. To implement efficient and effective environmental protection measures, it is essential to determine the background concentration of the critical elements (CE), and the biogeochemical processes affecting their mobility and transport processes, which can vary significantly depending on environmental conditions. This research aims to differentiate the anthropogenic sources of CEs including rare earth and platinum group elements from the background concentration in southeastern Ontario before the extensive development of CE-related sectors, (ii) determine the major environmental processes governing the geochemistry of CEs, and (iii) the seasonal variations in the sources and cycling trends. We collected water samples along the Otonabee, Trent, and Ottawa Rivers over the four seasons and determined elemental composition using ICP-MS. Across all rivers and seasons, we reported the concentration of 45 elements as well as dissolved inorganic (1.55 – 22.19 mg/L) and organic (4.12 – 8.06 mg/L) carbon content, reflecting the heterogenous water chemistry of the rivers draining the Canadian Shield and the southern interior platform. We hypothesize the mobility and concentration of CEs will increase as geologic background shifts from Precambrian shield rock to Proterozoic sedimentary rock. Preliminary data points to wastewater treatment plants (WWTPs) as a current source of Gd contamination, and the fact that a shift in background geology can increase geochemical weathering, causing concentrations that falsely appear as contamination. Overall, this study provides a data set of current background CE concentrations in three Ontario rivers, potential sources, and detail on the influence of seasonality on these sources and cycling trends.

Toxicity of three Rare Earth Elements (La, Gd, Y) in single and binary mixture exposures to two benthic organisms (PL)

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Rare Earth Elements (REEs) are used in multiple applications, including green technologies. Their growing demand has led to the development of numerous mining projects in Canada. In addition to mining effluents, there are other sources of contamination by REE mixtures, such as agricultural or municipal effluents. In aquatic environments, REEs are largely found in sediments where they can cause deleterious effects. However, their toxicity in benthic organisms is little known. To fill this knowledge gap, the objective of this study is to provide toxicity data on three REEs (La, Gd, Y) in *Chironomus riparius* and *Hyalella azteca*, then to evaluate whether there are interactions in binary mixtures in the most sensitive species. To do that, concentration-response curves were produced with sediments contaminated by La, Gd, and Y with both species using sub-chronic toxicity tests. Growth inhibition and mortality were assessed and NOEC/LOEC as well as ECx (e.g., EC20, EC50) were calculated. The results showed that *C. riparius* was more tolerant than *H. azteca*, and that there are slight differences in toxicity between the three REEs where EC20Y > EC20Gd > EC20La. In addition, toxicity tests with mixtures of La/Gd, Gd/Y and La/Y with H. azteca were also carried out and analyzed with the isobologram methodology to determine if there are interactions. According to our results, more-than-additive effects were found for La/Y and La/Gd and less-than-additive effects was found for Gd/Y. Analyzes of REE concentrations in sediments and in *H. azteca* are underway to get more insights about these potential interactions and the resulting effects. The next steps in this research will focus on understanding the metal-handling strategy of studied REEs using subcellular metal partitioning approach as well as determining their target biomolecules using metallomic coupling techniques.

Critical and strategic metals within cells: handling strategies by aquatic invertebrates and fish (PL)

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The subcellular metal partitioning approach allows researchers to address different questions to better understand the way metals are detoxified, the sensitive sites where they can cause deleterious effects as well as their potential trophic transfer in food webs. However, little is known about the subcellular distribution of critical and strategics metals (CSM) in field-collected aquatic organisms. The goal of this work is to provide key information obtained in the last decades regarding the subcellular partitioning of some CSM in various aquatic organisms. Applying enzymatic-validated protocols, we determined the subcellular partitioning of rare earth elements (REE), cobalt (Co), nickel (Ni), and copper (Cu) in various animal models including larvae of *Chaoborus*, *Hyalella azteca* and in liver of Northern pike (*Esox lucius*), American eel (*Anguilla rostrata*), European eel (*Aguilla Anguilla*), Yellow perch (*Perca flavescens*), and Lake whitefish (*Coregonus clupeaformis*). Our results showed that REE (as class A metals) were largely found (45%-65% of the total intracellular level) in the NaOH-resistant fraction, where granule-like structures are expected. In contrast to REE, Cu (with a class B behaviour) was consistently associated with the heat-stable protein fraction (up to 75 %), which includes metallothionein (MT), glutathione and other thermostable proteins and peptides. As borderline members, Ni and Co were similarly distributed between both detoxified fractions. Despite these differences, these CSM were all found in higher

proportion in the mitochondrial fraction compared to the other two sensitive sites (microsomes+ lysosomes; heat-denatured protein fraction). More analyzes based-on direct and indirect approaches related to metallomics are needed to identify the nature of their molecular targets as well as their interaction with subcellular structures. Such findings have the potential to provide new insights of the toxicological mode of action of these contaminants with potential early warning signals required for biomonitoring purposes.

Assessment of agricultural soil geochemistry and rare earth element concentrations across farm and production types in Southern Ontario (PL)

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Canadian fertilizer safety standards are not sufficient to address the potential for adverse metal accumulation in agricultural soils, as they do not account for past metal contamination, concurrent use of agricultural amendments, or the introduction of new metal contaminants. Current standards regulate metal contaminants for their ability to accumulate over 45 years, without taking site history or soil geochemistry into account. In addition, fertilizer regulations about metal content are limited to few widely studied metals (As, Cd, Cr, Co, Cu, Hg, Mo, Ni, Pb, Se, Zn), which ignores the risk of accumulation of emerging contaminants, such as the rare earth elements (REE). To determine whether there is widespread impact from fertilizer contaminants, the geochemical composition and trace metal concentrations of soils from 52 agricultural fields were analyzed using inductively coupled plasma mass spectrometry and energy dispersive X-ray fluorescence. Principal components analysis and clustering were used to determine trends in trace metal and total element concentrations. The REEs were not enriched in the study region, but multiple sites exceed the environmental standards of total metal concentrations set by the Canadian Council of Ministers of the Environment (V, Cr, As, Pb). In particular, chromium exceeds environmental safety standards on all farms (> 64 mg kg^{-1}), indicating potential contamination issues. Fields from the same farm often clustered together based on soil geochemistry. However, large differences in agricultural management between fields on a single farm altered this trend. Based on the measured concentrations of trace metals on farms, fertilizer regulations are not sufficiently protective and may result in unwanted metal contamination of soils.

The distribution of rare earth elements in rice plants (PL)

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Rare earth elements (REEs) are classified as priority critical minerals in Canada. In agriculture, commercial REE-based fertilizers, primarily available in Eastern Asia, can positively affect plant development, including rice (*Oryza sativa*). The distribution of REEs in rice plants is not yet fully understood, and the risk of REE translocation into edible rice parts can lead to food safety issues. This research, therefore, focuses on the bioaccumulation of REEs to evaluate how REE amendments in soil influence the uptake and translocation mechanisms among rice plants. Germinated rice seeds were exposed to a light REE (La). Elemental analyses showed that the highest La concentrations were in roots and husks (up to approximately 10 μg g^{-1} and 0.04 μg g^{-1}, respectively). The accumulation in roots and husks was also directly related to soil La concentrations. Nevertheless, rice grains had the lowest concentrations (0.001 μg g^{-1}) among the plant parts, which is unresponsive to soil La amendment.

Despite a common assumption in the literature of the biochemical analogy between REEs and Ca, given their similar ionic radii, we did not observe the enrichment of Ca and other divalent cations in roots and husks. The differential accumulation of La in different rice tissues suggests specific mechanisms of translocation of REEs into rice tissues and requires further assessment to explore the transport and storage processes. Ultimately, the results highlight the limited transfer of REEs from soils to rice grains, suggesting the limited exposure of REEs to humans from the practice of amending REE in rice paddies.

Growth inhibition of Boreal plants exposed to palladium (Pd) (PL)

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The rapid evolution of various energy transition and global technology sectors has precipitated an increased demand for critical and strategic minerals containing platinum group elements (PGEs). However, the current lack of comprehensive toxicological data hinders our ability to assess the risks posed by PGEs to terrestrial and aquatic organisms, particularly in boreal and northern environments where extensive PGE mining occurs in Canada. The objectives of this study were twofold:1) to devise a method for contaminating boreal-type soil with palladium (Pd), and 2) to investigate the ecotoxicological response of boreal plants exposed to this Pd-contaminated soil. To achieve this, we spiked artificial boreal soils with nominal $PdCl_2$ concentrations ranging from 0 to 1000 mg/kg. After aging the soils for 2 weeks, we leached them using an artificial rainwater solution to reduce leachate conductivity to below 1000 µS/cm. Subsequently, we exposed trembling aspen (*Populus tremuloides*) for 28 days and black spruce (*Picea mariana*) for 42 days to the Pd-contaminated soil. Preliminary results indicate a decrease in shoot and root growth (both dry weight and length) for both species, although trembling aspen exhibited greater growth inhibition compared to black spruce. Ongoing experiments aim to determine toxicity threshold values and Pd bioaccumulation in both Boreal plant species. These ecotoxicological data are crucial for the management PGE mining practices in boreal and northern regions.

Method for determination of Li toxicity to boreal plants in soil (PL)

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Lithium (Li), given its strong reactivity, very low mass, high specific heat capacity, and high thermal conductivity, is widely used in the production of glass and ceramics, lubricating greases, and, more recently, batteries. Environmental controls during exploration and mining operations have improved significantly over the past 25 years. However, surrounding terrestrial and aquatic ecosystems could be contaminated by residual Li contained in solid, liquid, and atmospheric emissions produced during extraction, refining, transportation, and solid waste storage and recovery. Once in the environment, this residual Li may potentially be taken up by organisms, circulate within the food web, and cause toxic effects. Ecotoxicological quality criteria, which are not currently available for the protection of terrestrial organisms from Li in soils in Quebec, nor in Canada, can then be generated, which in turn can be used in the management of mining facilities to limit the impact on the surrounding environment. The objective of this work was to develop and optimize a soil preparation method for creating a standard reference soil with elevated concentrations of different forms of lithium. The second objective of this work was to use a soil prepared in this method to determine range of Li concentration causing toxicity to a boreal species. OECD 'artificial' soil consisting of sand, peat and clay (70:10:20) was amended with dissolved $LiNO_3$ at

total Li doses ranging from 0 to 500 mg/kg. After aging, the EC of the soils was elevated (up to 19000 mS/m) as compared to control (490 mS/m); leaching with artificial rainwater returned the EC of all Li doses to control values but reduced soil [Li] to <10% of nominal. Amending soil instead with Li_2CO_3 did not elevate EC, but did elevate pH, confounded with soil [Li]. Dilute acetic acid was effective at reducing pH to values similar to the clean soil, but resulted in soils that didn't support growth of *Picea mariana* except at the lowest soil [Li], the treatment which also required the smallest acetic acid addition. As the highest soil pH before correction was between 7 and 7.5, a final assay used $CaCO_3$ to raise soil pH of the lower [Li] in the dose response so that all Li treatments had a similar and slightly basic pH, acknowledging that this is not the ideal range for boreal species. Emergence of radish (*Raphanus sativus*) and black spruce (*Picea mariana*) from OECD soil amended with Li_2CO_3 up to 350 mg Li/kg of soil, with pH standardized to between 6.5 and 7 with $CaCO_3$ were evaluated. These data indicated that this soil was supportive of plant growth generally and could be suitable for determination of Li toxicity thresholds for boreal species.

Assessment of platinum and palladium toxicity for three freshwater organisms (PO)

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Due to mining activities and anthropogenic uses, platinum (Pt) and palladium (Pd) now enter the environment in multiple ways. However, while their presence in the environment is now unequivocal, there are still gaps in our knowledge of their ecotoxicological risks. For example, there are no water quality criteria for these metals in Canada, due to the lack of available data. For this project, we want to determine how acute and chronic exposures to Pt or Pd will affect three freshwater organisms: the landlocked salmon (*Salmo salar*), the fathead minnow (*Pimephales promelas*) and the great pond snail (*Lymnaea stagnalis*). Our aim is to produce LCx and ECx values suitable to the establishment of acute and chronic quality criteria for the protection of aquatic life for Pt and Pd. Furthermore, the tissue-specific and intracellular partitioning of Pd and Pt and their potential to cause oxidative stress and lipid peroxidation will be investigated. So far Pd demonstrated a higher toxicity than Pt. Acute exposure (96 h) of landlocked salmons yielded an LC50 of 0.9 µM Pd whereas Pt did not cause any mortality even at a concentration of 51 µM Pt. Chronic exposure (28 d) of *Lymnaea stagnalis* led to an EC20 of 0.07 µM Pd and 0.22 µM Pt using specific growth rate, which were calculated using snail weight, as the endpoint. The effect of a chronic exposure to Pd and Pt on the expression of two oxidoreductase enzymes (catalase and superoxide dismutase) were evaluated and will be discussed.

Radionuclides in the Environment

Monitoring and quantification of naturally occurring radioactivity in rare earth bearing-ore processing (PL)

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Rare earth elements (REE) are crucial metals present in various technological advancements, such as in permanent magnets used in electric engines and green energy. These elements are economically

extracted from minerals such as bastnaesite, monazite, and xenotime, which contain naturally occurring radionuclides (NORs) like uranium and thorium due to isomorphic substitution in the minerals lattice. Consequently, extracting REE-bearing ore from the ground could mobilize these NORs, which may become present in both the products and the tailings during beneficiation processes. Uranium and thorium behaviors during hydrometallurgical processing of REE minerals have been studied in recent years. However, U and Th are only part of the story as they decay into progenies with distinct environmental behaviors. Sadly, uranium daughter nuclides, such as radium-226 (Ra-226), lead-210 (Pb-210), and polonium-210 (Po-210), are more challenging to monitor mainly due to their low concentrations which require complex analytical separation methods and high instrument sensitivity for their detection. Although separation methods exist for U-238, Th-232, Ra-226, Pb-210, and Po-210 in solid and liquid matrices, they may not always be suitable for the matrices or fluxes resulting from REE processing. In this research project, we successfully modified an existing method for the preconcentration of Ra-226, Pb-210, and Po-210 in solid matrices containing REE. The method utilizes commercially available chromatographic extraction resins, specifically Sr resin and HRa hybrid resin. This adaptation enables efficient separation and recovery of all three radionuclides. Additionally, we elucidated the mobility trends of Th-232 and U-238 during a flotation process. This project will enable a better understanding of the mobility and distribution of NORs in the context of REE exploitation.

Regional information and monitoring network related to nuclear activities in the Ottawa River watershed (RIMNet) (PL)

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The Regional Information and Monitoring Network (RIMNet) is an initiative to improve information sharing and documentation regarding the environmental aspects of past, existing and proposed nuclear facilities in the Ottawa River Watershed. RIMNet is an independent initiative co-led by Environment and Climate Change Canada (ECCC) and the Canadian Nuclear Safety Commission (CNSC) and is not tied to any regulatory issue or project in the area. The scope of this initiative includes information for both radionuclides and hazardous substances associated with past and current activities of existing and proposed nuclear facilities. Where information is available, medical radioisotopes and naturally occurring radioactive material will also be considered. RIMNet aims to strengthen partnerships and help foster trust with Indigenous Nations and communities and members of the public. The initiative is being conducted using a phased approach. As part of Phase I, ECCC and CNSC have collected existing data, information, and knowledge related to nuclear facilities in the Ottawa River Watershed. Where appropriate and with consent, Indigenous Knowledge will be incorporated in collaboration with interested Indigenous Nations and communities. The results from Phase I will include a public-facing report and preliminary database, while Phase II will continue the development of a publicly accessible database hosted through the Open Science and Data Platform, and Phase III will culminate in a 'Regional State of the Environment' Report which will summarize the available knowledge. The information collated for this initiative may be used to inform future environmental reviews, ongoing monitoring, and regulatory decisions related to proposed and existing nuclear facilities and activities in the region.

Analysis of radium-226 in environmental samples from uranium tailing management lakes by mass spectrometry (PL)

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In the city of Elliot Lake, once dubbed the "uranium capital of the world," several lakes were converted into disposal sites for uranium mining residues. However, in recent years, increased concentrations of radium-226 in these lakes have been reported but not explained. Understanding the geochemical processes influencing radium concentrations in these lakes is therefore crucial. To do so, sediments and porewater samples collected from 2 sites will be prepare and analyzed for radium-226.
As part of this project, efforts were made to develop reliable analytical methods to accurately quantify radium concentrations in various environmental components (sediment, water and porewater). To achieve this, different preconcentration and separation techniques using resins were developed, tailored to water and sediment samples. Subsequently, ICP-MS analysis was performed to rapidly, sensitively and efficiently quantify radium in the samples.
This project thus provides an integrated approach to evaluate and monitor radium levels in the lacustrine environment, thereby providing crucial data to better understand the geochemical processes affecting radium.

Deriving toxicity thresholds for three fish species exposed to radium (226Ra) from fertilization to swim-up (PL)

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Radium is a ubiquitous radioactive metal currently regulated as a hazardous substance in the Canadian Metal and Diamond Mining Effluent Regulations (MDMER). Discharge limits that were previously established based on historical treatment and technological capabilities are currently under review, to determine if exceedances would pose a threat to aquatic organisms. A federal water quality guideline is under consideration as part of this review, and our early life stage (ELS) work with fathead minnow (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), and white sucker (*Catostomus commersonii*) make it possible to establish toxicity thresholds for fish, including the possible use of non-traditional statistical approaches. In our evaluation, the embryos of *O. mykiss*, *C. commersonii*, and *P. promelas* were exposed to environmentally relevant activities of ^{226}Ra (0, 0.1, 0.5, 2.5, 12.5, and 62.5 Bq/L) in very soft (10 - 13 mg/L as $CaCO_3$; *O. mykiss* and *C. commersonii*) or hard (160 - 180 mg/L as $CaCO_3$; *P. promelas*) reconstituted water, using a modified EPS 1/RM/28 protocol for *O. mykiss* and *C. commersonii*, and an OECD method for *P. promelas*. Embryos were exposed at fertilization (*O. mykiss* and *C. commersonii)* or 4 hours post-fertilization (*P. promelas*) until swim-up under static renewal conditions to identify toxicity thresholds. Among all fishes, exposure to ^{226}Ra did not result in significant mortality, nor were there enhancements or delays in the time to eyed-stage, hatch, or swim-up in degree days, resulting in no observed effect concentrations (NOECs) of 62.5 Bq/L, the highest test concentration, for all species. However, length, condition, and mass of larvae were affected among the fishes. For example, there were significant increases in length for *O. mykiss* at 0.1 and 0.5 Bq/L ($p<0.05$) and for *P. promelas* at 2.5 Bq/L ($p<0.05$). Mass also significantly increased at 0.1 Bq/L for *O. mykiss* ($p<0.05$) and at 0.5 Bq/L for

C. commersonii ($p<0.05$). Similarly, condition increased for *O. mykiss* at 0.5 Bq/L on average, but decreased with cumulative time in degree days ($p<0.05$). For *C. commersonii* and *P. promelas*, condition was not significantly affected, but there were significant negative relationships among the radium treatments. These data indicate that the lowest observed effect concentration (LOEC) for length was 0.1 Bq/L in *O. mykiss* and 12.5 Bq/L in *P. promelas*. For mass, the LOEC was 0.5 Bq/L for *O. mykiss* and 2.5 Bq/L for *C. commersonii*, while for condition, the LOEC was 0.5 Bq/L for *O. mykiss*, and 2.5 Bq/L for *P. promelas*. Using non-traditional statistical approaches, the results imply that current limits are questionable and further investigations into molecular and structural toxicity are warranted.

Fate of radium-226 in aquatic toxicity tests (PL)

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The bioavailability and toxicity of radium-226 (Ra-226) is closely linked to its interaction and sorption to solid surfaces. In the present study, a 48-hr partitioning test was conducted to determine the solubility of Ra-226 in treatment waters to be used in Sphaerium toxicity testing. As the toxicity tests will involve a static renewal exposure, with solution being replaced approximately every 48 hours, the sampling regime of the partitioning test was designed to measure the change in Ra-226 concentration during a typical exposure period. The influence of various components of the toxicity tests (i.e., exposure water, sand, and food) on the potential loss of dissolved Ra-226 from solution were assessed in a tiered manner with 3 replicates per treatment, and 3 Ra-226 treatments (1, 5 and 50 Bq/L), plus a control (0 Bq/L). Preliminary results indicated that the solubility of Ra-226 in reconstituted soft water was high, with initial measured dissolved and total concentrations of Ra-226 at time 0 being at nominal concentrations. No loss of Ra-226 was observed in the water only treatment. In contrast, the loss of dissolved Ra-226 from solution occurred quickly, with a >50% loss occurring before the 6-hr mark in the 50 Bq/L media+sand treatment, and later before the 12-hr mark in the 50 Bq/L media+sand+food treatment. The loss of dissolved Ra-226 from solution reached ~70% at the 24-hr mark and remained so at the 48-hr mark for both the media+sand and media+sand+food treatments. It is hypothesized that quartz present in sand, which has a high affinity to Ra-226, is responsible for its decline in solution and that the presence of food limits Ra-226 complexation with the quartz present in sand. Surface complexation modelling will be used to confirm these hypotheses. Results from this test will hence help inform on the average exposure to Ra-226 in water-only, media+food, and media+food+sand toxicity tests.

Characterization of radium-226 fate in the green alga *Chlamydomonas reinhardtii* (PL)

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Radium is a radioactive isotope resulting from the decay of uranium naturally present in soils and rocks. Consequently, it can be found in mining residues and released into surface waters. However, its impact on organisms is still poorly studied. To assess the ecotoxicological risks and potential transfer in the aquatic food web, it is crucial to understand the accumulation and fate of radium-226. In toxicity experiments, we showed that the green alga *Chlamydomonas reinhardtii* was a sensitive species compared to several others. To get some insight as to where the radium ends up in *C. reinhardtii* cells, the distribution of radium-226 was determined through a subcellular fractionation scheme. Since radium is an analogue of calcium, we also used calcium-45 as a tracer for calcium and we compared the subcellular distribution of both elements. The cells will be exposed in MHSM-1 medium at pH 7 for 72

hours to a dose of 50 Bq/L of radium-226 (6.0 pM) and in the presence of 68 µM calcium. We hypothesize that radium accumulates in sensitive and tropically available fractions of the cell, indicating a potential ecological risk. The next phase of our project will focus on the underlying toxicity mechanisms, studying the effect of Ra-226 on gene expression in *C. reinhardtii* to better understand its biological impact.

Radium-226 toxicity to the early life stages of the great pond snail *Lymnaea stagnalis* (PL)

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The Anthropocene is associated with profound changes in natural ecosystems, including an increase in the presence of naturally occurring radioactive materials (NORM) in surface waters. In Canada, uranium mining has contributed to such an increase. Among these NORM, ^{226}Ra is of particular concern because of its long half-life (1600 years), its potential for bioaccumulation in biota and its radiotoxicity. To better understand the effects of this radioelement on aquatic organisms, exposures of the great pond snail *Lymnaea stagnalis*, a pulmonate snail common in Canadian fresh waters, to ^{226}Ra are conducted. This calciphilic organism is expected to bioaccumulate ^{226}Ra, particularly during early life stages, which could cause toxicity. We performed two chronic toxicity tests with early life stages of the great pond snail and aqueous ^{226}Ra activity concentrations up to 100 Bq/L : a 28-days toxicity test starting with newly hatched juveniles (responses: growth inhibition, mortality) and a 52-days multi-life stage test (embryo then juveniles) starting with freshly laid eggs (responses: developmental errors, growth inhibition, heartbeat, hatching success, mortality). Our results indicate that ^{226}Ra reduces survival in juvenile snails that have been exposed to ≥ 50 Bq/L ^{226}Ra since the embryonic stage, but not in juveniles only exposed since hatching. The heart rate of embryos increases by 1.28-fold with exposure to ≥ 0.1 Bq/L ^{226}Ra. Exposure to ^{226}Ra had no significant effect on the growth of embryos or juveniles in either of the two tests. We are currently assessing biomolecular damages (e.g. lipid peroxidation, DNA breakage) caused by exposure to ^{226}Ra.

Chronic radium-226 toxicity to the aquatic invertebrates *Daphnia magna* and *Chironomus dilutus* larvae (PL)

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The uranium mining and milling industry is crucial in Canada but produces by-products like radium-226 (^{226}Ra), which are harmful to aquatic life. Despite stringent discharge regulations, evidence is lacking that current regulatory thresholds for ^{226}Ra adequately protect all aquatic organisms. Consequently, there is no specific Canadian Water Quality Guideline for the Protection of Aquatic Life for ^{226}Ra, highlighting the need for research to identify protective limits. This need is further exemplified by the potential ecological hazards posed by ^{226}Ra, given its long half-life ($t_{1/2}$ ~1600 years), high mobility under typical environmental conditions, and bioaccumulation potential. Therefore, the objective of this research project was to collect data on the toxicity of ^{226}Ra to the aquatic invertebrates *Daphnia magna* and *Chironomus dilutus*. To this end, three 21-day chronic toxicity tests were performed with *D. magna*, with endpoints of survival and reproduction. Additionally, a multigenerational study and a bioaccumulation

assay were conducted. A partial life-cycle exposure with *C. dilutus* to ^{226}Ra was also conducted with survival, growth, pupation, and emergence as the endpoints. An additional experiment with *C. dilutus* was also performed to assess whether oxidative stress was the mechanism behind the observed toxicity. Collectively, these experiments demonstrated that high activity concentrations of ^{226}Ra can significantly impact the survival of *D. magna* (50 Bq/L) and the growth of *C. dilutus* (25 Bq/L). ^{226}Ra was also found to be bioaccumulative (BAF: 72.8) in *D. magna.* No deleterious effects were observed at activity concentrations below 25 Bq/L and there was no evidence of oxidative stress.

Emerging Contaminants and Biosolids: Characterization, Quantification, and Assessment of Risk

Fate of PFAS in sludge handling trains at two Canadian wastewater treatment plants (PL)

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While there is a growing body of evidence describing the fate of PFAS through the liquid treatment trains of wastewater treatment plants, there is little information on their fate through the associated sludge handling systems. Knowledge of PFAS fate in this regard has important implications for their presence in both recycle streams and the quality of the final biosolids product leaving the plants. Interstage mass balance analyses were performed to assess the fate of 15 PFAS in two full-scale wastewater sludge handling systems. Both systems consisted of thickening (rotary drum, gravity and dissolved air flotation), anaerobic digestion, and dewatering, while one of them also included primary sludge fermentation. On a mass concentration basis, PFOS was the most abundant compound in both systems, with concentrations as high as 12.9 µg/kg and 53 ng/L in solid and liquid samples, respectively. On a molar basis, PFOS was the most abundant compound in solid samples (up to ~ 26,000 picomol/kg), while PFBA was the most abundant compound in liquid samples (up to ~ 140 picomol/L). Fermentation (a sludge retention time of 4 days) did not cause a significant change in PFAS mass flows. In contrast, anaerobic digestion (sludge retention times of 45 and 20 days) resulted in mass flow increases for 12 PFAAs, and mass flow decreases for 3 precursors. Precursor transformation and PFAA formation increased with the sludge retention time of the digesters. In the liquid-solid separation processes (thickening and dewatering), PFAS with carbon chain lengths of < C7 were mostly present (between 50% and 86%) in the liquid recycle streams, whereas PFAS with chain lengths of ≥ C7 were mostly present (between 50% and 121%) in the solid streams. In terms of PFAS fate at a system-wide scale, over 50% of the mass of each <C6 compound exited both systems via liquid streams, while over 50% of the mass of each ≥ C6 compound exited the systems via the biosolids stream. Collectively, the results demonstrated that the fate of PFAS in sludge handling systems is likely dependent upon the processes employed and their retention time, as well as the physico-chemical properties of PFAS. The results also demonstrated that a thorough interstage mass balance analysis of an entire sludge handling system could provide insights into the impact of individual processes on PFAS fate and also identify opportunities where interventions might be introduced to remove PFAS from the urban water cycle.

PFAS and PPCPs in Canadian biosolids: results of 12 years of monitoring by Environment and Climate Change Canada (PL)

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Environment and Climate Change Canada (ECCC)'s wastewater monitoring program was initiated in 2008 to monitor chemical substances in wastewater systems in support of risk assessment and risk management activities under the Chemicals Management Plan. We have collected samples from over 80 wastewater treatment plants (WWTPs) across Canada which have been analyzed for many substances including per- and polyfluoroalkyl substances (PFAS) and pharmaceuticals and personal care products (PPCPs). The objectives of this study were to assess concentrations and time trends of 40 PFAS (2009-2021) and 136 PPCPs (2010-2022) in biosolids from Canadian WWTPs. PFAS with the highest maximum concentrations in 2018-2021 biosolids were the short-chain precursor 5:3 perfluorooctanoate, perfluorobutanoate, and perfluorooctanesulfonate (PFOS). Most WWTPs with biosolids collected in 2018-2021 contained PFOS concentrations below the proposed Canadian Food Inspection Agency standard of <50 ng/g. PPCPs with the highest concentrations in 2022 biosolids included fluoroquinolone antibiotics (e.g., ciprofloxacin), sertraline (an antidepressant), and triclosan (an antimicrobial that has been regulated since 2016). The time trends of most long chain-PFAS decreased over time in Canadian biosolids reflecting regulatory action and industrial phase-outs whereas concentrations of unregulated short-chain PFAS increased. Differing time trends were observed for PPCPs and were related to regulations (e.g., triclosan), Health Canada warnings (e.g., ciprofloxacin), and use to treat symptoms of COVID-19 (e.g., sertraline). Overall, the time trend data of PFAS and PPCPs provides needed information on community usage and changes to release of these chemicals to the environment via wastewater and biosolids.

Assessing the Microplastic Content of Biosolid-Amended Agricultural Fields (PL)

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Microplastics (<5mm) are an emerging ecological concern. Primary microplastics are intentionally created for commercial use (e.g., microbeads for cosmetic products) while secondary microplastics are created via the degradation of larger plastic materials. Due to their persistence in the environment, the unknown effects of microplastics are a potential threat to the health of various ecosystems. To date, the overwhelming majority of microplastic research has focused on their impacts on aquatic ecosystems, creating a need for research to be performed on their effects on terrestrial ecosystems. One potential significant source of microplastics to agricultural soils are biosolids. Biosolids are nutrient-rich field amendments that are derived from the processing of wastewater. Soil samples were collected from thirteen biosolid-amended agricultural fields and seven fields that have never had biosolids applied to them. Soil samples were digested with Fenton's reagent and density separated with NaBr to isolate microplastic content. Microplastics were counted and categorized based on size, colour, and shape. Biosolid-amended fields were found to have significantly more microplastics than non-amended fields. Additionally, biosolid-amended fields had a higher proportion of microfibres, which can be attributed by the high number of textile fibres found within biosolids. Microplastic characterization of soil samples identified polyester and acrylic as the two most abundant types of microfibre. In addition, HDPE, LDPE, and polypropylene were identified as the most abundant microparticles. Quantifying and characterizing

microplastic content of biosolid-amended fields is crucial for developing an accurate risk assessment of microplastics in terrestrial ecosystems.

Characterization of Microplastics Within Earthworms (*Lumbricus terrestris*) from Biosolid-Amended Fields (PL)

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Earthworms have numerous functions within the soil ecosystem, but their ability to cycle essential nutrients makes them extremely beneficial to agriculture. Conservation of earthworm populations supports sustainable agriculture, leading to higher crop yields and reduced costs for farmers. Unfortunately, agricultural practices such as the amendment of biosolids can introduce contaminants to agricultural soils, potentially damaging earthworm communities. One emergent contaminant of concern found within biosolids are microplastics. The risks associated with microplastics are largely attributed to their potential ingestion. To determine the type of microplastics that are being ingested by earthworms, a total of 200 earthworms from twenty agricultural fields (ten per field) were collected. Of the twenty fields, thirteen were amended with biosolids between 2017 and 2021, and the other seven have never been amended with biosolids. Earthworms were collected at night to ensure that all specimens were *Lumbricus terrestris*. Earthworms were flash frozen with dry ice, digested with 20% KOH, and filtered on 0.45μm PVDF filter papers. Microplastics were characterized based on their shape, colour, and size. Additionally, suspected microplastics were characterized via FTIR. Results showed that while biosolid-amended fields showcased elevated levels of microplastics, microplastic contamination within earthworms appear to be minimal. Of the identified microplastics, the majority were fibres or fragments. Additionally, earthworms showed an avoidance of larger microplastic particles. Microplastic concentrations are expected to continue to rise, so continued monitoring and mitigation of microplastic pollution is essential to protect earthworm populations.

Characterization of microplastics found in agricultural topsoils amended with municipal compost (PL)

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Microplastics (MP) have become an emerging contaminant detected, leading to it becoming a public concern. While most research is conducted in aquatic environments, there is growing concern for the effects it could have in our terrestrial ecosystems. Soil has been an identified sink for the microplastics. Several factors contribute towards the MPs accumulation into agricultural soils. A major source for the accumulation has been land application of urban-derived compost as organic soil amendments. While Ontario Compost standards ensure that plastics do not exceed 0.5% of its finished products dry weight, this can still contribute to a source of plastic contamination especially if usage rates of urban-derived compost increase. This study will focus on presenting results from microplastics extracted from municipally derived compost products, from soils receiving these amendments and eventually characterizing the microplastics. The topsoil will be of focus to compare the MPs found in the top 0-15cm layer and 15-30 and observe if there is a differ in size, abundance, shape, and potential degradation. This will help examine if soil texture along with repeated application of compost will play a role in the vertical distribution of MPs found in different agricultural soils.

Applications of Emerging Technologies in Aquatic Environmental Assessment and Management

Applications of emerging technologies, a case study on benchmarks for emerging contaminants (PL)

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There are enormous amounts of ecotoxicology data available through public or internal sources but making the data usable and accessible is a challenge. Digital tools such as R, R shiny and GitHub are a way to process, visualize and create accessibility with relative ease, transparency and minimal financial cost. R is an open-source programming language that is relatively easy to learn. R shiny is a system to build web applications using the R language. GitHub is a platform for collaboratively working on source code that allows users to inspect the source code, contribute, and comment on the project. As a case study we discuss a web application, shinywqbench, that we developed to quickly generate aquatic benchmarks for emerging contaminants using the data in the United States (US) Environmental Protection Agency (EPA) ECOTOX database. ECOTOX is a publicly available database that contains chemical environmental toxicity data. The project began by downloading and processing the ECOTOX data using R. Once the data were in a consistent and usable format, R shiny was used to create an application where users can select a chemical, view the associated data, and generate benchmarks. The application built upon other pre-existing R tools such as ssdtools which calculates HC5 values, and shinywqg, which displays pre-existing established guidelines. The code is publicly available on GitHub. Building on last year's exploration of the app's functionality, this presentation will focus on the development journey. Learning how to use these digital tools allows ecotoxicologist to automate workflows and increase efficiencies which facilitates monitoring and management of the aquatic environment.

A novel approach to developing water quality guidelines for polycyclic aromatic hydrocarbons (PAHs) (PL)

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Polycyclic aromatic hydrocarbons (PAHs) are a complex group of organic substances that are ubiquitous in the environment, including in surface waters, with thousands of individual non-substituted (parent) and alkylated PAHs possible. Due to their sources, PAHs do not occur as individual substances but rather as a mixture of PAHs in the environment. In addition, many PAHs demonstrate increased toxicity in the presence of ultraviolet light, a process called phototoxicity. Current Canadian Council of Ministers of the Environment (CCME) water quality guidelines are outdated, limited to eleven non-substituted PAHs, do not provide a means to consider the overall toxicity of PAHs as a mixture and have limited consideration of their phototoxicity. Therefore, CCME has initiated a project to update or derive new water quality guidelines for PAHs for a large number of parent and alkylated PAHs which will include consideration of phototoxicity and a means to derive guidelines for a mixture of PAHs. A novel approach using two models, the narcotic target lipid model (NTLM) and the phototoxic target lipid model (PTLM), will be

trialed as a method to address current challenges to PAH guideline development, including overcoming data limitations and consideration of phototoxicity. This presentation describes the proposed approach for deriving water quality guidelines for both low and high ultraviolet light conditions as well as how to apply them to mixtures.

Using *in silico* tools to predict ecological risks of PFAS to aquatic species (PO)

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Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in the environment. Although several studies have evaluated the potential ecological effects of certain PFAS, data gaps remain, especially for newer PFAS. In this study several publicly available *in silico* tools were used for a screening-level risk assessment of PFAS in aquatic species. The well-studied PFAS, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), were the focus to provide a better context to estimated values. The following software were used: (1) Ecological Structural Activity Relationships (ECOSAR) program (to estimate acute and chronic toxicity thresholds for several aquatic species), (2) Estimation Program Interface (EPI Suite™) (to estimate wastewater removal and bioconcentration values), and (3) Exposure and Fate Assessment Screening Tool (E-FAST) model [to derive estimated environmental concentrations (EECs) in surface water following hypothetical discharges from wastewater treatment plants (WWTPs)]. Subsequently, the predicted toxicity thresholds and EECs were compared to reported values, and risk quotients (RQs) were calculated. The findings indicated that (1) the estimated toxicity thresholds were typically lower (i.e., more conservative) than or in the range of reported values, (2) the EECs were mostly in the range of reported environmental concentrations, and (3) the RQs were mostly below the levels of concern.

Responsible use of generative AI for environmental professionals (PL)

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We are witnessing a rapid surge in technological advancements and the day-to-day integration of generative AI, such as ChatGPT, into fields ranging from customer service to marketing, to pure and applied sciences. Alongside this growth, there has been an increase in instances of the misuse of generative AI – for example, peer-reviewed journal articles starting with standard ChatGPT preambles or containing inaccurate, AI-generated figures, or a lawyer submitting a federal court filing citing non-existent cases. While these cases may suggest caution against adopting generative AI in the workplace, individuals and organizations are actively exploring ways to harness generative AI to enhance or even replace existing workflows. This talk will explore how to use generative AI responsibly in our daily activities as environmental professionals, discussing considerations such as legal implications, corporate policies, and professional and ethical obligations.

Refining risk assessments with EAS-E Suite: a case study from Health Canada's New Substances Program (PO)

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Health Canada's New Substances Assessment and Control Bureau evaluates the risk to human health and the environment, under the New Substances Notification Regulations (Chemicals and Polymers) of the Canadian Environmental Protection Act, 1999, of new substances used in Food and Drugs Act regulated products. A crucial aspect of these risk assessments is the use of robust models to fill in data gaps when empirical data are unavailable. This study focuses on the application of the on-line Exposure and Safety Estimation (EAS-E) Suite platform (www.eas-e-suite.com) which provides a combination of chemical property databases and models and ecological and human exposure models to inform CEPA risk assessments. We examine the use of specific EAS-E Suite tools in assessing physical-chemical endpoints and exposure estimates over a range of chemicals and chemical uses. We aim to demonstrate the utility of these tools in both conventional new chemical risk assessments and specialized scenarios, such as aquaculture exposure assessments. By including EAS-E Suite as an additional in silico tool for assessments, we seek to improve the reliability of predictions, improve decision making, and streamline the process in new substance assessments under CEPA. The findings of this analysis provide insights into the practical application of EAS-E Suite in CEPA regulatory frameworks and contribute to the development of additional robust risk assessment methodologies. This work helps the program in evaluating and managing the risks of new substances used in Food and Drugs Act products that are subject to notification under the New Substance Notification Regulations.

Exposure, Effects, and Management of Microplastic Pollution in Terrestrial and Aquatic Environments

Investigating wastewater treatment facilities as a source of microplastics to aquatic food webs (PL)

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Wastewater treatment facilities perform an important role in removing some contaminants and nutrients from wastewaters, yet they are suggested to be a major contributor of microplastics (plastic particles ≤5 mm) to aquatic systems. Once in aquatic ecosystems, microplastics may accumulate in key basal food web compartments such as sediments and biofilms, therefore posing a risk to benthic macroinvertebrates. This study examined the abundance and characteristics of microplastics in wastewater effluents, sediments, biofilms and benthic macroinvertebrates to assess exposure and uptake. Effluent samples were collected from eight wastewater treatment facilities in the Grand River watershed, Ontario, Canada, and sediment, biofilm and macroinvertebrate samples were taken upstream and downstream of each facility. Microplastics were filtered from effluent samples and extracted from sediment with $CaCl_2$ density separations and from biofilm and benthic macroinvertebrates using H_2O_2 digestions. Results show that some downstream locations contained higher abundances of microplastics in sediment, biofilm and benthic macroinvertebrates, however this

trend was not consistent across all eight wastewater facilities. Higher abundances of microplastics were not related to the size of the population served by the facilities. Concentration of microplastics ranged 8-78 particles/L in effluent, 0.11-7.42 particles/g in sediment, 0.99-299.27 particles/g in biofilm, and 0-0.35 particles/mg in macroinvertebrates. Overall, fibres were the most common type of microplastic found in all sample types. Particles have not yet been chemically confirmed as plastics. This research will provide key insights into sources and fate of microplastics in aquatic ecosystems and consider the potential risks posed to benthic macroinvertebrates downstream of some facilities.

Microplastics composition and loadings to Lake Ontario from an urban creek (PL)

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Urban streams are a dominant pathway for transporting land-derived microplastics (MPs) to adjacent waterbodies. Throughout a year of sampling, we assessed concentrations, composition and loads of MPs (>20 µm) under high and low-flow conditions in a highly urbanized creek containing a number of plastics-based industries. Sampling was conducted using different approaches to capture particles across a wise size range, including a drift net (250 µm-mesh; n=35), a pump and 50 µm net (n=50), and standard water quality pumping system (ISCO; n=50). The median concentrations of MPs (>250 µm) during rain and dry events were 1.3 items/L (n=25) and 0.08 items/L (n=10), respectively. The corresponding median loads for rain and dry events were 1.8×10^3 and 0.26×10^3 items/second, respectively, based on the mean discharge values on the sampling date. MP abundances and loads determined on a particle number basis from the pump/50 µm net were up to 300 times higher than measured via 250 µm mesh net. MP composition was different between rain and dry events. Concentrations of rubber particles >125 µm were strongly and positively correlated with stream flow rate ($p<0.05$, $r>0.7$) and thus abundant during rain events. In contrast, plastic pellet concentrations were associated with the first flush during rain events. Concentrations of fragments from commercial plastics activities were not related to flow volume. These results indicate the considerable loads derived from road wear and litter that are mobilized during rain events, but also industrial inputs entering the creek from, likely, direct discharges. The sampling method chosen influences the loads estimate, MP composition and interpretation of MP sources.

Microplastics contamination in Pelagic Fish and Surface Water from the St. Lawrence River and Estuary, Canada (PL)

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The St. Lawrence River and Estuary (SLRE) form an important ecosystem that can transport contaminants to the Atlantic Ocean. However, limited information is available on microplastic (MPs) contamination in this system, particularly for pelagic fish. To address this knowledge gap, the present study analyzed MPs

in the gastrointestinal tract (GIT) and gills of 5 pelagic fish species (n=114) and surface water (n=23) collected in the St. Lawrence around Montreal and Québec City. The average MPs abundance in fish GIT, gills and surface water was 1.0±0.19, 2.34±0.32, and 5.94±1.36, respectively. The dominant shape, color, and size of MPs in the fish and water were fiber, blue, and 403-1590μm, respectively. Polymer identification by spectroscopy indicated that polyethylene, polyester, polypropylene, rayon, and copolymers (e.g., vinyl) were prevalent in fish and water. The results showed that the abundance of MPs was higher in fish GIT and water collected around Montreal than Québec City, indicating greater exposure and ingestion of MPs by pelagic fish from the Montreal area. The gills of predator fish (e.g., northern pike and walleye) had significantly higher levels of MPs than those of prey fish (e.g., yellow perch, white perch, and rainbow smelt), suggesting that predator fish may have a higher passive ingestion of MPs than their prey fish, potentially due to their larger gills. This study demonstrates the spatial variation by species, and tissue variations of MP contamination in SLRE pelagic fish. It establishes a baseline for further investigation into the plastic pollution in the SLRE food web.

Assessing Microplastic Contamination in Benthic Fish from the St. Lawrence Estuary: Occurrence, Spatial Distribution, and Ecological Risk Assessment (PL)

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In Canada, microplastic contamination in the water and sediment of the St. Lawrence River and Estuary (SLRE) has raised concerns regarding the potential risks posed to aquatic species. However, understanding microplastic contamination in benthic fish is lacking, impeding risk assessment in this vital ecosystem. This study aimed to bridge this gap by examining microplastics in the gastrointestinal tracts (GIT) and gills of two key benthic species: Channel catfish (*Ictalurus punctatus*) and Atlantic tomcod (*Microgadus tomcod*) in the SLRE. Fish samples (n = 42) were collected from ten stations in the SLE and analyzed using KOH digestion, density separation, wet peroxidation, and spectroscopic methods. Results indicated an average abundance of 3.0±0.4 (mean±SE) microplastics per individual fish, consistent with global findings. Most detected microplastics were small (<809 μm) and fibers, with blue and transparent colors prevalent. Major polymers identified included polyethylene terephthalate and polyethylene. Catfish from the downstream of Québec City showed elevated microplastics and more variations of the characteristics of microplastics compared to the cospecies from the upstream, implying that urban activity affects the accumulation of microplastics in benthic fish in the SLE. Ecological risk assessment revealed medium to high risks for the habitat of the catfish close to the Québec City due to the prevalence of smaller microplastics <809 μm and highly toxic polymers (polymethyl methacrylate, polyvinylchloride, polyurethane, acrylonitrile butadiene styrene). This study establishes a baseline for plastic pollution monitoring in the fish in the SLRE and ecological risk assessment of microplastics in the habitat of catfish and tomcod.

Can we monitor aquatic microplastics from above? A review of remote sensing techniques and a pilot project using a drone (PL)

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Plastic pollution is a ubiquitous global concern and the study of the impacts of microplastics in particular presents many challenges. The development of new efficient, large-scale detection and monitoring methods, as could potentially be provided by aerial remote sensing, would be highly beneficial to complement existing monitoring activities. We conducted a review of the literature relevant to the detection, quantification and/or characterization of aquatic microplastics by means of aerial (i.e. satellite, crewed or uncrewed aircraft) remote sensing in order to ascertain techniques currently under development. We also assessed the potential of these approaches and determined ideal next steps. The review highlighted the preliminary state of this field of research: since 2016, only 22 variably relevant published research documents were uncovered (18 journal articles, 4 conference papers), as well as 10 literature reviews touching on the subject. To date, three different approaches have been explored, including remote sensing of aquatic microplastics based on (1) their spectral characteristics (n = 10 documents), (2) their effects on water surface roughness (n = 6) and (3) measurement of various proxies (n = 6), with a heavy focus on the use of satellite-borne sensors thus far. The most promising appears to be the first approach, which involves direct detection of microplastics and takes advantage of their unique spectral signature. Due to the small size of microplastics, better progress in this approach would likely be made by experimenting with image acquisition at much lower altitudes and finer spatial resolutions, as could be achieved using drones. Consequently, we will conduct a pilot study aiming to assess the detectability of microplastics in experimental outdoor pools with a drone-borne hyperspectral camera flown at varying heights up to 120 m above ground. This could open up possibilities for modest-scale drone remote sensing of aquatic microplastics, and provide a robust empirical baseline to model the upscaling of their detection in coarser satellite imagery.

Life in Plastic, is it Fantastic? Assessing Microplastics Toxicity and Accumulation in Freshwater Benthic Macroinvertebrates (PL)

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Microplastics (MPs) (< 5 mm) pollution has become one of the most pressing environmental issues to date. In freshwater ecosystems, particularly in riverine sediments, high levels of MPs have been reported, putting sediment-dwelling organisms such as macroinvertebrates at risk. However, the effects of MPs on macroinvertebrates remain unclear, despite their critical roles in freshwater food webs. To assess the toxicity and accumulation of MPs, we exposed two benthic macroinvertebrate species, *Tubifex tubifex* and *Planorbella pilsbryi*, to three MPs types (6 and 45 µm polystyrene microbeads, and 100 µm polyethylene terephthalate microfibers) across five environmentally realistic concentrations (0, 0.1, 1, 10, 100 and 1000 MPs/g dry weight sediment; or MPs/mL water). Using two MPs conditions, pristine and microbially colonized (biofouled), we assessed mortality, reproduction, accumulation, and alterations to whole-body microbiota in these species. Reproductive success was measured as the number of eggs and/or juveniles present. Whole-body microbiota were analyzed using the V3-V4 regions of the 16s rRNA gene. Accumulation of MPs was assessed following exposure, with a gut clearance period added to

determine excretion efficiency. No significant effects to reproduction or survival were reported for either species. Host microbiome analysis is underway. No significant MPs accumulation occurred in *T. tubifex*. In *P. pilsbryi*, significant accumulation of 6 µm and 45 µm microbeads were reported, however, we found that microbeads were able to be excreted, with retention proportional to exposure. No significant accumulation of microfibers was reported, but high levels were present in feces. While no significant toxicological effects were observed herein, high MPs accumulation in some benthic macroinvertebrate species with continuous exposures highlights a risk of trophic transfer to their predators.

Microplastics and mesocosms: investigating the effects of MPs on invertebrate communities (PL)

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As Canada strives to establish a risk assessment and management framework for microplastics, it remains critical to characterize the effects of microplastics on vulnerable taxa. While it is known that every level of the food web interacts with microplastics, the effects of these interactions remain under question. As bioindicators of ecosystem health, freshwater benthic invertebrates have long been used to understand the toxicological effects of contaminants. They are particularly suited to the study of microplastics, often being exposed to high concentrations of microplastics that settle in freshwater sediments and accumulate in biofilm, an important food source for many invertebrates. We sought to determine the effects of microplastic exposure on the invertebrate community in the littoral area of a lake. We exposed the native community contained within mesocosm enclosures to a range of environmentally relevant concentrations, spanning from zero to over 2 million particles/kg dry sediment. After an 8-week exposure period, we sampled the invertebrate community via sediment cores. We also aimed to characterize the fate and concentration of microplastics in the enclosures with sediment cores and biofilm samples. We report on the response of the benthic invertebrate abundance, diversity, and composition to the range of microplastic exposure.

Subtle effects of microplastics on zooplankton and emerging insect abundances in a littoral limnocorral experiment (PL)

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Although microplastic research has rapidly accelerated in recent years, our understanding of the effects of microplastics on freshwater invertebrates under realistic conditions remains limited. Using in-situ mesocosms (hereinafter 'limnocorrals') at the International Institute for Sustainable Development–Experimental Lakes Area, we investigated the effects of a microplastic mixture on zooplankton and emerging insect communities. We installed 12 open-bottom limnocorrals in the littoral zone of a boreal lake in June 2022 and added environmentally relevant microplastic concentrations (251—2511886 particles kg^{-1} dw sediment) in a regression-based design. Our microplastic mixture consisted of distinctively coloured polystyrene, linear low-density polyethylene, and polyethylene terephthalate fragments in equal parts. We sampled the zooplankton community at weeks 1, 5, and 8 after microplastic addition and collected emerging insects for 8 weeks. To evaluate possible longer-term effects, we sampled emerging insects again in May 2023 over the limnocorral footprints. We assessed

the relationship between nominal microplastic concentration and the abundance and community composition of zooplankton and insects, as well as the timing of insect emergence. We found evidence that microplastic additions negatively influenced total zooplankton abundance at weeks 1 and 5. For emerging insects, we found weak evidence that the total abundance was negatively affected by microplastic additions in summer 2022, but not spring 2023. Microplastics had little-to-no influence on community composition of zooplankton or insects. The timing of insect emergence was unaffected. Overall, our results suggest that current levels of microplastic pollution in freshwater environments may have subtle adverse effects on zooplankton and aquatic insect emergence.

Assessing effects of microplastics on microbial communities in outdoor mesocosms (PL)

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Microbial communities are the first living organisms to interact with plastic and play a key role in the fate of microplastic. At the same time, these microbial communities are integral in ecosystem health but can be sensitive to environmental stressors. I investigated the effects microplastics are having on these naturally occurring phytoplankton and biofilm communities. Our team conducted a multi-trophic ecotoxicology assessment at Queens Biological Station (QE3 Living lab), near Kingston ON. There, in the early spring (2024) we set up 21 mesocosms (330 L steel tanks) filled with 5 cm of aquarium sand as a substrate, 250 L of overlaying freshwater from a nearby mesotrophic lake and 40 tadpoles. We placed 1 terracotta flowerpot in each mesocosm as an artificial substrate for biofilm growth. Mesocosms were divided into 7 treatment concentrations ranging from 0 - 1,849,861 particles/L with 3 replicates per concentration. We added equal parts of three common polymers (LLDPE, PS, and PET) with a size range of 10 – 500 µm. After ten weeks of exposure, we conducted light and dark dissolved oxygen incubations to measure rates of primary production in the mesocosms. Additionally, bulk samples of the biofilm were collected from the terracotta flowerpots. We hypothesize that increasing concentrations of microplastics will alter microbial community structure and function.

The effects of microplastics on the growth, development, and body condition of wood frogs (*Rana sylvatica*) across a gradient of concentrations (PL)

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Microplastics are present in the habitats of amphibians around the world. However, there is limited information concerning the effects of microplastics on amphibians across a gradient of concentrations. To understand at which microplastic concentrations amphibians experience effects, we exposed wood frog (*Rana sylvatica*) tadpoles to a microplastic mixture that consisted of equal parts low-density polyethylene, polystyrene, polyethylene terephthalate, and their associated additives. The outdoor mesocosm experiment occurred at the Queen's University Biological Station where each of our six treatments and our negative control were triplicated. Wood frog embryos were initially exposed to nominal concentrations ranging from 18.5 particles/L to 1.85 million particles/L, followed by weekly

additions of microplastics for the first 12 weeks of the experiment at nominal concentrations ranging from 1.5 to 150 000 particles/L. Survival was assessed throughout the embryonic, larval, and metamorph stages. Wood frogs were photographed and weighed to assess hatching success, developmental stage (Gosner stage), growth (body length over time), and body condition (scaled mass index). Lethal sampling was used to quantify microplastic concentrations in wood frog tissues and to examine the intestinal tract for signs of inflammation and lesions. Our study will generate new knowledge on the effects of microplastics on amphibians across a concentration gradient to understand how wood frogs may be affected by microplastic pollution and at which concentrations those effects occur.

Quantifying responses of American toad tadpoles to microplastics in a freshwater lake using in-situ assays (PL)

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Globally, amphibians have been identified as the most threatened class of vertebrates, in part due to anthropogenic stressors such as pollution. Therefore, understanding how pollutants like microplastics affect amphibians is of great importance for their conservation. Therefore, starting in the summer of 2023, we began examining American toad (*Anaxyrus americanus*) tadpole responses to microplastic additions as part of a whole-lake manipulation study at the International Institute for Sustainable Development-Experimental Lakes Area. In this study, a mix of microplastic polymers (polyethylene, polystyrene, and polyethylene terephthalate) are being added bi-weekly to a natural boreal lake at environmentally relevant concentrations to examine the fate and effects of microplastics. To examine effects on amphibians, we collected American toad eggs and reared the tadpoles in flow-through enclosures within the microplastic-treated lake and a reference lake. We quantitatively compared organismal level endpoints (e.g., growth, developmental timing, mortality) between tadpoles from the two lakes, and the tadpoles reared in the microplastic-treated lake are also sampled for body microplastic content. From our preliminary results in 2023, we found that tadpoles from the microplastic-treated lake had shorter body (snout to vent) length and a slightly lower wet weight at metamorphic climax in comparison to those from the reference lake. Qualitatively, we found similar trends in time to metamorphic climax between tadpoles in the two lakes. Altogether, this study provides novel insight into how amphibian larval growth and development can respond to microplastics under natural conditions, bridging laboratory studies with the real world to better inform management policies and regulations.

The Fate of Ingested Biofouled Polyethylene Foam by Giant American Millipedes (*Narceus americanus*) (PL)

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Microplastics are a growing global concern due to their widespread presence in terrestrial and aquatic environments. Invertebrates, from Antarctica to the Arctic, have been found with microplastics in their

guts. These plastics can cause gut damage through oxidative stress, microbial depletion, and nutrient absorption blockage. However, little is known about what happens to these plastics once ingested by large invertebrate decomposers, such as millipedes. We hypothesize that biofouled polyethylene foams will be consumed by Giant American Millipedes (GAM; *Narceus americanus*), leading to the creation of micro and nano plastics as they are digested, as well accumulate plastic fragments in their guts. In this experiment, GAM were divided randomly into two groups: a polyethylene foam-fed group and an unfed control group. These groups were divided into 5 replicates, with 3 millipedes per replicate. Control and treatment groups were fed a 1 cm^3 piece of cucumber and 3 oat grains. Treatment groups were fed a 1 cm^3 piece of polyethylene foam that had been aged and accumulated biofilm over a two-week period in muddy water. From each replicate, one GAM was dissected to identify where microplastics may have accumulated in the body, and these were then digested to count the accumulated plastics. The remaining two millipedes in each replicate were fed for one week and two weeks, respectively, and then dissected and digested to assess the persistence of plastics in the body. The results of this experiment are pending. This study contributes to the growing body of knowledge on the environmental fate of microplastics and their impact on important soil-nesting decomposer invertebrates.

Assessing the Accumulation of Microplastics within Earthworms (*Eisenia fetida*) using Traditional Bioaccumulation Testing and X-Ray Microtomography (PL)

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Microplastics are ubiquitous in the environment, and their potential toxic effects are not yet fully understood. The physiological changes resulting from the ingestion of microplastics depend on their accumulation within the body and their ability to translocate from the gut to other parts of the body. According to Mehinto et al. (2022), plastic particles smaller than 83 µm are at risk of translocating into tissues. OECD 317 guidelines were used as a traditional testing method for microplastic accumulation. Earthworms (*Eisenia fetida*) were exposed to soils spiked with 10,000,000 microplastics/kg (dw) of varying morphologies (fiber, sphere, fragment, film, and foam). The results showed no evidence of microplastic accumulation within the earthworms. To test tissue translocation of microplastics, earthworms were introduced to soil spiked with 100,000,000 particles/kg (dw) of two sizes of barium-sulfate polyethylene microspheres (5-22 µm and 45-53 µm) purchased from Cospheric™. The earthworms were then preserved with formalin and dehydrated using an ethanol dilution series. X-ray microtomography was performed using the BMIT-BM beamline from the Canadian Light Source synchrotron in Saskatoon, Saskatchewan. The resulting images showed little evidence of tissue translocation for both the 5-22 µm and 45-53 µm polyethylene microbeads. Additionally, there was no clear location within the earthworm gastrointestinal tract that indicated microplastic accumulation for either size fraction. Nevertheless, X-ray microtomography proves to be a valuable tool for assessing microplastic deposition and movement within organisms.

Zooming in on the interactions between microplastics and terrestrial organisms (PL)

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While plastic pollution in the marine and freshwater ecosystems has received fairly broad attention, plastic pollution in soil ecosystems has been somewhat overlooked. As the urgency surrounding plastic pollution intensifies, it becomes increasingly important to understand the biological interactions and subsequent effects of plastics in both aquatic and terrestrial organisms. Despite recent reports on the macroscopic effects of plastics on terrestrial fauna, the microscopic interactions of MPs with earthworms are still poorly understood due to the limitations of analytical techniques currently available to localize microplastics in organisms. In this project, adult earthworms (*Eisenia andrei*) were exposed to MPs of various shapes, sizes, nature, and degree of weathering. Various exposure and fixing techniques were compared and lethal concentrations or sublethal effects were examined. Scanning electron microscopy was used to characterize the bare MPs before or after exposure. A label-free method, *i.e*., optical photothermal infrared (O-PTIR) microscopy, was used for the localization, identification and imaging of plastic particles within organisms using histological sections of worm models. Additionally, lightsheet microscopy, a fluorescence imaging technique that allows for 3D imaging of biological samples with high resolution, was employed. This technique provides a complementary approach to O-PTIR, enabling a more comprehensive understanding of the distribution and impact of plastic particles within the organisms. The aim of this research is to deepen the understanding of the interactions between microplastics and terrestrial organisms and to demonstrate the versatility of using cutting-edge imaging techniques such as the O-PTIR for visualization of microplastics in organisms that have been potentially exposed to plastics in the environment.

Do microplastics originating from biodegradable plastic mulch adsorb more organic contaminants when they are weathered? (PL)

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Plastic mulch is commonly used in agriculture to suppress weeds and conserve water, and there as been an increase in the use of biodegradable plastic mulches. Biodegradable plastics like polylactic acid (PLA) require conditions of industrial composting to fully biodegrade, leaving microplastics in the soil. These microplastics can undergo extensive weathering under environmental conditions, resulting in significant change to their chemical and physical properties. These properties determine how microplastics interact with other environmental organic contaminants, such as pesticides, plasticizers, or polycyclic aromatic hydrocarbons (PAHs). This study explores how these changes to physiochemical properties affect their sorption to organic contaminants, in comparison to conventional plastics like polyethylene (PE). Microplastics made from PE and PLA plastic mulch films were subjected to accelerated laboratory weathering through UV-B irradiation. The microplastics were then characterized by attenuated total reflectance – Fourier Transform Infrared Spectroscopy (ATR-FTIR) for changes to surface chemistry, and differential scanning calorimetry (DSC), for changes to crystallinity. The sorption of pristine and weathered microplastics was compared to model contaminants: phthalic acid (plasticizer), 2,4-D (herbicide), atrazine (herbicide), and naphthalene (PAH). These contaminants represent common environmental contaminants with diverse hydrophobicity – a key determinant of sorption to microplastics. High-performance liquid chromatography (HPLC) was used to measure the sorption and

modelled sorption isotherms and kinetics of each contaminant. This study provides insight into the interactions between weathered microplastics present in agricultural soils and common organic contaminants.

Shape matters: exploring the impact of microplastic polymers on soil carbon cycling dynamics using stable carbon isotopes (PL)

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Microplastic (MP) pollution in soils is a burgeoning concern with significant implications for soil carbon (C) cycling. While physical alterations to soil structure associated with MP have been recognized, their chemical effects and consequent impact on C dynamics remain underexplored. This study aimed to elucidate these complexities by evaluating two common MP polymers with contrasting shapes: low density polyethylene (LDPE) beads and polyacrylic (PA) fibers. Incubation experiments were conducted using a Grey Brown Luvisol soil from Ontario, Canada, and MP additions at 0.1% and 1% w/w, alongside glass controls shaped as beads or fibers and a negative control (soil only). Natural abundance $\delta^{13}C$-isotopic analysis was used to assess the proportion of CO_2 derived from plastic versus soil C mineralization over 63 days. In addition, soil physicochemical properties (pH, dissolved organic carbon and ammonium and nitrate) were assessed at the end of the incubation period. We were able to measure a small plastic-derived CO_2 emissions, revealing that PA fibers degraded at a faster rate than LDPE beads. Traditional calculations of soil carbon priming effects (PE) using negative controls showed significantly greater negative PE in PA treatments compared to LDPE treatments, suggesting that changes to soil physical properties induced by shape are driving alterations in soil carbon mineralization. However, accounting for physical changes in soil induced by MP using glass controls to calculate PE revealed no significant difference between LDPE and PA treatments. Notably, a dose-dependent increase in PE was observed with higher MP concentrations, irrespective of polymer type. Overall, this study demonstrates a novel approach for quantifying the contribution of MP to soil C cycling dynamic and highlights that physical alterations to soil induced by MP shape drive changes in soil C emissions.

Leveraging organismal traits to predict microplastics environmental concentrations within the context of quantitative risk assessment (PL)

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A significant body of ecotoxicological data have been developed for micro- and nanoplastics (MNPs). However, there remain challenges in synthesizing and interpreting these data within the context of ecological risk assessment. While many of these challenges are being addressed, particularly those around study design, QA/QC, and reliability, one significant challenge remains - identification of relevant species for use in the development of ecological protection criteria (i.e., PNECs, HC5). Previous studies have attempted to derive these values, however significant differences in species sensitivity have been observed for micro- vs. nano-sized particles. This results in (1) significantly different PNEC values as a function of the selected particle size / size range, and (2) uncertainty as to the relative sensitivities of different aquatic species to different MNP size, shape, and polymer types. This work summarizes results of a systematic quality review of over 500 MNP biomonitoring studies using criteria previously developed by Hermsen et al., with minor modification to integrate relevance and reliability more clearly for risk

assessment & prioritization of potential future environmental monitoring programs. For studies that met the criteria, trait-based descriptors and particle characteristics and distributions were compiled for all species to provide guidance on (a) species selection and (b) environmentally relevant exposure profiles for informing future biomonitoring and risk assessment studies. The long-term objectives of this work are three-fold – first, to systematically compile and evaluate physiology and behavioral data for a wide range of freshwater and marine species into a searchable database for use in identification of sentinel species for ecosystem health & quality monitoring, and ecological risk assessment. Second, this work is intended to provide a biologically-relevant framework against which the relevance of existing (and developing) MNP reference materials may be evaluated and additional studies prioritized. This can provide a systematic basis for the inclusion or exclusion of species or materials for the purpose of quantitative risk assessment in various environmental compartments. Third, available information on ingestion and egestion rates as well as behavior and habitat can inform the selection of relevant and efficient species for integrated biomonitoring programs in sea surface, subsurface, estuarine, and sediment environments.

Navigating Microplastic-Biota Interactions: Uptake and Bioaccumulation in *Hyalella azteca* in a Recirculating Open Channel Flume (PO)

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Microplastics, which are plastic particles ranging in size from 1 to 5000 µm, pose a persistent, bio-accumulative, and toxic threat to the environment. Their ubiquitous presence in water and sediment within aquatic ecosystems worldwide makes them a growing environmental concern, particularly due to their impact on various organisms in these habitats. To date, our understanding of their impact on organisms under varying environmental conditions and hydrodynamic influences remains limited. Under various flow conditions, turbulence could affect microplastic particle transport, settling, and resuspension, which in turn could alter exposure levels and subsequent ingestion by benthic organisms within the water-sediment interface. In this study, we will test the hypothesis that turbulent flow conditions can enhance the uptake of microplastics by *Hyalella azteca* via dietary pathways. *H. azteca* will be exposed to various concentrations of 27–75 µm polyethylene microbeads and fragments. We will assess the uptake, bioaccumulation, and health impacts over time under both quiescent and turbulent conditions, using a dynamic recirculating open channel flume. Overall, our results will elucidate the complex interactions between microplastics and biota under turbulent conditions, highlighting the interplay between hydrodynamics and microplastic exposure. This study emphasizes the role of hydrodynamics in microplastic distribution and its impact on benthic organisms, underscoring the need to consider hydrodynamic factors in ecological risk assessments. Ultimately, it aims to guide the development of more effective mitigation and management strategies to address the pervasive threat of microplastics.

Streamlining plastic reclamation using microbial degradation and Raman spectroscopy (PO)

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Electronic waste (i.e., e-waste) is the fastest-growing solid waste stream in the world, increasing 3 times faster than the world's population (according to the WHO). As plastic constitutes about 20% of e-waste, it is critical to find proper recycling approaches. Current practices of recycling plastic (e.g., photodegradation and incineration) are slow, inefficient and release toxic pollutants. These methods are challenging with e-waste plastic due to the release of additives such as brominated flame retardants (BFRs). BFRs have been found to pose environmental and health risks including being potential endocrine disruptors and reproductive toxins. Recent discoveries demonstrating that bacteria can metabolize plastic have provided a sustainable alternative to physical and chemical plastic recycling strategies. Model microorganism, *Ideonella sakaiensis*, has been shown to degrade polyethylene terephthalate (PET) into monomers as more biodegrading organisms are being discovered annually to a wide range of polymer types. Physical and chemical evidence of biodegradation are critical to the integrity of experiments but high throughput and rapid testing methods have been understudied. Classification of plastics in e-waste based on the type of polymer and BFR should be reached to support the selection and optimization of the recycling approach. Analytical methods such as Raman spectroscopy allow rapid and non-destructive analysis of the types of plastics and the presence of BFR has great relevance to e-waste recycling, as well as detecting the monomers from the degraded plastic. Overall, the goal of this work is to investigate and compare the biodegradation capacity of several model biodegrading organisms for plastics and BFR in e-waste under controlled conditions and develop a rapid Raman spectroscopic method to characterize plastics and BFRs in e-waste.

The fate of microplastics in an adult freshwater mussel: from ingestion to excretion (PO)

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Plastic debris polluting waterways has been a concern for decades. Recently increased attention has been placed on microplastics (MPs) in aquatic ecosystems. Small plastic particles (<5 mm) have been observed in marine and freshwater ecosystems globally. There is a need to assess environmental risk of MPs by increasing our understanding of how these particles interact with important biota. Freshwater mussels are filter-feeding organisms that have experienced a decline due to habitat destruction and poor water quality, yet they are under-represented in MPs research. In this study, adult *Megalonaias nervosa* (washboard) mussels were exposed to polyester microfibres and polystyrene spheres in two separate 7-day tests. Mussels were exposed through spiked algal food stocks, and tissues dissections occurred every day during the 7-day exposure, as well as 2 days after exposure ceased. Digestive gland, gonad, foot, and hemolymph samples were collected at each time point to determine whether MPs tend to concentrate in a particular tissue of the mussel anatomy. To determine the efficiency of MP excretion by adult mussels, additional 7-day tests were run to capture pseudofeces and feces daily. This study serves as a preliminary assessment of the fate of microfibers and microspheres, MPs that are commonly detected in Canadian surface waters. Furthermore, this study investigates the potential of MPs to accumulate in an organism that is a critical component of freshwater ecosystems.

Impact of microplastic (polyethylene, polyester, tire particles) consumption and exposure on terrestrial isopods (PO)

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This study explores the potential toxicity of common microplastics such as polyester fibers and tire wear particles on terrestrial isopod species *Porcellio laevis, Porcellio scaber,* and *Porcellionides pruinosus*. In previous experiments, I found that these species will readily consume weathered polyethylene plastic fragments and generate microplastics with no adverse health effects measured after 28-days. Though the isopods prove formidable when ingesting polyethylene plastic, other microplastics may lead to potential hazards. Polyester fibers and tire particles can be jagged and may have the potential to damage the digestive tract or accumulate over longer duration exposures. Tire wear particles also contain compounds to ensure their durability, reduce wear, and inhibit weathering by exposure to the elements, some of which may be toxic to isopods. Experiments in this study expose isopods to microplastics through soil or spiked into their food in a gradient of concentrations that reflect clean, environmentally relevant, and worst-case scenarios. Should isopods continue to consume different forms of microplastics without any detrimental health effects, we should investigate the potential for microplastic generation by organisms within soil ecosystems, and not simply those produced through anthropogenic interactions.

Enhancing Pesticide Risk Assessment: Integrating Field Data and Modeling

Pesticides in Manitoba streams, rivers, and lakes: 2015 to 2021 (PL)

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The widespread use of pesticides in a variety of agricultural, industrial, commercial, and urban settings has been a concern for decades owing to a large body of research detailing negative environmental effects to exposures. Since 1972, the Manitoba government has carried out pesticide monitoring in provincial surface waters (i.e., streams, rivers, and lakes) to protect the environment and human health. The objective of the current study is to provide a comprehensive analysis and risk assessment of the extent and nature of pesticide contamination in Manitoba surface waters for data collected between 2015 and 2021. Over this time period, 526 water samples from 36 sites across the province were analyzed for herbicides, insecticides, and fungicides. In total, 28 out of 62 pesticides analyzed were detected. In general, pesticide concentrations in Manitoba surface waters are low; often below the detection limit (815 detections out of 16, 475 analyses conducted; detection rate of ~4.9%) and rarely exceed water quality guidelines. The most frequently detected pesticides were the most commonly used herbicides (i.e., dicamba, 2,4-D, glyphosate and its metabolite AMPA) and neonicotinoid insecticides (thiamethoxam and clothianidin) for agricultural food crop production. Specific challenges and limitations can impact our knowledge and understanding of pesticides in Manitoba surface waters. The pesticide industry is rapidly evolving and surface water monitoring programs must adapt to new pesticide use patterns, innovative application technologies, and more modern chemistries and pesticide products. Recommendations are proposed to improve the efficiency and efficacy of Manitoba's pesticide monitoring, interpretation, and reporting program.

The Sorbed, the Degraded, and the Unprocessed; Three Validated Pesticide Extraction Methods for Biomixture of a Manitoban Biobed (PL)

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The prairie region of Canada is a large contributor to the Canadian agricultural production. Most farmers rely on pesticides to manage pest populations in crop and livestock production systems which also results in frequent detections of pesticides in surface waters. Point-source pollution of pesticides via sprayer tank rinse material (rinsate) plays a significant role in pesticide leaching and run-off into the environment. Biobeds, a form of on-farm remediation system, were invented to reduce point-source pollution by first processing rinsate through a 2:1:1 volumetric mixture of wheat straw: peat: topsoil to enhance sorption and degradation of pesticides pre-disposal. Although many studies have focused on quantifying the reduction in pesticide concentrations in the water phase, fewer studies focus on quantifying pesticides retained within the biomixture. Biomixtures are complex matrices that require care to ensure valid quantification of pesticide concentrations to ensure proper management techniques can be established for biobeds. Three extraction method were developed and validated to focus on three physicochemically distinct pesticide groups (acidic herbicides, broad-spectrum method, and glyphosate-glufosinate-AMPA) utilizing ultra-high performance liquid chromatography – tandem mass spectrometry with isotope dilution. Biobed cell 1 and 2 concentrations were monitored for 2022 and 2023 pre- and post-operation, in tandem with water concentrations, to determine which pesticides were degraded, sorbed, or unprocessed by biobeds. Through analysis of this, we will be able to quantify the benefit of biobeds within Canada and help determine future research required to enhance biobed operations to support farmers.

Using algal biofilms to monitor pesticide contamination in agricultural streams (PL)

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Pesticides monitoring in surface waters generally relies on water sampling, which may not accurately represent actual exposure concentrations nor potential for pesticide bioaccumulation in aquatic organisms. Algal biofilms (periphyton), a consortium of microorganisms (algae, bacteria, fungi, and micro-meiofauna) embedded within a matrix, play a crucial ecological role in nutrient cycling and serves as food for primary consumers. Biofilms also accumulate various xenobiotics that may further be transferred to primary consumers. Thus, objectives of this research were 1) to determine if biofilms from agricultural streams in the province of Quebec, Canada, accumulated pesticides that are monitored in surface water by the provincial government, 2) to establish the relationship between pesticide concentrations in the water and in the biofilm and 3) to assess the relationship between microbial community composition (bacteria, fungi, algae, and micro-meiofauna) and pesticide concentrations accumulated in the biofilms. Biofilms were sampled in 26 streams that have been monitored by the Quebec government for several years. Pesticides were extracted from the biofilms using a modified QuEChERS method and analyzed by HPLC-MS/MS. Pesticide concentrations in the water were analysed by the Quebec ministry of the environment (Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs, MELCCFP). Biofilm community structure was

assessed by metabarcoding of the 16S (prokaryote) and 18S (eukaryote algae) rRNA genes, ITS (fungi), and CO1 (metazoa). Preliminary results showed that biofilms accumulated pesticides in several streams, particularly S-metolachlor and chlorantraniliprole, which were detected at the highest concentrations in both water and biofilms.

Pesticide presence in stream water, suspended sediment and periphyton is strongly linked to upstream catchment land use and crop type (PL)

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Pesticide pollution presents a high ecological risk to aquatic ecosystems. Small streams are particularly susceptible. There is a need for reproducible and readily available methods to identify aquatic regions at risk of pesticide contamination. There is currently a limited understanding of the relationship between upstream catchment land use and the presence of pesticides in multiple aquatic matrices. The aim of this study was to develop empirical relationships between different land uses and the levels of pesticides detected in multiple aquatic matrices. The inclusion of periphyton and suspended sediment as monitoring matrices has recently been proven effective for the characterization of pesticide exposure in stream ecosystems. Ten streams in southern Ontario with a variety of upstream catchment land uses were sampled in 2021 and 2022. Grab water, suspended sediment and periphyton were collected and analyzed from each site for the presence of approximately 500 different pesticides. Each of the three matrices exhibited distinctive pesticide exposure profiles. We found a significant relationship between the percentage of agriculture and urban land use and the detection of multiple pesticides in water, sediment and periphyton (logistic regressions, $P<0.05$). Statistically significant probabilistic models capable of predicting pesticide detections based on upstream catchment land use were developed. High-resolution cover crop maps identified soybeans, corn and other agriculture (e.g., vegetables, berries, canola) as the key variables associated with individual pesticide detection frequencies in each of the three matrices (linear regressions, $P<0.05$). Soybean land use was also the strongest predictor of site-wide pesticide pollution. This modelling approach using upstream catchment land use variables has the potential to be a powerful tool to identify streams at risk of pesticide pollution.

Bioaccumulation and Physiological Effects of a Common Pesticide Mix on Pond Snails (*Lymnaea stagnalis*) (PL)

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Modern agricultural practices heavily rely on agrochemicals which can accumulate in soil, water, and living organisms, leading to potential ecological risk. Many organisms are exposed to and accumulate chemicals through polluted water or contaminated food sources. While eco-toxicologic analyses are often conducted in the laboratory on individual chemicals, mixtures from agricultural runoffs could have complex interactive effects on organisms living in natural waterways. We studied the great pond snail (*Lymnaea stagnalis*) in semi-natural outdoor mesocosms to assess chronic (28 day) toxicity of a pesticide mix commonly observed in water surrounding corn/soybean farming of Quebec. The snails were exposed to a mixture of seven pesticides (atrazine, metolachlor, glyphosate, clothianidin, thiamethoxam,

imidacloprid, and chlorantraniliprole) at varying environmentally relevant concentrations. Snail body mass, reproduction and bioaccumulation were evaluated. The bioaccumulation of the snails was analyzed compared to the exposure concentration, water concentration, and biofilm concentration of individual chemicals. Small *Lymnaea stagnalis* had lower survival in higher concentrations of pesticides compared to larger snails. This selection resulted in an apparent increase in the contaminated population's average body mass. Snails in pesticide treatments also laid more total eggs than controls that decreased over time. Egg sacs in pesticide treatments also started off denser with eggs and then decreased rapidly to control levels. Our findings support evidence-based decision making for agronomists to transition away from pesticide usage. This research project serves as an important step towards modeling the true effects of environmentally relevant agro-chemical mixtures and their effects on non-target resistant taxa.

Using transcriptomics and early life stage bioassays to generate hazard data for pesticides in endangered fishes (PL)

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Species at risk may be disproportionally affected by environmental contaminants such as pesticides. However, due to practical and ethical considerations, hazard data are seldom available for these vulnerable species. Early life stage (ELS) toxicity testing strategies may be used to address this data gap in species that produce many offspring. For example, some fishes produce 10s to 100s of thousands of eggs, making it possible to obtain a significant number of embryos and larvae for toxicity testing without impacting the population. Here we combine transcriptomics and ELS exposures to assess sensitivity to pesticides in two listed fishes; copper redhorse (*Moxostoma hubbsi*, endangered), and river redhorse (*Moxostoma carinatum*, special concern). Fertilized embryos were obtained from a rehabilitation program run by the provincial government (MELCCFFP) and incubated in clean water until hatching. Within 24 hours of hatch, larvae were loaded into 24-well plates (one individual per well) and exposed to solvent control or 11 different concentrations of five different current use pesticides. The dose ranges spanned 5 – 7 orders of magnitude and were designed to capture concentrations that were predicted to cause mortality at the high end, and more subtle transcriptomic effects at the low end. After 24 hours of exposure, larvae were flash-frozen on dry ice and preserved for transcriptomic analysis via RNASequencing. A preliminary analysis found that the highest tested concentrations of atrazine (30 mg/L), thiamethoxam (1000 mg/L), and clothianidin (100 mg/L), and the second highest concentrations of glyphosate (100 mg/L) and metolachlor (30 mg/L) had no impact on larval survival in either species. Ten-fold higher concentrations of glyphosate and metolachlor were associated 100% mortality. These pesticides are all detected at ng/L concentrations in the Richelieu River, the only known spawning site of the copper redhorse. Ongoing analysis will investigate the transcriptomic response to each pesticide at environmentally relevant concentrations and evaluate the effectiveness of toxicity testing in a 24-hour ELS assay.

From risk assessment to laboratory testing, a sediment toxicity study in the Northwest Territories (PO)

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The Tuktoyaktuk Harbour is an active marine transportation site located in the Northwest Territories (NT). Two risk assessments (RAs) were carried out for a site within the Tuktoyaktuk Harbour and identified potential risk for ecological receptors. The Ras concluded that the pesticide dichlorodiphenyldichloroethane (DDD) in sediment could cause adverse effects to the populations of benthic invertebrates, while arsenic, copper, lead, petroleum hydrocarbons fractions one to three (PHC F1/F2/F3), dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyltrichloroethane (DDT) were less likely to cause widespread adverse biological effects. To further investigate the potential sediment toxicity, additional sediment samples were collected during the 2022 field season at five locations historically characterized by elevated concentrations of DDD, DDE, and DDT, and a sediment sample from a reference area located approximately 2 km northeast of the site. Sediment samples were analyzed for pesticides, metals and PHCs. Based on the results of historical and current chemical analyses, four sediment samples (including one reference sample) were selected to assess sediment toxicity using two standardized biological test methods: 1) amphipod survival, and 2) echinoderm larval survival, and normal development. The results indicated that two sediment samples may reduce the survival and development of sensitive benthic invertebrate species, such as amphipods and echinoderms. DDD, DDT and possibly PHCs and copper may have contributed to the observed effects. Study limitations do not allow to draw definitive conclusions with respect to the toxicity that site sediment may pose to benthic invertebrates. These limitations and challenges will be discussed.

Method Development and Application - Novel Approaches and Applying Environmental Relevance to Standardized Aquatic Toxicity Testing

The application of rainbow trout acute lethality test variants for effluent discharge compliance (PL)

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Industrial discharge of effluent to the receiving environment is governed by both federal and provincial regulations, stemming from the Fisheries Act. The Rainbow Trout Acute Lethality test method, was first published by the Environment Canada in 1990 (EP1 1/RM/13) as a standardized reference test method that could be employed to assess the acute toxicity of effluents, and replace previous generic guidelines dating back to the 1970's. Dischargers must meet the stipulated "pass" criteria outlined in their permit. Typically, a test is found to have failed if the mortality in the undiluted effluent sample is >50%. Within the methods cited for regulatory use (EPS 1/RM/13 and EPS 1/RM/50), dischargers may opt to run a single or multi-concentration test. In cases where effluents have a long history of meeting compliance goals, a multi-concentration test provides no additional information, yet is more expensive, and requires 3 times the number of juvenile fish than its counterpart. Despite this, one reason dischargers may opt to run the multi-concentration test is due to the language used in their permit, which infers they must obtain an LC50 result, and this precludes the use of the single concentration

approach. Other reasons for employing the multi concentration test option may stem from an overabundance of caution or simply a lack of familiarity with the method and its outcomes. Increasingly, there is international pressure on industry to decrease their use of vertebrates in testing. Mandated compliance testing with larval or juvenile fish remains a significant component of all animal testing conducted in North America. In December 2023, further revisions were made by ECCC to the reference method EPS 1/RM/13, with the intent to reduce the number of fish used in these tests. This presentation is geared towards providing data specific to Acute Lethality test outcomes for point source discharges, estimating the potential reduction in juvenile fish numbers required for compliance testing in Canada, plus recommendations for how permit language could be revised to better align with existing federal regulatory and test method language.

Reducing fish use: Impacts of rainbow trout control sharing in commercial laboratories in Canada (PL)

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Fish acute lethality tests are frequently used to assess wastewater effluent toxicity for compliance with federal regulations. To address the need to reduce vertebrate animal testing, the Method Development and Applications Unit (MDAU) of Environment and Climate Change Canada (ECCC) has recently amended the rainbow trout acute lethality reference method (RM/13) to permit control sharing, under certain conditions. The method originally required each effluent test to include one control per test, such that for each effluent tested in a lab there would be an equivalent treatment exposing fish to control/dilution water. In this scenario, if a lab tested 5 different effluents in one day, they would set up 5 unique controls. Control sharing permits a lab to set up multiple effluent tests in one day with one shared control, therefore reducing the number of fish used per day. In order to determine the impact that control sharing has on reducing fish use in practice, we surveyed commercial laboratories across Canada that are accredited for the rainbow trout acute lethality method and that have implemented control sharing. We collected data on fish use during control sharing from several laboratories and estimated what the fish use would have been in the absence of control sharing for RM/13 single-concentration and multi-concentration tests. The results of this survey will provide insight into the actual reduction in vertebrate testing as a result of the amendment to the ECCC method, and further signal ECCC's commitment to reducing vertebrate testing.

The use of the EcoToxChip test system for regulatory decision-making: A case study with a 24-hour transcriptomic rainbow trout assay (PL)

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Global efforts are underway to reduce vertebrate toxicity testing and integrate new approach methods (NAMs) into regulatory chemical risk assessment. In Canada, recent amendments to the *Canadian Environmental Protection Act, 1999* recognize the need to replace, reduce or refine the use of vertebrate animal testing when prioritizing and assessing the potential harm that substances may pose to human health and the environment. This presentation will highlight a case study from a partnership between academic researchers and government regulatory end-users that aims to overcome barriers to the

integration of NAMs into regulatory risk assessment activities. The objective was to establish a high-throughput toxicity test method for larval fish that yields data comparable to both acute (i.e., 96-hr LC50s) and chronic (i.e., long-term) fish toxicity tests. Twenty-five chemicals with diverse physical and chemical properties were selected. Rainbow trout alevins (1-2 day post-hatch) were placed in individual wells (24-well plates) and exposed for 24 hr to 12 different concentrations of the test chemicals. Results indicate good concordance between acute toxicity data generated from the case study and the literature. The repeatability of the results was assessed using copper sulfate and yielded consistent LC50 values. For the chronic endpoint, a transcriptomic point of departure (tPOD) was derived for five compounds and appears to be lower than or similar to values associated with chronic toxicity tests. The findings thus far are promising in terms of establishing a NAM, although work continues to yield more tPOD data, test more compounds, and deepen studies into exposure measures.

Replacing *in vivo* acute wastewater toxicity testing with the RTgill-W1 cell line (PL)

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Recently, the Canadian Environmental Protection Act was amended to phase out whole animal toxicity testing by 2035. Currently, whole animal toxicity tests are used to provide critical information to risk assessors, monitoring programs, and regulatory bodies. The acute fish lethality is one of the most prevalent whole animal tests and uses millions of fish each year to assess industrial and municipal wastewater samples. Despite the efforts to replace, reduce, and refine animal use in science, there is no standardized, validated, and regulatory acceptable alternative to this acute lethality test in Canada. Our research examines an alternative approach, based on the OECD 249 and ISO 21115 methods, that uses cell viability indicators in a rainbow trout gill cell line (RTgill-W1) to assess the acute toxicity of wastewater samples. We demonstrate how complex wastewater samples may influence the sensitivity of the *in vitro* method through matrix interactions, and present ways to test if these interactions influence sample toxicity. Wastewater samples tested with both the *in vitro* and *in vivo* techniques will be compared to demonstrate the accuracy of the RTgill-W1 assay on a range of variable, real-world wastewater samples. This is an important step in providing data to support the use of the RTgill-W1 assay to replace whole animal testing for wastewater toxicity under the Canadian Environmental Protection Act.

Development of a mummichog (*Fundulus heteroclitus*) Early-Life Stage Bioassay as an alternative to adult fish field studies in Environmental Effects Monitoring for Pulp and Paper mills in Canada (PL)

Andrea Lister[1], Deborah MacLatchy[1], Tim Vickers[2], Mary Murdoch[2]
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A mummichog (*Fundulus heteroclitus*) early-life stage bioassay (ELSB) has been developed to address constraints to environmental effects monitoring (EEM) field studies for fish for several pulp & paper mills in eastern Canada. The mummichog ELSB can be used to assess the EEM endpoints of growth and survival and matches the fish species used in existing adult reproductive tests for which research conducted over past EEM cycles offers some guidance on performing mummichog adult reproductive tests. The ELSB is a reproducible test with criteria established for sample size requirements, performance, and test validity, as well as transferrable and standardized conditions, and is developed on

a relevant species that is endemic to estuarine receiving environments in Atlantic Canada. A technical paper describing the protocols established for the ELSB is being developed.

The effects of neurochemical manipulation on the behaviour of *Capitella teleta* (PL)

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Chemical contaminants are being increasingly found in marine environments, potentially posing risks to species within these ecosystems. These chemicals can act as neurochemical modulators, altering the behaviour of organisms. The development of an efficient, low-cost assay is needed to quantify neurochemical disruption to identify potential impacts these contaminants are causing. Invertebrates represent excellent candidates for testing, as they contain conserved molecular machinery and hold important ecological positions. *Capitella teleta* are marine annelid worms that live in the sediment of estuarian environments. Past work on *Capitella* has shown they exhibit conserved locomotory behaviours, which can be manipulated by exogenous chemicals. The objective of this study was to create a high-throughput assay for contaminants with *Capitella*. We modified an existing locomotory assessment for use in a 6-well plate, testing locomotory behaviours in different life-stages. Juvenile (2-weeks), and adult (>8-weeks) worms were exposed to nicotine; juveniles were exposed to fluoxetine , apomorphine, or phenobarbital. The endpoints assessed were distance moved (mm), velocity (mm/s), and time spent at edge (s). *Capitella teleta* were responsive to serotonergic, dopaminergic, cholinergic, and gabaminergic manipulation, implying that neuromodulatory contaminants may alter behaviour of this critical marine worm. Differences were observed in juvenile and adult *Capitella* behaviour within the 6-well plate, implying the assay can be used for investigations across life stages. This study improves marine toxicology testing by creating a high-throughput assay to measure the impact of chemical contaminants on invertebrates.

Development of a behaviour assay in the freshwater snail, *Planorbella pilsbryi*: assessing the effects of selective serotonin reuptake inhibitors (PL)

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Pharmaceutical and personal care products (PPCPs) are ubiquitously detected in surface waters. Selective serotonin reuptake inhibitors (SSRIs) are currently the most prescribed psychotropic medications, and their increased use and production globally has become a growing concern for aquatic toxicology and environmental biology, as increasing evidence suggests that several aquatic organisms are affected by chronic exposure to these contaminants. Previous studies have shown that SSRIs increase activity and movement in freshwater mussels, and induce foot detachment in freshwater gastropods, and that they may have more profound effects on populations than is currently understood, or that standardized toxicity tests can detect. The objective of this study was to assess the chronic effects of a mixture of four SSRIs in the ramshorn snail *Planorbella pilsbryi* (Family Planorbidae), using apical endpoints (survival, growth) and through the development of a behaviour assay. Exposure concentrations included an aqueous mixture corresponding to the LC25 for each chemical, diluted by a

factor of two (LC25 – 0.03·LC25), a mixture corresponding to environmental concentrations in southern Ontario, and a water control. Juvenile snails were exposed to the SSRI mixture for 28 days, and their behaviour was assessed weekly using timelapse videography, with and without feeding. Endpoints considered include distance travelled, average speed, time to first movement, and righting time (when applicable). Preliminary data analysis suggests chronic exposure to SSRIs affected snail growth and locomotory behaviour. The results of this research will help identify whether chronic exposure to SSRIs triggers behaviours detrimental to the survival of freshwater gastropod populations.

The need for alternatives to field-based studies in Environmental Effects Monitoring for Pulp and Paper mills in Canada (PL)

Tim Vickers[1], Mary Murdoch[1]
[1]*Stantec Consulting Ltd.*

Pulp and Paper mills in Canada must undertake aquatic environmental effects monitoring (EEM) programs to evaluate the potential effects of their effluents on fish, fish habitat, and the use of fisheries resources. The Pulp and Paper Effluent Regulations (PPER) under the *Fisheries Act* provides a tiered approach to EEM programs that begins with an assessment of potential mill-related effects on the receiving environment and, if mill-related effects are confirmed, a determination the magnitude and extent of the effects, followed by the identification of effluent-related causes and potential solutions. Environment and Climate Change Canada has indicated that the scope and compliance requirements of the PPER may be expanded and could include increased requirements to implement solutions to mill-related effects identified in EEM programs. The financial and operational implications of such requirements on Pulp and Paper mills underscores the importance of definitively confirming that observed effects are mill-related, and not the results of confounding influences in complex receiving environments. Laboratory-based studies such as bioassays and indoor mesocosms could provide a more robust assessment of mill-specific effluent-related effects on fish and benthic invertebrate communities as compared with field-based studies. Establishing scientifically defensible laboratory-based alternatives to field studies could elucidate mill-specific effects on fish and fish habitat and mitigate unnecessary upgrades to process water infrastructure in Pulp and Paper mills.

Sunscreens are better together: understanding the differences in whole sunscreen mixture toxicity in *Daphnia magna* compared to isolated UVF toxicity (PL)

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Ecotoxicological research has played a critical role providing insight to inform policy & regulation for the environment. This has made standardized methodologies commonplace to provide enhanced replication & consistency in results. Although these standard methods are important, a consequence of this increased control is decreased relevancy of a given toxicant to dynamic natural environments. One such example is studies involving ultraviolet filters (UVFs), as typical UVF research focuses on the isolated UVFs rather than the sunscreen mixtures which introduce UVFs into the environment. To address this, 5 commercial sunscreens (SS1 – SS5) were acquired & tested on *Daphnia magna* over the course of a chronic exposure (21 days) at low (0.00005% v/v), medium (0.0001% v/v), and high (0.0005% v/v) concentrations. Changes in survivorship between control & 0.0005% sunscreen mixture concentrations were observed for SS3, SS4 & SS5, with 50%, 100%, and 90% mortality compared to 0% in the controls.

The mortality observed upon completion of the chronic exposure differed from the expected mortality from the isolated UVF literature. Specifically, 50% mortality was observed for isolated octocrylene at 5.35 µg/L, but octocrylene at concentrations within the medium sunscreen mixtures ranging between 12.4 – 23.7 µg/L only resulted in ≤20% mortality. Differences in mortality were also observed for oxybenzone (isolated: 50% mortality at 53.5 µg/L; mixture: ≤20% mortality at 40.3 – 62.9 µg/L). These results indicate that we may not be getting the whole picture when we remove toxicants from the context of the exposure occurring within the environment.

Effects of repeated pulsed exposures of road salt on early life-stage coho salmon (PL)

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The use of road salt (NaCl) is increasing across Canada, with an average of 5,500 tonnes applied in Vancouver alone every year. Road salt enters nearby freshwater streams through stormwater runoff, leading to salinization of these systems. These salt inputs are highly seasonal, with the highest frequency and magnitude of road salt contamination typically occurring at the end of December/early January, coinciding with spawning and early development of local Pacific salmon species, including coho salmon. In order to understand the effects of road salt contamination on early life-stage coho, we conducted modified bioassays designed to mimic the exposure regimes that these fish face in nature. Based on data from a network of conductivity loggers throughout Vancouver's Lower Mainland, we have confirmed that acute inputs of road salt result in brief (~24-h) peaks in stream chloride concentrations, as high as 10x the provincial acute water quality guideline. Although acute salt exposures are known to be toxic to juvenile salmonids, the consequences of repeated pulsed exposures are not well understood. In this study we exposed coho salmon (Oncorhynchus kisutch) to up to three repeated 24-h salt pulses at an environmentally relevant concentration at either <1-hr post-fertilization or 50% hatch. When exposed to acute road salt pulses starting just after fertilization, we saw increasing mortality with multiple pulses, and increased deformity across all road salt exposed groups. No significant increase in mortality was observed in alevins exposed at 50% hatch. In all treatment conditions, mortality was significantly influenced by family.

Photomodification Of Low-sulfur-fuel-oils: Investigations of Toxic Effects (POLITE) (PL)

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The 2010 Deepwater Horizon oil spill changed our understanding of the significance of photomodification as a fate pathway following an oil spill. It revealed a considerable portion of non-volatile, non-soluble surface oil was rapidly oxidized by sunlight, resulting in the formation of complex mixtures of tens of thousands of water-soluble oxygenated compounds. The toxicity of these photo-products is largely unknown, and their propensity to form varies with oil type, however for new fuels like very low-sulfur fuel oils (VLSFO) it is not known whether they will behave in the same manner as traditional crude and fuel oils. The POLITE project sought to improve the understanding of the photomodification potential of VLSFOs and their relative toxicity to species that are important to aboriginal, commercial and recreational fisheries. We determined the toxicity of 13 VLSFOs both with and without photomodification to a variety of early-life stages of marine organisms including, American lobster, Atlantic cod, Green-sea urchin, Common periwinkle, and Red abalone. Bioassays were conducted using low energy water accommodated fractions (WAFs) that were prepared and mixed either in the

dark or under full spectrum UV light exposures for an 18-hr period using an Atlas Solar Constant lamp. Exposure media were characterized using a variety of analytical techniques to characterize the formation of photoproducts. The results highlight a wide range of responses across fuel types, significant differences in sensitivity across species, and the importance of addressing and incorporating modifying factors such as UV light when determining the toxicity of complex mixtures.

Testing the applicability of standardized laboratory tests to natural environments by investigating UV filter toxicity to *Daphnia* (PL)

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To accommodate the growing need for toxicology research due to the continued anthropogenic change of natural environments, standardized test guidelines have been developed that use simplified models to simulate complex real-world environments. These methods require that test organisms are reared in stable laboratory conditions to control life history traits in order to minimize the effects of biological variation across research groups. By using these highly specific tests, critical assumptions are made that exposing organisms raised in stable laboratory environments for a set duration of time produces results that are representative of real-world outcomes. These assumptions were tested by exposing *Daphnia* to organic ultraviolet filters (UVFs), emerging contaminants of concern that have been demonstrated to cause toxicity at environmental concentrations. *D. magna* exposed to UVFs experienced 60% mortality and 40% decreased reproduction over the standard first generation of exposure but were capable of gradual acclimation to continuous exposure across subsequent generations until no impairment was evident by the 4th generation. Additional studies comparing the responses of the laboratory lineage of *D. pulex* to a wild population revealed that each population differs in sensitivity to UVFs on a case-by case basis, as each tested chemical severely impacted one population type but not the other. This research identifies several challenges in applying standardized research to real-world environments and highlights the necessity to understand how the methods used to conduct biological research impact the conclusions drawn from resulting data.

C-sniff: Cultured *Rana [Lithobates] catesbeiana* tadpole olfactory epithelium is a sensitive system for detecting thyroid hormone induction and disruption (PO)

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The amphibian olfactory system is highly distinct between aquatic tadpole and terrestrial frog life stages and therefore must remodel extensively during thyroid hormone (TH)-dependent metamorphosis. Furthermore, developmentally appropriate functioning of the olfactory epithelium is critical for survival. While endogenous THs initiate metamorphosis in wild premetamorphic tadpoles, THs are also a common wastewater contaminant. Previous studies in *Rana [Lithobates] catesbeiana tadpoles* showed that the olfactory epithelium is one of the most TH-responsive tadpole tissues investigated to date, positioning it as a useful bioindicator tissue for TH-disruption. Herein, a system for culturing tadpole olfactory epithelium *ex vivo* was developed and validated. Olfactory sacs were excised from *R. catesbeiana* premetamorphic tadpoles and cut in half to yield four tissue fragments which were exposed to 10 nM 3,5,3'-triiodothyronine (T_3) or solvent control for 24 hours at 24 °C in culture. RT-qPCR analysis showed that the cultured tissue fragments were enriched in olfactory tissue marker *omp* and

demonstrated high TH-responsiveness as measured by the induction of classic TH bioindicator transcripts *thra*, *thrb*, and *thibz*. TH response was equivalent among each of the four tissue fragments. Additionally, the olfactory epithelium transcriptomes of tadpoles exposed to TH at 5°C and 24°C were analyzed by RNA-seq, revealing TH-induced postembryonic processes that may be targets for disruption by environmental contaminants. The highly TH-sensitive C-sniff system is a promising tool for the screening and detection of TH-disrupting substances, allowing for the easy manipulation of variables like temperature, duration of exposure, and chemical dosing, while reducing animal usage.

Protect Your Metal and the Environment: Assessing the Ecotoxicity of an N-Heterocyclic Carbene Species in Brown Flatworms (PO)

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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.

An improved method to assess growth rates of freshwater diatom *Navicula pelliculosa* for mayfly toxicity testing (PO)

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The Ephemeroptera, Plecoptera and Trichoptera taxa, or "EPT" are widely used as indicators of aquatic ecosystem health due to their sensitivity to waterborne toxicants and environmental change, but are typically excluded from standardized testing as they represent a challenge to culture in the laboratory. It is critical to develop standardized methods that are representative of the most sensitive species present in aquatic ecosystems in order to accurately quantify risk and develop protective guidelines. Recently, research has begun exploring the potential of using the triangle small minnow mayfly *Neocloeon*

triangulifer as a candidate species for laboratory-based toxicity testing. While progress has been made to develop toxicity test protocols with *N. triangulifer*, toxicological endpoints such as body weight, growth and survival have relatively high intra-treatment variability, which inhibit the ability to elucidate treatment effects. A methodological gap that has been identified as a potential source of variability is culturing of their preferred food, diatoms from the genus *Navicula*. Nutritional composition and age of the *Navicula* culture may influence the sensitivity of *N. triangulifer* to contaminates. The objective of this study was to develop a method to characterize growth curve profiles for *Navicula* using live cell counts. It is hypothesized that the nutritional composition (protein, fatty acid and total caloric content) of the *Navicula* diatom culture changes at different growth stages (lag, exponential, stationary, and death phase). *N. triangulifer* will be fed a diet of *Navicula* from each of these different growth phases. Developmental endpoints including head capsule width, body length and weight will be assessed to determine the optimal diet for these insects. While growth stages of *Navicula* diatoms are currently unknown, we hypothesize that feeding *N. triangulifer* diatoms exclusively from the exponential growth phase will optimize mayfly health.

Using Transcriptomic Point-of-Departures to Compare Sensitivity Across Fish Species (PO)

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Chemical regulatory assessors require hazard data from multiple species spanning a range of sensitivities as the foundation for developing environmental quality guidelines. Due to lengthy exposures, high costs, and ethical concerns, chronic chemical hazard data for diverse species are currently lacking. New Approach Methods (NAMs) have been touted as potential alternatives that could provide toxicity data quickly and more ethically. Our group is currently developing a NAM that uses early life stage fish exposures to obtain transcriptomic points-of-departure (tPODs). Previous studies support the possibility of substituting chronic apical PODs (aPOD) with tPODs generated from short-term exposures given their high degree of correlation. The overall objective of our project is to validate tPODs as suitable toxicity data for the derivation of environmental quality guidelines. As a first step, the aim of this study is to assess the reliability of tPODs to predict sensitivity differences across fish species. The oxidative product of 6PPD, 6PPD-Quinone (6PPD-Q), was chosen for this study because it is known to cause variable toxicity in different species of fish with a larger impact on salmonids. Correspondingly, a range of fish species such as salmonids, listed native species and model lab species were included in our study. Eleutheroembryos (0 to 24 hours post-hatch) were exposed for 24 hours to 6PPD-Q of 12 different concentrations (10-8 to 10 mg/L). The concentration range spanned across 9 orders of magnitude to cover the transcriptomic response and to potentially include the apical response of lethality. RNA was isolated from pooled eleutheroembryos, followed by RNA sequencing. Further processing was then done to generate tPODs using ExpressAnalyst. Our current results show that a difference of sensitivity of at least 3 orders of magnitude was observed among species. Similarly, a difference of 3 orders of magnitude was observed between acute LC50 and tPODs for at least one species. More exposures are currently underway to obtain acute LC50 and tPODs data, which will be presented and discussed. Future work will focus on comparing tPODs across fish species relative to chronic apical endpoints, paving the way for the potential use of tPODs in guideline development for chemicals of concern.

Establishing rainbow trout (*Oncorhynchus mykiss*) as a neurotoxicity model for screening environmental contaminants: From behavioural outcomes to molecular mechanisms (PO)

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With the increased occurrence of neurotoxic chemicals in freshwater ecosystems, it is vital to assess the effects of these compounds on resident wildlife, in particular fish. However, traditional toxicity endpoints and current regulatory testing frameworks often exclude techniques to measure neurotoxicity, focusing primarily on lethality and general physiological damage. Neurotoxic compounds can induce subtle changes in behaviour, which can be indicative of a more serious health effect. Given that neurotoxicants can have a long-lasting impact on non-target organisms, it is important to determine the influences of these chemicals. This study aims to fill this knowledge gap by using an ecologically relevant fish species in North America, rainbow trout (*Oncorhynchus mykiss)*, to evaluate the neurotoxic effects of emerging aquatic contaminants through a combination of behavioural assays and molecular techniques. The findings from this study will be compared with data obtained from zebrafish (*Danio rerio*), a well-established model species in neurotoxicity research. Behavioural assays measuring locomotion, chemosensory reactions, novel environment responses and social interactions will provide a direct measure of neurotoxic impacts. To complement behavioural data, molecular techniques such as RNA-seq and utilization of a focused transcriptome qPCR array (EcoToxChips), will be applied to analyze changes in gene expression levels. Additionally, specific biochemical markers will be quantified, along with the application of neuronal imaging techniques, to identify molecular and cellular changes underlying the behavioural responses. This research will provide a comprehensive screening tool for measuring neurotoxicity using rainbow trout, which can be applied to future environmental monitoring and risk assessment of chemicals.

Ceriodaphnia dubia inter-laboratory results with naphthenic acid mixture (PO)

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The *Ceriodaphnia dubia* standardized toxicity test (RM/21) is used in federal regulations, and has been successfully performed in consulting laboratories across Canada for over 30 years. When conducted as part of a test suite, *C. dubia* is often one of the more sensitive species. We launched an inter-laboratory comparison to: (i) characterize current lab-to-lab variability in toxicity endpoints; and (ii) provide toxicity information for a group of chemicals of regulatory interest. Laboratories were provided with a mixture of commercially available naphthenic acids in an oil (Merichem). Technicians prepared a stock solution, performed pH adjustments as necessary, conducted a range-finder, and conducted a definitive test. Tests were performed according to prescriptive conditions outlined in the test method, but each laboratory followed their typical laboratory practices in line with the flexibility offered by the test method. For example, each laboratory followed their in-house culturing practices and used their own control/dilution water (flexible processes), but each laboratory performed the actual exposure under stringent conditions and QA/QC limits (prescriptive processes). All participating laboratories are accredited to the international quality standard ISO/IEC 17025. The mortality and reproduction endpoints were within the expected range of agreement among the five labs, with coefficients of variation of 35% for the LC50 endpoint and 40% for the IC25 endpoint. A review of the QA/QC data submitted by the laboratories

showed that *Ceriodaphnia dubia* is performing as expected in all laboratories. The results can be used to further evaluate the toxicity characteristics of Merichem (e.g., comparison with other species).

Does sociality matter? Assessing the influence of social context on physiology and toxicology (PO)

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The social context of a species influences its way of life, impacting factors from predation to metabolism. The presence of conspecifics is known to moderate physiological responses of individuals which can affect toxicological outcomes; however, in studies of this nature, sociality is seldom considered. To broaden our understanding of the importance of sociality we performed social manipulation experiments on a social marine species, threespined stickleback (*Gasterosteus aculeatus*). We investigated whether changing the social context altered physiological traits of individuals and affected sublethal and lethal toxicity of Cu, an anthropogenically relevant contaminant. It was hypothesized that fish in isolation would experience social stress potentially leading to an elevated metabolic demand and ventilation frequency, allowing more Cu to encounter and accumulate in the gills. This would result in a greater Cu burden, impairment of traits susceptible to Cu, and higher mortality. Isolated sticklebacks demonstrated reduced foraging and locomotion, with the latency to food consumption 130% longer and distance travelled 109% less than grouped stickleback. Stickleback gill, intestine, and liver Cu burdens were 144%, 114% and 90% higher on average within the isolated stickleback compared to stickleback tested in groups of two or six. Changes to the social context did not lead to differences in upper thermal tolerance (critical thermal maximum), nor Cu's 96-hour acute median lethal concentration, with LC50 values of 560 [454, 639] µg/L, 596 [491, 751] µg/L, and 537 [453, 605] µg/L calculated for isolated, paired, and grouped fish, respectively. These findings highlight social context as a biotic factor that alters physiological and toxicological outcomes.

Investigating the mechanism of intragenerational oocyte maturation inhibition in Japanese medaka exposed to (TBCO) via maternal transfer (PO)

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The brominated flame retardant, 1,2,5,6-tetrabromocyclooctane (TBCO), is an endocrine-disrupting chemical that impairs the reproductive performance of fish. Previous studies demonstrated decreased fecundity of female Japanese medaka (*Oryzias latipes*) exposed as embryos to maternally transferred TBCO. Results to date suggest that early life-stage exposure to TBCO decreased fecundity by impairment of maturation-inducing hormone (MIH)-induced oocyte maturation, which is the final stage of oogenesis. The objective of the present study was to investigate the molecular basis of decreased oocyte maturation leading to decreased fecundity in Japanese medaka exposed as embryos to maternally deposited TBCO. Fecundity of F1 generation Japanese medaka reared in fresh water from F0 generation fish given a diet of 100 or 1000 ugTBCO/g food was decreased by 11.3% and 31.4%% relative to the control. Using an ex vivo assay, maturation of stage IX oocytes to stage X oocytes from the F1 generation females was decreased by 16.1% and 22.3% relative to the control. To elucidate mechanisms of decreased oocyte maturation, high-throughput RNA sequencing is being used to compare the transcriptome of stage IX oocytes that fail to mature and stage X oocytes that mature from control and

TBCO-exposed female fish. In addition, whole genome bisulfide sequencing is being used to determine if changes in DNA methylation are altering expression of genes that regulate oocyte maturation. The goal is to identify genes, and pathways, that are dysregulated by embryonic exposure to TBCO thereby leading to oocyte maturation inhibition.

Chronic toxicity and bioaccumulation of trace elements in daphnids exposed to water and sediment from an oil-sands tailings pit lake (PO)

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Lake Miwasin is a constructed pilot-scale oil sands pit lake containing treated tailings, generated by the extraction of oil from Alberta's bituminous sands. It is expected that such lakes will help to ameliorate any toxic effects of tailings as a part of their return to the surrounding landscape. The long-term goal of this research is to understand how the hydrological and geochemical processes that occur over time in a pit lake setting will affect the bioavailability, bioaccumulation and toxicity of the trace elements in treated tailings and overlaying waters. The objective of these early studies was to determine toxicity of Lake Miwasin water and sediment to the model freshwater invertebrate species *Daphnia magna* in year 1 and 2 following lake construction, and to analyze trace metal body burdens for trace elements that may contribute to the observed effects. Acute 48 h toxicity tests were conducted with mortality as the endpoint, and chronic 21 d toxicity tests were performed using reproduction and growth as endpoints. No mortality was observed over 48 h; however, final body masses were significantly larger in daphnids exposed to Lake Miwasin water. Daphnid reproduction was also affected, with reduced total neonate production observed in both Lake Miwasin water and water/sediment groups relative to controls. Exposure to Lake Miwasin water and water/sediment increased trace metal (i.e., Mo, Ni, Al, Co) burdens in daphnids. To mimic the effects of riparian runoff into the lake that will occur as this constructed ecosystem evolves, additions of commercially available sources of dissolved organic matter (DOM) were made to exposure waters. The inclusion of DOM ameliorated the effect of Lake Miwasin water on reproduction in chronic exposures. Developing a better understanding of the evolving toxicity associated with trace elements in Lake Miwasin is important for assessing the safety of future pit lakes and water releases.

Environmental DNA (eDNA): Research and Applications to Assess Biodiversity and Supporting Aquatic Ecosystem Health Management

A comparison of environmental DNA metabarcoding and five conventional methods (PL)

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Currently a wide variety of fish sampling methods exist and are commonly used, yet it is unclear how these methods compare due to a lack of statistically rigorous comparisons. To address this eDNA data was collected alongside environmental consultants collecting conventional fish assessment data. 12S metabarcoding, COI metabarcoding, electrofishing, hoop netting, seine netting, minnow trapping, and gill netting samples were taken at 10 sites across four waterbodies from June 8th to June 13th, 2023. Typically, during sampling method comparisons, diversity or species richness is used as the metric. However, this strategy has significant limitations which commonly result in the misinterpretation of

results. In order to rigorously statistically compare the methods, four metrics were used: congruency, consistency, accuracy and diversity. The study outlines a novel statistical framework for conducting thorough methodological comparisons by utilizing the four metrics. By conducting a thorough statistical analysis, a variety of novel insights are highlighted; however, three 3 main findings were discovered: 1) COI outperformed 12S on 3 out of the 4 metrics 2) seine netting is largely redundant in the presence of COI, and 3) eDNA captures a more complete picture of biodiversity and is best compared to a combination of conventional methods.

Effects of assay selection, sequencing depth, and PCR stochasticity on detected Grand River amphibian communities using eDNA metabarcoding (PL)

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Predicted future human population growth will disproportionately affect taxa such as amphibians, which require connected, high-quality terrestrial and aquatic habitats to complete their lifecycles, often by breeding in vernal pools. Environmental DNA (DNA) can facilitate species conservation through detection of species-specific DNA barcodes in the genetic material shed by organisms into their surroundings. Using high-throughput sequencing, metabarcoding can characterize eDNA shed from multiple local amphibian species. The objective of this research was to evaluate the optimal eDNA metabarcoding assay, sequencing depth, and number of PCR replicates to characterize amphibian communities present in vernal pools within the Grand River watershed. eDNA was extracted from six 1 L water samples gathered from four local vernal pools. After *in silico* evaluation, one published and two *de novo* assays were optimized *in vitro* before amplifying each eDNA extract with twin-tagged primers in 12 technical PCR replicates. PCR replicates were purified, quantified, pooled, prepared into libraries and sequenced on an Illumina MiSeq flowcell. While all assays detected amphibian eDNA, assay choice influenced species richness and community composition. Species accumulation curves emphasized the importance of high PCR replication and although most amphibian detections occurred before 6000 reads per replicate, select PCR replicates required much higher read depth to fully characterize the species present. These results suggest that despite careful *in silico* evaluation and *in vitro* optimization, considerable PCR stochasticity is present in each of the selected amphibian metabarcoding assays, which should be explicitly considered when implementing amphibian eDNA monitoring programs. [Funded by CFREF-Global Water Futures]

Optimizing eDNA protocols for a fish biodiversity assessment in a cold freshwater habitat (PL)

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Robust baseline assessments at industrial sites are critical for mitigating ecological impacts and supporting sustainable practices. By establishing comprehensive baseline data, eDNA metabarcoding can enhance our understanding of how environmental impacts affect population dynamics over time. Environmental DNA (eDNA) metabarcoding has emerged as a transformative tool in biodiversity monitoring, enabling non-invasive, sensitive assessments of ecosystem health. We aimed to optimize

eDNA metabarcoding protocols for evaluating fish biodiversity in a cold (~6°C) freshwater habitat at a mine site in Northern Ontario. We focused on several key areas: a high-capacity filtration system capable of processing large water volumes in triplicate, the efficiency of two eDNA extraction protocols, and the performance of three bioinformatic approaches for sequence identification. Evaluating five volumes of filtered water (1.00, 6.25, 12.50, 18.75, 25.00 L), we demonstrated that larger filtration volumes significantly enhance detection sensitivity; however, the Preserve Precipitate Lyse and Precipitate (PPLPP) method required lower volumes to achieve the same, if not superior, results compared to higher volumes extracted using the Qiagen DNeasy Blood and Tissue kit. Additionally, our bioinformatic analyses indicated that a beta MiFish pipeline had superior accuracy and reliability compared to consensus BLAST or VSearch. Compared to traditional biomonitoring techniques (electrofishing and minnow trapping), eDNA metabarcoding identified a species of minnow, the Lake Chub (*Couesius plumbeus*), that would have otherwise been missed and identified a hybridized population of dace: all were phenotypically Northern Redbelly Dace (*Chrosomus eos*) but genetically Finescale Dace (*Chrosomus neogaeus*). Importantly, eDNA and traditional methods did not detect Brook Trout (*Salvelinus fontinalis*), a prized recreational fishing species that is experiencing population declines due to habitat fragmentation and climate change. Our findings provide valuable insights into optimizing eDNA protocols and highlight how eDNA can enhance the accuracy and efficiency of biodiversity assessments.

Environmental DNA as a tool to detect phylogenetic lineages of Arctic grayling (*Thymallus arcticus*) (PL)

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Arctic grayling (*Thymallus arcticus*) is an iconic fish species found in Canada, that can have important cultural, economic, and ecological value. However, they are facing anthropogenic stressors and are thus considered a Species of Special Concern in Alberta. Distinct microsatellite loci have been observed to separate this species into two phylogenetic lineages, the “Beringia” lineage of North America/Eurasia, and the “Nahanni” lineage which is locally distributed in the Nahanni, Liard, and Hay watersheds. Northern Alberta represents a potential contact zone between the lineages, but this is not well characterized. Further research is needed to investigate whether these genetically distinct lineages require separate conservation considerations. Traditional fisheries inventories are time consuming and difficult due to the extreme remoteness of the Hay River watershed. Using environmental DNA and a newly designed and validated rhPCR tool, we assayed for the presence of either Beringia, Nahanni, or both in 14 sites in the Peace and Hay River systems in June of 2024. These results, coupled with existing eDNA samples from within the Athabasca River Watershed, will provide insight into the possibility of differential conservation requirements and will aid government agencies, industry, and Indigenous communities in identifying at-risk populations.

Differences in detection of fish presence between environmental DNA and conventional fish capture methods as it pertains to mine development (PL)

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Environmental DNA is a promising tool that is gaining recognition as an efficient and cost-effective method for detecting freshwater fish species. Confidence in the detection of fish species can vary widely based on sampling methodology and fish abundance in the watercourses and waterbodies being sampled. There is also uncertainty regarding acceptance by regulators. When developing a mine, obtaining accurate fish presence/absence data is required to accurately site project infrastructure, inform subsequent permitting and provide baseline data to support the environmental assessment. Pairing novel and conventional fish capture methods can provide a weight of evidence approach to determine whether a waterbody is fishless or fish bearing, which can increase regulator acceptance and reduce overall risk to our clients. On the Island of Newfoundland, environmental DNA and conventional fish capture data was compared to determine the occurrence of freshwater fish species in headwater ponds at a proposed mine. There were differences in the perceived presence of fish species in ponds between eDNA and conventional methods. Most notably, species were detected more frequently by eDNA than conventional fish capture methods. These results suggest that eDNA is capable of detecting fish at low abundance in headwater waterbodies where fish presence may be influenced by connectivity, flow, beavers and ice cover. By pairing the two methods to assess fish presence, we can provide context as to when eDNA can be an effective and robust tool for use in the mining sector.

Biomonitoring of the endangered redside dace (*Clinostomus elongatus*) over a 10-year period in Southern Ontario using environmental DNA (PL)

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Environmental DNA (eDNA) methodologies have revolutionized species detection in aquatic environments, offering sensitive and non-invasive approaches to monitor rare or at-risk species such as redside dace (*Clinostomus elongatus;* RSD). RSD is a federally protected minnow that is classified as endangered in Canada by COSEWIC due to significant population declines and increasing urbanization threats. Currently, almost 80% of the species' Canadian distribution is situated within the rapidly developing Greater Toronto Area. Historically, RSD was found in 25 water bodies across Ontario, but as of 2019, the species is thought to be extirpated from 9 of these water courses while their population status is poor in an additional 12. Biomonitoring changes in the species distribution and abundance is crucial to inform ongoing conservation and re-introduction efforts. In this study, we utilised a species-specific qPCR assay to assess the presence of RSD within 90 sampling sites across 17 Southern Ontario water bodies over the course of a decade. RSD eDNA was detected in 72% of sites (21/29) sampled in 2013/2014 but only 26% of sites (17/66) sampled in 2023. To assess RSD presence in Southern Ontario over a decadal interval, we intend to re-sample the remaining 14 stations that were surveyed in 2013/2014 10 years later. Results from this sampling effort will further inform the current status of RSD populations within Ontario and reveal trends in population distribution over the past decade. This research will provide valuable insights for RSD conservation and recovery efforts, as well as assist in identifying potential re-introduction sites.

Maximizing eDNA quantification: the essential impact of sample clean-up for accurate gene copy estimation (PL)

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Environmental DNA (eDNA) analysis is a valuable tool used in biodiversity monitoring as it enables identification of species presence and abundance through analysis of genetic material in environmental samples. However, the presence of inhibitors in an environmental sample can interfere with the efficiency of quantitative polymerase chain reaction (qPCR), which reduces the accuracy of eDNA quantification. In this study, we assessed the impact of sample clean-up process (OneStep™ PCR Inhibitor Removal Kit) on the detection and quantification of eDNA samples, using the Eulachon (*Thaleichthys pacificus*) assay eTHPA6 as a case study. The need for sample cleanup is determined by the cycle threshold (Ct) from the IntegritE-DNA™ test. We observed that samples requiring additional clean-up due to high levels of inhibitors showed significant improvement in the qPCR efficiency after clean-up, leading to higher and more reliable estimates of gene copies. In addition, some samples with low levels of inhibition yielded strong detections and more accurate quantification after clean-up. This comparison shows the critical importance of inhibitors' removal for enhancing the sensitivity and accuracy of eDNA-based qPCR assays. Our findings indicate that proper sample preparation, especially through effective clean-up, is necessary for obtaining accurate gene copy estimation in eDNA studies. By optimizing clean-up procedures, laboratories can better support ecological studies and biodiversity assessments, ensuring high quality data for decision making and conservation efforts.

Comparative analysis of eDNA barcoding and conventional monitoring methods to detect amphibians in southern Ontario vernal pools (PL)

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Ongoing monitoring is vital for conservation of amphibian species and is typically conducted through conventional surveys (audio/visual). Environmental DNA (eDNA) surveys may offer a more sensitive method of species detection that negates the need for direct species observation. In this study, we performed a comparative analysis of eDNA barcoding versus non-invasive conventional species detection methods for six amphibian species in southern Ontario. We hypothesized that eDNA barcoding would offer equal or greater species presence detections compared to conventional methods. Conventional and eDNA surveys were conducted in three vernal pools from April-July 2019 in collaboration with ***rare*** Charitable Research Reserve (Cambridge, ON). Conventional surveys included daily recorded audio surveys and weekly/biweekly visual encounter surveys. To obtain eDNA alongside conventional methods, duplicate water samples were collected at multiple sampling locations around three vernal pools. eDNA was concentrated by filtration, extracted, and quality controlled before being processed using optimized quantitative PCR eDNA barcoding assays. Comparative analysis between conventional methods and eDNA barcoding contradicts a one-size-fits-all model of amphibian monitoring and suggests that while eDNA barcoding offers a reliable, effective method of species detection for five of the target amphibians, it failed to detect *Pseudacris crucifer* despite detections by conventional surveys. We propose the use of eDNA barcoding alongside conventional method(s) of species detection to optimize detections across a

spatiotemporal scale, with selection of the conventional method dependent on the target species. eDNA barcoding is a species detection method which can aid in ongoing amphibian monitoring and therefore, the conservation efforts of this declining taxa.

Application of molecular tools to monitor health, abundance, and distribution of spawning oolichan (*Thaleichthys pacificus*) (PL)

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Protection of aquatic species relies on timely, reliable, and detailed monitoring data, which can be challenging for species whose life histories and distributions are not well described. Oolichan (*Thaleichthys pacificus*), a culturally and ecologically important anadromous smelt, develop in the ocean and return to fresh water along the Pacific Coast of North America to spawn in the general river system in which they hatched. Their populations have steeply declined since the 1990s, and supporting their recovery relies on accurate knowledge of spawning locations, yearly abundance, and organismal health. We are using environmental DNA (eDNA) and transcriptomic approaches to improve our understanding of this species while decreasing survey invasiveness. We have developed a highly sensitive and specific eDNA assay targeted to oolichan and used it to determine the yearly relative abundance and locations of spawning runs from 2019 to 2023. We have also assembled a novel transcriptome and developed bioindicator assays targeting gene transcripts that are differentially expressed in oolichan tissue in the presence of oil contaminants. Using these two cutting-edge tools in conjunction with conventional monitoring techniques in Haisla Territory near Kitimat, British Columbia, we demonstrate the capacity of harmonized approaches to provide an enriched view of population health, while advancing the capabilities of each individual method.

Assessing soil microbiota adaptations to brine disturbance using prokaryotic and eukaryotic eDNA Sequencing (PL)

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How soil microbiomes respond and adapt to conditions of elevated salinity associated with inadvertent brine releases is a relatively underexplored means of evaluating risks to soil health and ecological function associated with these events. This study investigates the impact on soil microbiota of brine released more than 30 years ago from a buried pipeline at a > 30-hectare site in Northern Alberta, Canada. Portions (undisturbed) of the site are forested whereas the pipeline right of way and other historical work areas are dominantly vegetated with graminoids and forbs. Soil sampling of both A and B horizons was carried out along four 300-meter transects that spanned from the forested areas to the pipeline. Electrical conductivity (EC) values of the soils ranged from 0.1 to over 40 dS m^{-1}; soils with increased salinity also showed higher sodicity (Sodium Adsorption Ratio up to 41) and pH (from 4 to 8). Microbiome analysis was conducted via both 16S rRNA and 18S rRNA amplicon sequencing to characterize prokaryotic and eukaryotic communities, respectively. A negative correlation was observed between microbial alpha and beta diversity and the levels of brine contamination. High brine impact zones were dominated by salinity-adapted prokaryotes from the phyla Desulfobacterota, Bacillota,

Crenarchaeota, and Halobacterota, as well as eukaryotes from Labyrinthulomycetes, Ciliophora, and Phragmoplastophyta. In contrast, Acidobacteriota, Verrucomicrobiota, and Mixococcota (prokaryotes), along with fungi from Basidiomycota and Ascomycota, were depleted. Notably, community composition experienced significant shifts at EC values of 1.4 and 4.2 dS m^{-1}, indicating ecological inflection points. We identified differential niche adaptations based on brine impact rather than changes in vegetation cover: 27.3% of taxa were suited to low EC environments, 38.8% thrived in high salinity soils, and 20% displayed generalist profiles, indicative of their halotolerance. Network analysis revealed increased connectivity among halotolerant microbial communities, yet these networks were less robust, suggesting a higher vulnerability to environmental perturbations. These findings highlight the adaptive capacity of both prokaryotic and eukaryotic microbial communities to brine-induced stress and underscore the need for remediation strategies that enhance microbial diversity and restore ecological functionality in brine-contaminated landscapes.

Unveiling a Century of Change in Lake Eukaryotic Communities: Insights from Sedimentary DNA and Indigenous Knowledge (PL)

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Sedimentary DNA (sedDNA), a form of environmental DNA (eDNA) shed by aquatic organisms and preserved in sediment, is essential for reconstructing historical community compositions in aquatic ecosystems. In Cowpar Lake, Alberta, a historical landslide around 1950 CE significantly impacted the lake's geochemistry and fish communities. This event was documented by Indigenous Knowledge from the Chipewyan Prairie First Nation and supported by targeted fish sedDNA analyses. This study employed 18S DNA metabarcoding of lake sediment cores to reconstruct past and present community assemblies of eukaryotic functional trophic groups (photoautotrophs, mixotrophs, parasites, and consumers), corroborating these historical accounts. Sediment chemistry data revealed a significant sedimentation event from 1941 to 1956, recorded in the core at depths of 17-19 cm, with peaks in organic matter and sulfur, a redox-sensitive element. A notable shift in community assemblage occurred between 1948 and 1956 CE, with an observed decline in overall alpha diversity. The dominant eukaryotic communities included fungi, rotifers, diatoms, dinoflagellates, photosynthetic protists, arthropods, and terrestrial plants. An increase in primary productivity and terrestrial organic input post-1950 led to a significant rise in the diversity of phototrophs and mixotrophs, indicating potential algal blooms. Parasite diversity remained constant, while consumer diversity declined, likely due to heightened microbial respiration of organic matter, which reduced oxygen levels and made the lake less habitable for consumers like whitefish, which eventually disappeared from the lake. The reconstructed eukaryotic community records from sedDNA data were consistent with Indigenous accounts of natural-mediated changes around the lake. This study underscores the substantial potential of integrating sedDNA data with Indigenous Knowledge to reconstruct long-term historical changes in aquatic communities, providing high-resolution baseline data for environmental monitoring and a comprehensive understanding of freshwater system responses to natural and human-mediated impacts.

Assessing the performance of environmental DNA (eDNA) to estimate the presence of white sucker (*Catostomus commersonii*) during a spring spawning survey in a northern Alberta tributary (PO)

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Environment and Climate Change Canada's (ECCC) Strategic Application of Technology of Genomics in the Environment (STAGE) supports the development of environmental DNA (eDNA) technology to detect and quantify the presence of species of interest within the aquatic environment. In collaboration with Alberta Environment and Protected Areas, ECCC periodically conducts spawning surveys in northern Alberta. This study aims to monitor the movement of large-bodied fishes, including the target white sucker (*Catostomus commersonii)*, as the fish move from the lower Athabasca River region up-river to the spring spawning area in Muskeg River near Fort McKay, Alberta, and back down the river post-spawn. This survey required the installation of a fish fence across the whole Muskeg River to funnel fish movement into a receiving cage so that they can be enumerated, their length and weight can be recorded, and condition assessed. During the 2023 spring spawning survey, surface water was collected and filtered downstream of the Muskeg River fish fence, and filters were stored until analysis. In the laboratory, a 110-bp cytb target was amplified using a new eDNA assay designed specifically for white sucker. The concentration of white sucker eDNA over a temporal scale was determined via quantitative polymerase chain reaction (qPCR). Preliminary data suggests strong congruency of targeted sucker eDNA collected during the spawning survey to the actual fish fence enumeration of white sucker moving upstream and downstream in the Muskeg River. This data will assist in the identification and quantification of species of interest using eDNA technology to assess its potential as a method for aquatic monitoring.

Appropriateness of sampling media to detect eDNA and assess fish diversity in a small stream (PO)

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Electrofishing has been used alongside other conventional sampling methods used in the estimation of fish community diversity for aquatic biomonitoring in Canada. Increasingly, environmental DNA (eDNA) metabarcoding is paired with electrofishing efforts and it has been shown that these methods can be complimentary for aquatic biomonitoring. Sedimentary eDNA is also used when monitoring rivers but is infrequently paired with electrofishing. In this study, we used a common universal 12S primer set for fishes on three different eDNA sampling media (water, deposited sediments, and suspended sediments) over three sampling campaigns to determine which most similarly estimated fish community diversity with paired electrofishing efforts in a recently restored creek in Guelph, Ontario, Canada. A mock community comprised of DNA extracts from fishes originating in the system was used as a positive control. Estimates of species richness were most similar between electrofishing and suspended sediments, though water samples estimated the greatest overall species richness. However, all three

eDNA sampling media were found to generate estimates of community diversity that were more similar to each other than they were to estimates from electrofishing. Differences in community diversity were associated most strongly with sampling method, weakly with sampling site, and were not associated with sampling period. Additionally, an indicator species analysis revealed that the taxa discriminating between eDNA and electrofishing methods were all taxa which could not be amplified from the mock community. Diversity and indicator species analysis suggest that the dissimilarity observed between eDNA and electrofishing sampling methods are being primarily driven by methodological limitations relating to primer specificity and resolution.

Addressing community concerns surrounding char populations (*Salvelinus alpinus* and *Salvelinus malma*) near Kugluktuk, Nunavut, using environmental DNA (eDNA) (PO)

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Over the last decade, community members in Kugluktuk, Nunavut, have described warmer temperatures and decreased water levels in the Coppermine River, along with decreased catches of Arctic char (*Salvelinus alpinus*) and Dolly Varden (*Salvelinus malma*). The apparent decline in char is of great concern as they are an essential subsistence fish species, and hold economic, social, spiritual, and cultural value to the community. This study will provide insight into the composition and habitats of the anadromous char populations near Kugluktuk, by developing and employing an environmental DNA (eDNA) assay to non-invasively identify char habitat. In the laboratory, primers have been designed and tested for their effectiveness at identifying and differentiating between the two char. In collaboration with local community members, 15 sites (5 with only Arctic char, 5 with only Dolly Varden, and 5 with both fish) have been identified and sampled for eDNA to evaluate the assay. This eDNA assessment will be critical in achieving effective community-based stewardship of char in Kugluktuk.

Environmental DNA metabarcoding for biodiversity characterization of a harmful algal bloom (PO)

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Harmful algal blooms (HABs) pose a major threat to the health of freshwaters and the humans, animals, and other organisms that rely on them, in part due to the production of toxins by cyanobacteria. With climate change, overgrowth of toxic or otherwise harmful cyanobacteria and microalgae is increasing, and the development of tools to assess HABs is crucial for freshwater conservation. Here, we describe a novel approach for high-throughput biodiversity characterization of microbial diversity in a small inland lake (Fairy Lake, Acton ON). We describe a tool for high-throughput isolation and amplicon sequencing of select microalgal strains from Fairy Lake to build 1) a working culture collection for further physiological characterization and 2) a molecular reference library for environmental sequencing. We present methods and preliminary results of paired molecular and morphological assessment of environmental samples collected during the summer of 2024. High-throughput amplicon sequencing of ribosomal RNA genes (16S, 18S, and 23S) is coupled with a novel plate-based isolation strategy and a FlowCam 6000 to describe the microalgal community throughout the HAB season. This research aims to compare molecular and morphological tools for phytoplankton community assessment and generate a culture collection for further physiological analysis of strains. This research will significantly advance the field of

aquatic ecosystem monitoring and create opportunities to better characterize and understand shifts in freshwater microbiomes.

Statistical workflow for environmental DNA cross-assay comparison: A use-case example with threatened Oolichan (*Thaleichthys pacificus*) (PO)

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Targeted environmental DNA detection approaches have broadened in application in recent years, to the extent that disparate research groups may be studying the same taxa using different protocols even in overlapping ranges. While this represents a positive surge in growth and acceptance of the tools by several fields of research communities, it is desirable to enable comparison of results using a variety of methods while retaining statistical rigor. A particular challenge lies in the finding that the eDNA concentration results made using one assay do not necessarily match estimates made using a second assay in environmental samples. We use weighted orthogonal regression and error propagation statistics to permit the transformation of DNA concentration estimates from one qPCR assay into predicted values of a second assay. We produced standard curves for two robust eDNA assays targeted to Oolichan (*Thaleichthys pacificus)* in two laboratories using synthetic DNA sequences of known starting copy number, then tested eDNA samples containing a range of Oolichan abundance with both assays simultaneously. With these data we demonstrate the use of our workflow to predict DNA copy number estimates from one assay given the qPCR results of the other assay. Using statistically robust approaches we permit direct comparison between eDNA datasets, which can be applied to individual projects using multiple assays or separate research groups producing independent data. We hope this toolset will lead to increased confidence in eDNA results, and encourage harmonization of data in the eDNA community at large.

Monitoring native lamprey populations with environmental DNA (eDNA) to inform lampricide application (PO)

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Native lamprey species in the Laurentian Great Lakes tributaries are fast declining, and efforts towards their conservation are hampered by an insufficient knowledge of their distribution. Two native lamprey species, northern brook lamprey (*Ichthyomyzon fossor*; NBL) and silver lamprey (*Ichthyomyzon unicuspis; SVL*) have been listed as Special Concern on Schedule 1 of the Species at Risk Act (SARA) in Canada. The cause of this decline remains contested, but the addition of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2',5-dicholoro-4'-nitrosalicylanilide (granular Bayluscide) to stream reaches for the purpose of invasive sea lamprey (*Petromyzon marinus; SL*) control is often credited with having substantial unintended consequences for native lamprey populations. Currently, over 50% of streams with known SVL and NBL populations have been acutely exposed to one of these lampricides at some point. To help direct lampricide additions away from native lamprey populations, we developed a novel

quantitative PCR (qPCR) assay designed to identify SVL and NBL. We implemented this assay during routine electrofishing surveys for larval SL at 54 locations across 12 Great Lakes tributaries throughout the summer and fall of 2022 and conducted additional, unpaired eDNA sampling in the winter. We found that the eDNA-based method of detection was more sensitive than targeted electrofishing, identifying *Ichthyomyzon* species in 6 instances where electrofishing did not. Conversely, electrofishing outperformed eDNA on only 3 instances. Building off this work, we intend to sample more novel waterways across Ontario with the purpose of identifying native lamprey hotspots and reducing unintended lampricide toxicity to these at-risk species.

Application of a multiplex eDNA barcoding assay to detect brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) in remote Karukinka Natural Park, Tierra Del Fuego Island, Chile (PO)

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Environmental DNA (eDNA) barcoding and metabarcoding approaches are valuable biomonitoring tools to detect fish species, especially when the use of conventional methods such as electrofishing may not be suitable for the target species or location. A multiplex probe-based barcoding (RT-qPCR) assay was developed to test for the presence of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) DNA at eleven sites along the Rasmussen River, including Despreciado Lake and El Cura Lake in Karukinka Natural Park, Tierra Del Fuego, Chile. In general, three 2-L grab samples of water were collected and filtered on-site with a Smith-Root 1.2 µm PES eDNA filter and later extracted in the laboratory for DNA with a DNeasy Blood & Tissue kit. The DNA extracts were quantified using qPCR against a gBlock standard with a sequence for each species, plus an 18-base synthetic insert to check for cross-contamination. Accompanying the eDNA approach, a combination of backpack electrofishing, gill nets, and fly fishing was employed as a method to capture brown trout and rainbow trout, which showed a transition from no-fish to brown trout only, and then a mix of brown trout and rainbow trout moving downstream. In aquatic systems that are remote and difficult to sample with traditional approaches, eDNA barcoding can detect species presence and provide insights into the fish community that is present, targeting future sampling or research.

Mining and the Environment

Investigating the linkage between biological activity and changes to selenium speciation in mine sedimentation ponds (PL)

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Algal and bacterial metabolism have been implicated as the source of the range of inorganic and organic selenium species detected in and downstream of mine sedimentation ponds. We investigated the factors

that facilitate these processes, with the objective of informing management action to reduce the generation of highly bioavailable organic species, and thereby manage selenium risk. Monitoring upstream and downstream of more than 20 ponds over 3 years revealed variable effects on speciation seasonally, among years, and among ponds. We used bivariate and multivariate analysis to evaluate whether differences in physical, chemical, and biological characteristics of the ponds could explain this variation. Characteristics such as hydraulic residence time, total selenium concentration, and sediment redox conditions varied widely among ponds, but were not able to explain differences in speciation. Higher concentrations of reduced selenium species tended to be associated with dense aquatic vegetation, high orthophosphate concentrations, and either increases or decreases in dissolved oxygen. These patterns suggest a role of both algal productivity and bacterial metabolism, with the relative importance of these processes differing among ponds (and among seasons).

Characterization of selenate (SeVI) uptake rates in natural periphyton communities from anthropogenically influenced lotic systems (PL)

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Selenium (Se) is a naturally occurring element essential to organismal health that can become toxic when its concentrations rise above tolerable biological thresholds. Environmental Se concentrations can be elevated by anthropogenic activities, including agriculture and mining. The critical step of Se accumulation aquatic food webs is its uptake via microbial and algal communities, particularly periphyton. Variations in Se speciation and type of aquatic system can result in differences in measured Se uptake rates. To examine if selenate dynamics in lotic systems could be expanded upon by characterizing periphyton SeVI uptake kinetics with respect to community composition, natural periphyton communities were collected from three lotic systems in the Elk Valley of British Columbia representing reference and elevated Se exposure conditions. Periphyton communities were then exposed to radiolabeled SeVI-spiked media in the laboratory to quantify SeVI uptake rates as a function of community composition. The uptake rates of natural periphyton showed a concentration-dependent increase over a 4-hour exposure period irrespective of sampling site. Moreover, SeVI uptake rates in periphyton from one reference site and the elevated Se site did not differ, while the second reference site had significantly lower uptake rates compared to both the other reference and elevated site. Community subsamples are currently being analyzed using eDNA metabarcoding to quantify the periphyton community composition. Exploring the differences in community composition will help explain the variations in SeVI uptake rates across different study sites.

Advancing mine dust monitoring and characterization techniques (PL)

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Dust generated throughout the life cycle of metal mining has not been extensively studied, resulting in knowledge gaps of this contaminant transport mechanism. With projects across Canada including Nova Scotia, Quebec, Northwest Territories and most recently, Nunavut, this research program aims to address the gaps associated with mine dust composition and the spatial-temporal distribution of fugitive dust emissions to the near-mine environment. For new projects in the Arctic, the bridging of western science and Indigenous Knowledge to work towards a more comprehensive understanding of fugitive mine dust is guiding research development and collaborations. Innovative dust monitoring techniques have been evaluated at both abandoned and operating mining sites including the deployment of passive dry deposition collectors (Pas-DDs). Through geochemical and mineralogical characterization of dust captured by the Pas-DDs, the mineralogical controls on the metal(loid) inventory of the dust can be evaluated, providing insight on both the environmental mobility of contaminants and likely metal(loid)/dust source(s). With new ground-based dust capture and characterization techniques, coupled with Earth Observations, this project aims to provide industry with options to bolster current dustfall monitoring programs. In addition to the evaluation of new monitoring and characterization techniques, this presentation will also describe work being conducted on the fate of dust in the environment in media such as surface water, soils and peat.

Mine-related modifications of a small Canadian sub-Arctic lake – changes in lake water chemistry due to the flooding of tundra (PL)

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The Gahcho Kué Mine is a diamond mine established within a headwater watershed in the sub-Arctic Canadian Shield that has been isolated and dewatered to allow mining. As a component of Mine's water management plan, several small upstream lakes were diverted from the mine area to an adjacent watershed. These diversions resulted in increased water levels and surface areas in these lakes, which were expected to alter aquatic habitat and biota. While changes were predicted, their extent and magnitude were qualitative and reliant on studies of larger, lower latitude impoundments. The largest raised lake, Lake D2/D3, has been monitored for eight years following the construction of the downstream dyke, as well as multiple years when it existed as two distinct but connected lakes. Lake D2/D3 reached full supply level four years after diversion with a doubling of surface area and created a new outflow to a small headwater lake in an adjacent watershed. Water quality changes in the raised lake relative to pre-raised condition have been observed due to the release of nutrients and metals from the flooding of the tundra vegetation, especially during seasonal ice-cover when decomposition processes and anoxic conditions are prevalent. Four years after reaching full supply level, Lake D2/D3 remains in transition with early signs of water quality stabilization. Our work supplements the limited information regarding effects of rising lake levels in sub-Arctic environments, and provides insight into potential changes to Canadian Shield lakes in the event of future water level increase from climate change.

Analysis of petroleum hydrocarbons in oil sands mine waters (PL)

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The study was completed to evaluate laboratory methods for the analysis of "petroleum hydrocarbons" (PHC) in oil sands mine water (OSMW) samples. The reference method for PHC under the Canada-Wide Standard uses a silica "cleanup"(SGC) to remove polar organics so that only PHCs are measured. The standard method allows for either in-situ cleanup, where the silica gel is added directly to the sample, or ex-situ (column) cleanup where the sample extract is dispensed through a glass column packed with silica gel. The column cleanup is considered more robust, particularly for waters with high concentrations of polar organics, such as OSMWs, but is not routinely offered by commercial laboratories. Over 20 samples were collected from a range of OSMWs including groundwater, reclamation water, and process water over several years. Samples were analyzed using both the in-situ and column SGC methods. A capric acid reverse surrogate was added prior to SGC that should be 100% retained by the silica gel. The analysis using the column SGC resulted in lower measured concentrations of PHC relative to the in-situ method and PHC concentrations were typically below detection using the column SGC for a range of OSMWs including tailings pond water. For the in-situ SGC, there was substantial variability in the measurement of hydrocarbon concentrations and high capric acid breakthrough. Based on the study results, the measurement of PHC using the in-situ SGC method is not considered a reliable measure for OSMW samples and is not an effective measure of potential toxicity.

Assessment of polymer-treated tailings from a pilot-scale pit lake in the Alberta Oil Sands Region (PL)

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The reclamation process is a major challenge for companies mining in the Alberta Oil Sand region. One potential reclamation strategy involves the creation of pit lakes that incorporate waste materials such as fine tailings and oil sands process-affected water (OSPW). However, these lakes may pose ecological risks due to the presence of toxic constituents. Suncor Energy has employed the Permanent Aquatic Storage Structure (PASS) approach in Lake Miwasin, a pilot-scale pit lake in northern Alberta. This structure utilizes polymer flocculants to manage tailings sedimentation and dewatering. However, there were unknowns regarding the potential toxicity of Lake Miwasin bottom substrate. This study assessed the toxicity of polymer-treated tailings used as bottom substrates in Lake Miwasin. Sediment toxicological assessment and sediment Toxicity Identification Evaluation (TIE) were conducted to assess the toxicity of the sediment to aquatic organisms. Water and sediment samples were collected from the littoral and limnetic zones of Lake Miwasin from 2020 to 2023 to capture spatial and temporal variability in sediment toxicity. Sediment metal concentrations and organic compounds, including polyacrylamide polymers from treated tailings, were quantified to identify potential toxicants (some analyses remain outstanding). Toxicity testing was conducted using larvae of *Chironomus dilutus* that were acclimated to reconstituted saline water (RSW), formulated to simulate Lake Miwasin bottom water. The larvae were exposed in 14-day static-renewal bulk sediment toxicity tests to littoral and limnetic sediments in combination with either RSW or Lake Miwasin bottom water. Results showed toxicity for the limnetic ($\leq$ 5% survival) and littoral ($\leq$ 15% survival) sediments. Whole-sediment TIEs revealed that no single class of contaminant

was the sole cause of toxicity. Coagulation polymer concentrations in Lake Miwasin surface water ranged between ~5 – 40 mg/L. Polymer toxicity testing was carried out across this concentration range using static-renewal 10-d exposures of *C. dilutus* larvae. The results showed no polymer toxicity. A sediment toxicity test comparing core (top 2-cm) versus grab (top 10-cm) samples was conducted using limnetic sediment collected in 2022. Results showed a survival range of 86–100 % for the top 2-cm sediment layer and 60–80 % survival for the grabs consisting of the top 10-cm of sediment. A follow-up sediment vertical gradient toxicity test was carried out in 2023, comparing the Top 2-cm, Top 2-5-cm, and grab (top 10 cm) samples. The result revealed a potential increase in toxicity down the vertical gradient. These findings will help inform future sampling of sediments in this and similar pit lakes in the AOS region.

Do we have the right tools in our toolkit? Insights into the complexity of designing aquatic effects monitoring programs from Canada's North (PL)

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The development of the Environmental Effects Monitoring (EEM) program for metal mining began in 1993 with the Assessment of the Aquatic Effects of Mining in Canada (AQUAMIN). The final AQUAMIN report made recommendations to Environment Canada for the design of an EEM program for metal mining. The consultation on the EEM program culminated with a consensus agreement on proposed EEM requirements and the completion of a guidance document (Environment Canada 2012), which includes information on fish and benthic invertebrate monitoring, and the testing of effluent, water and sediment using chemical and toxicological measures. This 2012 guidance document forms the current basis of how Aquatic Effects Monitoring Programs (AEMPs) are designed in the North, and specifically how the endpoints that are studied to determine mine-related effects are selected. Here, we provide an overview of future challenges and complexities of designing AEMPs in the North, including melting permafrost, climate change, and the growing lack of taxonomists. We provide examples from two long-term monitoring programs in northern Canada, Giant Mine and Faro Mine. We highlight the use of multiple lines of evidence as a tool to properly detect mine related effects for monitoring programs in the North, and pose the question – does the EEM program need to be re-visited in light of our changing environment?

Scientific monitoring and traditional knowledge: transparency, equivalency and the rattlin' worm (PL)

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In the fish, there was a worm (not a rare worm), but a rattlin' worm! Aquatic environmental monitoring studies typically quantify biological change by performing statistical tests on relevant endpoints, often comparing an exposure area and a reference area, or using a gradient design. In recent years, monitoring studies often incorporate some form of adaptive management into the overarching monitoring strategy. This approach has gathered consensus and is widely considered a strong, defensible approach in the regulatory and scientific community for monitoring long term effects in pristine aquatic environments such as the Canadian arctic and subarctic. There is, however, a shift toward 'two-eyed seeing', in the spirit of reconciliation, to better incorporate the more subtle and equally relevant (but less quantifiable)

traditional knowledge of these complex ecosystems held by indigenous communities familiar with the land and water since time immemorial. This presentation will consider fish health data collected over the life-of-mine at a Canadian diamond mine through both the lens of science and traditional knowledge, and will consider the implications and importance of how such information is perceived. The presentation will highlight the particularly challenging topic of parasite presence and abundance, including observed changes over time and predictive trends, in the fish of a large sub-arctic lake.

Hair screening tool: detection of elevated exposure to copper (PL)

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In this study, single human hairs were examined as a potential biomarker of elevated copper exposure. Hair has long been used as a tool to assess copper exposure; however, the high likelihood of external contamination of the hair strand upon scalp emergence precludes hair from being fully accepted, as it is difficult to distinguish between endogenous and exogenous copper sources. Here, we use laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) on hair strands from 50 individuals above and below the scalp. The main objectives were: 1) to determine if a double ablation technique (i.e., ablating the hair twice) removes copper contamination on the exposed hair strand; 2) to calculate a normal reference range of copper levels in the cuticle and cortex layers; 3) to determine if a single hair can represent multiple hairs from the same individual; and 4) to determine if hair is sensitive enough to record elevated copper exposure. This latter objective was conducted using grizzly bear hair following elevated exposure to dietary copper. Copper concentrations were, on average, 4.5 times higher in hair above the scalp than below the scalp, indicating significant external contamination in most individuals. Double ablation was successful at burning off the contaminated cuticle layer in all cases, leaving only endogenous copper concentrations within the inner cortex layer. The resulting (non-contaminated) reference range (10th to 90th percentile) for copper was narrow at 7.3 to 15.2 mg/kg. A subset of the 50 individuals were used to determine if a single hair can represent any hair strand from an individual. Multiple hairs were taken from 6 people and the copper concentrations were compared. The relative percent difference was low ranging from 5.3% to 15%, suggesting high replicability among hairs. The grizzly bear hair showed elevated copper at various points along their strands reflecting dietary exposure, and results were replicated in hairs from the same bears. All results together strongly indicate that single hair strands could provide effective and accurate monitoring of elevated copper exposure in wildlife and humans.

A Nisga’a Nation-led water quality monitoring program in the Nass Area (PL)

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The Nisg̲a'a Nation holds Treaty rights to the Nass Area – a vast region comprising the Nass River watershed and others. Exercising Treaty rights such as the harvest of Pacific salmon requires a healthy environment – with water quality being a primary concern. Mineral exploration and development in the Nass Area date back to the 1800s and continue today. Accordingly, it is essential that the potential for direct, indirect, and cumulative environmental effects on Nisg̲a'a rights and interests be understood. The goal of this study was to understand whether mineral exploration and development activities affect ambient water quality in the Nass Area. To accomplish this, the Lands and Resources Department of Nisg̲a'a Lisims Government (NLG) began monitoring key waterbodies that might be affected by mineral

exploration or development activities (potentially impacted sites) and those that are unlikely to be affected (reference sites). NLG staff collected in situ water quality data and water samples monthly from May through October 2023 at two potentially impacted sites and three reference sites. Freshwater quality concentrations were compared among sites and with available water quality guidelines. Significant differences in the concentrations of some metals between the reference and potentially impacted sites were observed. The information generated from this, and any future studies, will inform present and future decisions concerning natural resource management in the Nass Area.

Peat-based soil amendments enhance vegetation recovery of mining-affected areas (PL)

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The mining industry has been seeking various means to amend mine till, to restore the affected biome surrounding a mine to its natural state. To address these challenges, an amendment trial to monitor vegetation development for reclamation planning was implemented at a gold mining site in the boreal forest ecosystem in Northern Ontario. This study evaluates the necessity and benefits of amending mine till with 8 treatments (Control, Fertilizer, Oats, Peat, Biosolids, Oats + Fertilizer, Peat + Fertilizer, Biosolids + Peat) on the establishment and growth of native plant species from 2016 to 2020. Generalized Linear Mixed Models (GLMM) were used to analyze the impact of fixed effects (e.g., Treatment and Time) and their interactions, and random effects (e.g., Group). Least squares means were generated for each treatment, and Tukey-adjusted multiple comparisons were conducted to determine significant differences between treatments. Results show that: 1) Treatments with peat significantly improved plant survival across all species. Prairie Willow (*Salix humilis*) and White Spruce (*Picea glauca*) showed the highest survival rates, demonstrating resilience compared to other species; 2) Treatments with peat significantly enhanced tree growth, with higher median heights and greater variation. Prairie Willow and White Spruce exhibited particularly high growth rates, reinforcing the beneficial impact of peat; 3) Treatments of Peat + Fertilizer and Biosolids + Peat were most effective in enhancing diversity index, and year-over-year analysis indicated positive biodiversity recovery. These findings provide strong evidence for the effectiveness of peat-based soil amendments in promoting ecological recovery of mining-affected areas.

Sources and fate of inorganic and organic selenium species in a mine-affected watershed (PL)

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Selenium occurs in natural surface waters as a variety of inorganic and organic chemical species, which complicates the evaluation of fate and risk. We studied spatial and seasonal patterns of aqueous selenium speciation over 7 years at more than 100 locations near coal mine operations in southeast British Columbia, Canada. In addition to the ubiquitous oxyanions selenate and selenite, we detected two inorganic selenides and five methylated organic species. Organoselenium species, although hypothesized to be more bioavailable than oxyanions, have rarely been identified or quantified in natural waters and little is known about their fate or bioaccumulative potential. Most reduced species were infrequently detected and appeared to be highly labile, but two (methylseleninic acid and dimethylselenoxide) were relatively persistent and associated with significant increases in bioaccumulation in downstream biota. Spatial and temporal patterns implicate algal and/or microbial

selenium metabolism, primarily in mine sedimentation ponds, as the source of reduced species detected in downstream areas.

Efficacy of Clearflow treatment technologies in treatment of coal mine effluent (PO)

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Coal mining is a common source of environmental pollutants, including suspended sediments, metals and excess nutrients, which can harm aquatic ecosystems. To minimize the release of pollutants associated with coal mine process water, this water is often treated with cationic polymer within settling ponds, which clarifies the water through flocculation of suspended sediments. However, following flocculation, residual cationic polymers are themselves toxic to aquatic life, in particular causing impaired gill function in fish. This toxicity of cationic flocculants can be effectively ameliorated by a treatment with a neutralizing agent developed by the Clearflow Group and tested in previous studies under laboratory conditions. The present project follows up with field verification of the effectiveness of the amelioration of cationic polymer toxicity using Clearflow's neutralizing agent. This field study examines the effects of treating mine water with both cationic polymer and neutralizing agent on nutrient and metal contaminant removal, invertebrate community impacts, and toxicity to fish. The first season of this study demonstrated that the use of neutralizing agent 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 two-step treatment process. The invertebrate community compositions in the treated mine effluent stream vs the receiving river appears to promote the abundance of mollusk species and reduce the abundance of some insect taxa.

Weight of evidence approach as an integration tool for a small Canadian sub-Arctic lake (PO)

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[1]WSP Canada Inc

The Gahcho Kué diamond mine is located approximately 280 kilometres northeast of Yellowknife, Northwest Territories. The Aquatic Effects Monitoring Program (AEMP) is one of several monitoring programs and management plans for the Mine that employs an adaptive approach to reduce the magnitude, frequency, and extent of effects of the Mine on the environment. A weight of evidence (WOE) approach has been implemented in the AEMP as a transparent and systematic tool to integrate changes and patterns identified in individual AEMP components to inform conclusions regarding the type(s) of impacts that may be occurring in the aquatic ecosystem around the Mine. Although individual AEMP components involve quantitative analysis, a qualitative integration using narrative criteria for WOE has been successful in providing an indication of the strength of evidence for one or more of the impact hypotheses. Construction of Dyke F to prevent flow towards the mine area raised the water level in Lake D2/D3. Changes to aquatic habitat and biota were observed, including altered water quality due to the release of nutrients and metals from the flooded tundra vegetation. The WOE indicated weak evidence for toxicological impairment to the lower trophic communities, moderate evidence for nutrient enrichment to the plankton community but negligible to the benthos community, and moderate evidence for physical habitat alteration to the benthos community but weak evidence for the plankton community. These results highlight the complexity of the causes and types of impacts to aquatic biota due to the increased water levels and subsequent flooding of tundra.

Latest Advances in Fate and Effects of Metals in the Natural Environment

Study of nickel toxicity on phytoplankton of the Arctic Ocean (PL)

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The Arctic and its exceptional ecosystem face numerous environmental challenges. These include the growing exploitation of its mineral resources, particularly nickel (Ni), a key metal for the energy transition which appears on the list of strategic minerals drawn up by the Quebec government. However, the environmental risks associated with nickel mining in these regions are poorly understood, particularly its impact on polar organisms. Most available ecotoxicological data pertain to organisms from temperate regions, and there is little information on northern species. As a result, environmental risk management for Ni in polar environments relies on data from “southern environments”. However, polar organisms have certain physiological and metabolic adaptations (e.g., slower growth and development times) that could make them more or less susceptible to Ni. In order to address this data gap and remove these uncertainties, we will study the effects of nickel on marine phytoplankton, which is a vital component of the polar ecosystem. The project involves culturing several species of phytoplankton from the Arctic Ocean and conducting toxicity tests to measure nickel bioaccumulation and its effects on algal growth rates. The study will also examine how an increase on temperature and salinity can influence these parameters. Then, we will compare the nickel sensitivity of polar and temperate species, based on literature findings for the latter. The results of this project will contribute to the development of an ecotoxicological database for nickel in polar regions. In this platform, the methodology and the first results of the toxicity tests will be discussed.

Effect of pH on metal bioaccumulation in freshwater biofilms (PL)

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In Quebec, the mining sector is of crucial importance. In 2022 alone, investments in this sector reached $4.8 million. However, mining activities can lead to an increase in metal concentrations in surface waters, resulting in additional stress to biota. The current rise in demand for minerals will thus likely increase the overall geochemical mobility of metals. It has previously been demonstrated that pH plays a key role in metal bioaccumulation and our ability to predict their effects requires a better understanding of this parameter's influence. To study this phenomenon and its impacts, this research focuses on the bioaccumulation of Cd, Pb, Zn, Cu, and Ni in freshwater biofilms. Biofilms mostly contain algae, bacteria and fungi and can be used as a model biological target for its ability to quickly respond to environmental changes and its advantageous position at the base of the trophic network. Rocks naturally coated with biofilms were collected from the Cap-Rouge River (Quebec, Canada), a river with low metal concentrations, and were exposed to the selected metals in the laboratory at different pH levels for 96 hours. Biofilm metal contents were then determined in order to develop a predictive model of Cd, Pb, Zn, Cu, and Ni bioaccumulation in river biofilm as a function of pH. The development of this biomonitoring tool will help to more efficiently identify environmental conditions (e.g. pH) that are likely to result in high metal bioavailability in ecosystems affected by mining activities.

Reproductive effects of arsenic-contaminated natural diet in zebrafish (*Danio rerio*) during chronic exposure (PL)

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Very little is known about the reproductive effects of arsenic in fish. The current study investigated the reproductive toxicity of environmentally relevant chronic arsenic exposure *via* an oligochaete worm (*Lumbriculus variegatus*) in adult zebrafish. Worms exposed to waterborne arsenic (0, 2.5, and 5.0 mg/L as arsenite) for 14 days had body burdens of 0.3 (control), 35.9 (low), and 78.0 (high) μg/g wet weight. For 60 days, experimental fish were fed either of these arsenic-contaminated worms at 3.5% body wet weight twice daily. Fish reproductive performance was evaluated after exposure by assessing mating behavior, gonadosomatic index (GSI), fecundity, fertilization and hatching success, larval survival, and deformities. In addition, brain, liver, gonads, and blood were collected to assess the relative expression of genes associated with the hypothalamic-pituitary-gonadal (HPG) axis and circulating sex steroid levels in males and females. Gonads (testes and ovary) were collected for histopathological analysis to examine the structural integrity of reproductive tissues. Finally, male fish sperms were collected to assess the effect of arsenic on sperm abundance and motility. Chronic dietary arsenic exposure showed impaired mating behavior, decreased fecundity, GSI, fertilization rate, hatching success, larval survival, and increased larval deformities in a dose-dependent manner. Gene expression analyses suggested chronic arsenic exposure disrupted the HPG axis irrespective of gender. Arsenic also reduced sperm density and motility dose-dependently. Gonadal histopathology and plasma sex steroid levels are currently being analyzed. Overall, our findings imply that arsenic is a potent endocrine disruptor and causes reproductive toxicity in zebrafish by disrupting the HPG axis.

Exposure to metals induces beahvioral changes in fathead minnows (*Pimephales promelas*) without affecting the hypoxic ventilatory response (PL)

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Eutrophication from nutrient pollution can result in hypoxic waters leading to overall stress of aquatic ecosystems. Fish have adapted strategies to cope with hypoxia, such as increasing their ventilation rate and changing their behaviour. However, fish are often exposed to more than one stressor in the wild. Nickel (Ni) has been included on Canada's critical mineral list because of its importance in clean technology and the significant reserves that are being mined in Canada. Lead (Pb) is also mined in Canada and has great economic value due to large scale mining and manufacturing operations. This study examines the effects of Pb and Ni on the oxygen regulation and behavior in fathead minnows *(Pimephales promelas)* by observing differences in ventilation rate and activity of the fish exposed to the metals. Results showed that exposure to Ni (150 μg/L) and Pb (100 μg/L) caused an increase in activity in both normoxia and hypoxia, although there was no significant impact on ventilation rate. Ongoing experiments assessing the partial pressure where the fish loses equilibrium demonstrated that metal exposure has no impact on P_{LOE} signifying no significant difference in hypoxia tolerance. Pb and hypoxia caused a significant increase of metal accumulation in the gills. Emphasizing a multiple stressor approach, this research is essential for developing Canadian Water Quality guidelines that can be used to

effectively protect our aquatic species, as it reflects environmentally relevant challenges faced by Canadian aquatic ecosystems.

Does temperature modify the accumulation of metals and olfactory-mediated behaviours in salmonids? (PL)

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Metal pollution impacts aquatic environments across Canada and the globe. Salmonid species, in particular, are sensitive to metal exposure, such as cadmium (Cd) and copper (Cu). The goals of this study were to determine the relative sensitivity of northern fish species to closely related temperate counterparts and to understand the potential influence of climate change on the effects of metal exposure. In a chronic dose-response exposure to Cd and Cu, fish were exposed at optimal temperatures for their species. Arctic charr (northern distribution) and lake whitefish (temperate to northern distribution) both experienced impacts on olfactory-mediated behaviours at environmentally realistic metal concentrations. Assessments of metal load revealed that certain tissues, such as the gill and liver, accumulated both Cd and Cu. However, only lake whitefish accumulated Cd in the brain. Subsequent experiments examined whether temperature modified the behavioural response to metals. Fish were exposed to Cd (0.25 and 2.5 ug/L) or Cu (10 and 40 ug/L) for 6 to 8 weeks; rainbow trout (temperate species) and lake whitefish were exposed at 14 or 17°C, while Arctic charr were exposed at 8 or 11.5°C. Fish behaviours were then monitored in a Y-maze that contained a food odour in one arm. There were species- and metal-specific impacts on behaviour. For example, rainbow trout showed decreased activity (total distance travelled during the 15-minute trial) with Cd exposure and hyperactivity with Cu exposure. In contrast, Arctic charr showed changes in exploratory behaviours following metal exposure. Of the three species, lake whitefish was less sensitive to metals. Finally, temperature did not appear to modify the impact of metals on behaviour greatly, suggesting that metal concentrations, but not exposure temperature, influence metal accumulation and animal performance.

The acute osmoregulatory effects of copper on *Apistogramma agassizii*, in Rio Negro blackwater and Rio Solimoes whitewater at circumneutral and acidic (PL)

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Dissolved organic carbon (DOC) is central to the structure, function, and diversity of aquatic ecosystems and often the most abundant dissolved component in freshwater. DOC has been shown to protect against ionoregulatory disturbances and metal toxicity, particularly at low pH. However, little is known about the effects of DOC and trace metals, such as copper, on the water transport pathways. Water moves across the gills of fish through two distinct pathways, paracellularly through tight junctions and by diffusion, via the transcellular pathway through aquaporins. In the present study, the effects of copper (200 µg/L) on diffusive water flux rate (a proxy for transcellular water movement), paracellular permeability (measured by [^{3}H]-polyethylene glycol-4000 clearance), ion transport (net sodium, potassium and chloride fluxes) and nitrogenous waste (ammonia and urea) excretion rates were evaluated in the dwarf cichlid, *Apistogramma agassizii,* in two DOC sources (Rio Negro blackwater and

Rio Solimoes whitewater) at two pHs (4, 7). Our results show, for the first time, that copper tends to inhibit urea-N excretion rates, as well as ammonia excretion rates. It provides the first measurements of both water transport pathways in Amazonian fish, demonstrating that copper decreases water transport both transcellularly and through tight junctions. The protective effects of DOC against the disturbances caused by copper were dependent on the source of the DOC and the water pH. Overall, this work describes the damaging effects of copper on osmoregulation at the gill as a function of DOC source and water chemistry.

Assessing the multigenerational toxicity of lead from fishing gear to the freshwater snail, *Planorbella pilsbryi* (PL)

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Lead is a highly toxic element with no known biological function. It is estimated that over 460 tonnes of lead fishing gear are lost in Canada's waterways annually, posing a threat to aquatic organisms. Despite their importance and abundance in aquatic environments, freshwater snails (*Planorbella pilsbryi*) lack representation in environmental risk assessments. Although the toxicity of lead is well known, the toxicity of lead from fishing gear remains unknown. This project has two main objectives: (i) to determine the toxicity of lead from fishing gear to *P. pilsbryi* through a multigenerational exposure; (ii) use metabolomics to investigate the mechanisms of lead toxicity to *P. pilsbryi*. Chronic 28-d exposures were conducted with adult snails, and 21-d embryo exposures were conducted with eggs from unexposed (F_0) and exposed (F_1) adults. The lead leaching from fishing gear did not affect the reproduction or growth of adult snails, however we observed decreased growth in treatments with lead sinkers added directly to the jars, and in the positive control, 1.2 mg/L Pb. We observed decreased hatching success in the F_0 embryo generation at the highest concentrations (0.25 mg/L) of lead from fishing gear, and in the F_1 generation at lower concentrations (0.02 – 0.25 mg/L). Ongoing metabolomic analysis will provide insight into the mechanistic toxicity of lead and the physiological pathways disrupted. This work will provide fundamental knowledge for metabolomics in freshwater snails, contributing relevant data on the risk of lead fishing gear, and will provide baseline data for future efforts restricting the use of lead fishing gear in Canada.

Investigating the potential toxicity of copper nanoparticles from agricultural run-off on *Daphnia magna* (PL)

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Agricultural pesticides leach into the environment, causing detrimental effects on surrounding aquatic biota. Nanopesticides (e.g., copper nanoparticles (CuNPs)) are cost-effective with improved dispersibilities with a greater surface area for application, ensuring a more targeted pesticide delivery. While these nanoformulations are effective in pest reduction, they exhibit novel properties with unknown effects compared to conventional counterparts. CuNPs tend to aggregate and fall out of suspension, raising concerns about the long-term impacts on aquatic organisms and environmental

deposition. Understanding the toxicity of nanopesticides used in agriculture is crucial for assessing the potential risk associated with NPs and allows for better regulatory limits to protect sensitive species. This study aims to compare the acute and chronic toxicity of CuNPs with what is already established about dissolved Cu in agricultural pesticides, as CuNP toxicity is unclear. The 48-hour lethal median concentration (LC50) of CuNPs was tested using the freshwater crustacean *Daphnia magna*, as they represent a critical species in aquatic food webs. The observed acute LC50 concentration for ionic Cu was 38.5 µg/L (95% CI 32.48-46.68), while only partial mortality was observed for CuNP exposures. Furthermore, toxic effects of CuNPs on *D. magna* reproduction and growth were also observed and compared to that of ionic Cu. Data obtained seem to suggest that CuNPs are acutely less toxic compared to ionic Cu across similar concentrations and aging periods. This project is funded by the Alberta Conservation Association.

Sublethal effects of rare earth elements (REEs), Nd, Pr, and Y in *Daphnia magna* (PL)

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Neodymium (Nd), Praseodymium (Pr), and Yttrium (Y) are three rare earth elements (REEs) that occur in the mineral ore bastnaesite, the primary ore of Canada's first REE mine on Thor Lake, Nechalacho, NT. Usage of these elements is an expanding market as they are key components in various green energy equipment such as wind turbines & electric vehicle batteries. In recent years, there have been several announcements regarding the development of electric battery plants across Canada. As a result, there is a growing concern for the potential environmental risk due to anthropogenic contamination. Minimal data is currently available regarding toxicity of individual REEs and even less data is available on mixtures. The objective of this study was to investigate the bioaccumulation of Nd, Pr, and Y mixtures in *Daphnia magna*. Daphnids were initially exposed to sublethal concentrations for 24-h in an artificial soft water medium with a pH of 6.8. Mixture exposures were designed using a toxic unit (TU) approach, based on converting the EC_{50} concentrations from previously conducted acute toxicity tests to TUs and applying a matrix isobologram approach. For example, at 0.4 TUs of each element (81 µg/L Nd, 342 µg/L Pr & 364 µg/L Y), a significantly lower amount of Nd was observed in mixture exposures compared to individual REE exposures indicating that Nd accumulation was influenced by other REEs. This research is supported via an NSERC Alliance Grant with additional funding from Environment and Climate Change Canada, Stantec Inc and Cheetah Resources.

Understanding the environmental mobility and potential toxicity of elements associated with Nb tailings (PL)

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As the niobium market is expanding into renewable energy production and storage, the environmental hazards associated with niobium mining need further attention. In addition, there is limited understanding with regards to Nb toxicity to aquatic organisms. Surficial tailings samples from an active niobium mine were investigated to understand the leachability of elements and their potential toxicity. Geochemical analysis of these tailings was conducted alongside solubility tests, following the transformation/dissolution protocol, at three pH levels (6, 7 and 8.5) to measure elements of potential

environmental concern in leachates. To evaluate effects on aquatic organisms, the leachate from the pH 7 solubility test was used in chronic toxicity tests with *Ceriodaphnia dubia* and the algae *Raphidocellis subcapitata* and *Chlorella kislerii*. The results demonstrate that Ca, Mg, S, Fe, Ba, Mn, and P are the most mobile, and pH has a moderate but variable effect on leachability of elements in the tailings. Our results indicate the possibility of many elements leaching from niobium mine tailings, with some (e.g., La, Nd) being above the draft guideline values. P concentrations in the leachate are also above concentrations found in hypereutrophic lakes. Niobium was shown to be insoluble in water, therefore comparative water-only toxicity testing with Nb was not possible. Therefore, additional tests to evaluate the toxicity of particulate Nb to aquatic organisms, using spiked-sediments, were conducted with *Chironomus dilutus* to evaluate growth, survival and bioaccumulation. Results from the solubility and toxicity tests will be presented.

Evidence of eutrophication as a cumulative environmental impact problem for watersheds across Northern Ontario (PL)

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Fish population monitoring in Northern Ontario has found increased fish tissue concentrations of methylmercury. This has been attributed to contributions of total phosphorus (TP) from various sources. Increased TP loading can lead to eutrophication via enhanced primary production. General TP management is via Ontario's 1979 interim-PWQO for TP, focusing on aesthetics: preventing algal blooms, excessive macrophyte growth, and oxygen depletion. Guidance for lakes naturally below 10 µg TP/L specifies that levels should remain below this level. The 2010 Ontario Lakeshore Capacity Assessment (LCA) model provides an alternative for lakes on the Precambrian Shield allowing a 50% TP increase a model-estimated historical background level. Nevertheless, increases in TP loading in low productivity watersheds can trigger eutrophication and result in increases in fish methylmercury levels to the point where human and/or wildlife fish consumption is hazardous. Where problematic elevated fish tissue methylmercury levels are found PWQO Policy 2 provides guidance: "*Water Quality which presently does not meet the Provincial Water Quality Objectives shall not be degraded further and all practical measures shall be taken to upgrade the water quality to the Objectives.*" The LCA methodology identifies lake-specific, loading-based TP objectives, that can address PWQO Policy 2 and reduce elevated fish tissue methylmercury levels. Limitations of the aesthetics-based interim-PWQO for TP in Northern Ontario lakes, and the advantages of the LCA methodology, will be discussed using examples where fish populations show elevated tissue methylmercury compared with similar habitats with lower fish methylmercury levels, including lakes in different watersheds and lakes within a watershed.

Refining ecological risk assessment using multilevel concentration-response models: a case study on effects of copper to salmonids (PL)

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Risk assessors commonly use concentration-response models to estimate the effect magnitude at specific exposure concentrations or doses, or to derive site-specific effects concentrations or benchmarks for decision-making. Fitting concentration-response relationships to data for individual experiments is routine work for toxicologists. However, risk assessors are typically more interested in the

range of possible relationships reflecting variability across multiple studies. Multilevel models, such as mixed-effects or hierarchical models, are suitable for such analyses because they fit a global mean relationship while accounting for variability among subsets of the data. This case study uses multilevel concentration-response modeling to assess the potential effects of copper exposure for salmonids. Using data from studies supporting aquatic life guideline derivation in Canada and the US, we extracted raw concentration-response data from the original publications or through direct contact with authors. Our final dataset focused on survival, encompassing 21 experiments from 7 studies. We fit several generalized linear mixed-effect models to the data, allowing for varying intercepts or slopes among studies and experiments. After identifying a preferred random-effects structure, we incorporated known copper toxicity modifying factors (dissolved organic carbon, hardness, pH) as covariates and selected a suitable final model. To more fully account for uncertainties, we also re-fit the preferred model within a Bayesian framework. This Bayesian approach estimates a full probability distribution for the effect magnitude at specific copper concentrations and particular values for toxicity modifiers, while accounting for variability in concentration-response relationships across studies and experiments.

Subcellular fate of metals, alone and in mixtures, in the unicellular alga *Chlamydomonas reinhardtii* (PO)

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The rapid development of new technological tools worldwide is leading to an increase in emerging contaminants such as platinum (Pt), lanthanum (La) and cerium (Ce) in aquatic environments. These metals are rarely considered in toxicological studies, especially when combined with other contaminants such as copper (Cu), silver (Ag), cadmium (Cd), cobalt (Co), uranium (U) and nickel (Ni). This limits our ability to predict their toxicity and anticipate their ecological impact. To address this gap, the detoxification efficiency of these metals, their presence in sensitive cellular components potentially leading to their toxicity and the interactions of these metals are examined using the unicellular green alga *Chlamydomonas reinhardtii*. This study focuses on the intracellular fate of metals in five distinct subcellular fractions through the use of a subcellular fractionation by differential centrifugation after cell lysis. In this project, the cells were exposed to the nine metals cited above, both individually and in mixtures, for a duration of 2 hours in a simplified exposure medium, with a hardness of 80 mg $CaCO_3$/L. The distribution of bioaccumulated metals is then determined and compared between treatments. It is anticipated that "soft" metals (Ag, Cd, Cu and Pt) will be more bioaccumulated and sequestered by stable proteins in the cytosol, rich in thiol groups, while "hard" metals will be less bioaccumulated, but causing more deleterious effects due to their affinity for sensitive sites such as organelles. The next phase of our project aims to examine protein- and peptide-metal complexes in the cytosolic fraction.

Copper speciation, bioavailability and toxicity to local fish in the mixing zone of the Rio Negro and Rio Solimões along the Amazon River (PO)

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The two main tributaries of the Amazon, the Rio Negro and the Rio Solimões, converge in Manaus (AM, Brazil) and flow side by side for tens of kilometres before mixing. This remarkable phenomenon is attributed to the different characteristics of the rivers in terms of current speed, temperature, and

composition. The dark coloration of the Rio Negro ("black waters") is due to its elevated dissolved organic matter (DOM) content, while the sandy Rio Solimões ("white waters") is rich in suspended sediment. Additionally, they exhibit distinct pH and ion compositions, making them markedly distinct habitats. Such parameters are of great importance for the speciation of trace metals, i.e. their chemical composition in solution, and therefore their bioavailability, uptake and potential toxicity to aquatic organisms. The effects of copper contamination were studied on the tambaqui (Colossoma macropomum), a local fish species found in both river systems. Acute exposures were carried out in the waters of the Rio Negro, the Rio Solimões and two different ratios representative of the mixing zone (1:3 and 3:1). Copper speciation (dissolved vs. particulate, Cu-DOM complexation), bioavailability (Cu bioaccumulation in gills) and physiological effects (net fluxes of sodium, chloride, potassium and ammonia) were measured.

The influence of organic carbon on mercury food web dynamics in remote Patagonian ecosystems (PO)

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In aquatic ecosystems, dissolved organic carbon (DOC) of terrestrial origin can influence mercury (Hg) exposure by complexing with Hg and transporting it to lakes to directly increase aqueous Hg exposure or by altering diet/resource use to indirectly increase exposure and bioaccumulation of methylHg (MeHg). However, little is known about the dynamics of DOC and MeHg in freshwater ecosystems in the southern hemisphere, especially in remote ecosystems at high latitudes. Here, we investigate mercury bioaccumulation across three aquatic habitats (a river, a lake, and a wetland) in Tierra Del Fuego, Chile, and use dietary markers (carbon and nitrogen stable isotopes and fatty acid composition) to infer the influence of different organic carbon sources (allochthonous versus autochthonous) on MeHg bioaccumulation across three distinct habitats. In January 2024, we collected a range of food web compartments (biofilm, particulate organic matter, aquatic vegetation, benthic macroinvertebrates, zooplankton, and fish) to measure dietary markers and methylmercury concentrations. We will present results of preliminary analyses associating carbon resource use and habitat (inferred using stable isotopes and fatty acids) to bioaccumulation and biomagnification of MeHg. This work will contribute to a better understanding of the DOC and MeHg dynamics in under-studied aquatic ecosystems in the southern hemisphere, as well as the relative importance of autochthonous/allochthonous processes contributing to MeHg bioaccumulation in aquatic food webs.

General Ecotoxicology: Soil, Sediment, Water, Air, and Biota

Contaminants and nutrients in fishes along a large, dammed river: Do benefits outweigh risks? (PL)

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Fish provide an important source of nutrients including the omega-3 fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, contaminants, such as metals (e.g., mercury (Hg))

and organic pollutants (e.g., polychlorinated biphenyls (PCBs) and pesticides), can bioaccumulate in fish and undermine the nutritional benefits to consumers. In rivers, dams can alter the distribution and biogeochemical cycling of contaminants and nutrients, which can sometimes increase their availability and uptake in fish, thereby affecting the risks vs benefits of consuming fish at different locations along the same river. This study examined spatial and species differences in the risks and benefits of consuming fish along the Wolastoq | Saint John River (New Brunswick), which has a large hydroelectric dam and supports recreational and subsistence fishing by Indigenous communities. Smallmouth Bass, Yellow Perch, American Eel and Striped Bass were collected from locations upstream and downstream of the dam and analyzed for Hg and 30 other trace elements, organochlorine pesticides (OCPs), PCBs and FAs. Contaminant levels were compared to provincial and/or federal consumption advisories, while FA levels were compared to a daily intake of 250 mg of EPA+DHA, recommended to reduce risks of cardiovascular disease. Hg was the contaminant of greatest concern as it triggered consumption advisories, unlike the other contaminants. Species differed in their risks and benefits where higher-trophic-level Striped Bass had the highest levels of Hg concentrations, which limited consumer EPA+DHA intake if these fish were consumed safely at their Hg consumption advisory. Across sites, fish farthest upstream from the reservoir were advised for more frequent consumption based on their lower Hg concentrations and as such, allowed for greater potential intake of EPA+DHA. Nonetheless, these fish did not provide optimal intake levels of EPA+DHA if they are consumed at frequencies that are considered safe for Hg intake. Overall, this research will help inform local recreational and First Nation fishers of the risks and benefits of consuming fish along the Wolastoq | Saint John River.

Simultaneous analysis of drugs of abuse in fish tissue: Extraction optimization (PL)

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Drugs of abuse and their metabolites are concerning contaminants due to their potential adverse effects on the environment. Their presence in surface waters is primarily attributed to the limited removal efficiencies by municipal wastewater treatment plants. Their continuous release leads to persistent low-level exposure for organisms in these environments, significantly impacting the physiological functions of aquatic life. An extraction method using ultrasonic solvent extraction (USE) was optimized to enhance the extraction efficiency of 23 drugs of abuse and their metabolites. The optimization involved two steps: USE and lipid clean-up. The analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The optimized protocol involves extraction with 1% formic acid solution in acetonitrile and a clean-up step with oasis HLB solid phase extraction cartridges. Absolute recoveries ranged from 13% to 82% for normorphine and tramadol, respectively.

Development of the optimal extraction method of chiral pharmaceuticals from rainbow darter and application to an in-lab bioaccumulation exposure (PL)

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Pharmaceuticals are widespread contaminants that enter the aquatic environment mainly via wastewater effluent. Over 50% of common pharmaceuticals are chiral, and this is notable due to its

impact on the distribution, fate, and toxicity of these compounds. Historically, chirality has been overlooked when completing risk assessments. The chiral antidepressant venlafaxine (VEN) and its major metabolite o-desmethylvenlafaxine (desVEN) are pseudo-persistent through wastewater treatment and have been shown to impact aquatic biota in receiving environments. Sentinel darter species (e.g., rainbow darter, *Etheostoma caeruleum)* live downstream of wastewater treatment plants in the Grand River and are chronically exposed to effluents containing VEN and desVEN. An enantiomerically unbiased method was developed and validated for the extraction of R- and S-enantiomers of VEN and desVEN from whole, small-bodied fish. Accelerated solvent extraction (ASE) and oasis hydrophilic–lipophilic balance (HLB) solid-phase extraction (SPE) was used for the extraction and subsequent lipid cleanup for fish tissue. High recoveries and satisfactory reproducibility have been achieved, with no enantiomeric bias. The extraction method was applied to rainbow darter collected from the Grand River, to determine if there were differences in bioaccumulation of enantiomers of venlafaxine in small-bodied fish in controlled lab experiments. To do this, a 14-day exposure of wild-caught rainbow darters to R- and S-VEN was conducted. S-VEN appears to bioaccumulate more in fish than R-VEN. The metabolite S-desVEN was found in fish exposed to S-VEN for longer periods of time, however R-desVEN was not found in fish exposed to R-VEN.

Identifying developmental windows of sensitivity to diluted bitumen exposure in sockeye salmon (*Oncorhynchus nerka*) (PL)

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Subpopulations of sockeye salmon (*Oncorhynchus nerka*) in the Fraser River Watershed (FRW) are currently designated as threatened. Previously, we have shown that Pacific salmon are sensitive to diluted bitumen (dilbit), which can enter sockeye habitats in the FRW by pipeline failure. However, little is known about how the timing of exposure relative to embryonic developmental stage influences the magnitude and scope of negative outcomes. This study aims to compare key fitness metrics in sockeye embryos exposed to low, realistic concentrations of water-soluble fractions of dilbit during discrete developmental windows. The fitness metrics included cardiorespiratory performance at the swim-up stage, morphometrics, and mortality. We found that cardiorespiratory performance was compromised in sockeye salmon exposed to dilbit (6 µg/L total initial PAC), however the extent of this impairment was dependent on the developmental window in which they were exposed. Specifically, sockeye exposed at early stages of development and subsequently reared in clean water to swim-up showed no difference in performance relative to unexposed controls. However, those exposed in later stages experienced a marked reduction in cardiorespiratory performance when measured at swim-up stage. These findings suggest that sockeye salmon may be more vulnerable to dilbit exposure during the period from hatching to the swim-up stage, and that a sufficient period of depuration may mitigate the negative effects of dilbit on cardiorespiratory performance. This data is valuable for informing evidence-based decision-making in the event of oil spills and for enhancing the accuracy of risk assessments regarding the impact of such incidents on salmon populations.

A model naphthenic acid reduces oxidative phosphorylation through selective impacts on complex activity (PL)

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Naphthenic acids (NAs) are thought to be a primary contributor to the toxicity of oil sands process-affected water. Despite this, few studies have examined the mechanisms of the toxic action of NAs. Recent findings indicate that the model quaternary naphthenic acid, 3,5-dimethyladamantane-1-carboxylic acid, reduced mitochondrial membrane potential and oxygen consumption and resulted in increased emissions of ROS in isolated mitochondria in an identical fashion to extracted NAs. The purpose of the present study was to determine if a primary carboxylic acid, the moderately hydrophobic naphthenic acid, 3,5-dimethyladamantane-1-acetic acid, would also affect mitochondrial oxidative phosphorylation. It was hypothesized that this effect occurred through the inhibition of specific complexes in the electron transport chain. Mitochondria isolated from rainbow trout (*Oncorhynchus mykiss*) liver were exposed to commercially available 3,5-dimethyladamantane-1-acetic acid. The effects of these compounds on States 3 and 4 respiration and mitochondrial membrane potential were quantified using respirometry. Subsequently, each mitochondrial complex was isolated using inhibitors, and the activity of each complex was measured in response to graded doses of the substance using spectrophotometric methods. The compound 3,5-dimethyladamantane-1-acetic acid inhibited states 3 and 4 respiration and uncoupled mitochondrial membrane potential with an EC50 of 174.847 mg/L. The activity of all complexes was impaired by the NA but there was a 10-fold range in sensitivity. Complex CIV was more sensitive to naphthenic acids than CI and CII; CIII was the least sensitive. These results suggest that toxicity and mitochondrial oxidative phosphorylation are influenced by adamantane 3,5-dimethyladamantane-1-acetic acid NAs via protein complexes in the electron transport chain (Kalvani et al., 2024 Env. Tox. Pharm 107:104386).

The life-stage effects of metformin exposure in zebrafish (PL)

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Metformin (Met), a medication used to treat type 2 diabetes mellitus, regulates glucose metabolism by targeting mitochondrial pathways, enhancing glucose utilization and reducing hepatic glucose production. Recent fish studies indicate possible impacts on steroidogenic pathways that are life-stage specific, although the exact mechanisms remain unclear. This research examines how Met affects stress responses in larval and adult zebrafish following exposure to 0, 4 and 40 µg/L. Exposure of zebrafish embryos and larvae from 0 hours post-fertilization (hpf) to 24 hpf led to increased mortality and induced early hatch in the metformin groups, but the mRNA abundance of steroidogenic genes (MC2R, StAR and p450scc) was unaffected. We are currently exploring effects on the later embryonic and larval life stages at rest and following an acute stressor exposure. In the adult experiments, the metformin exposure was done over 30 days in triplicate tanks, with 30 fish in a 1:1 male:female ratio. After exposure, fish underwent acute stress via net chasing, and muscle samples were collected pre-stress (0h) and post-stress (1h, 6h). Met exposure did not significantly alter muscle lactate levels across sexes. However,

males exposed to Met showed sustained elevation in muscle glucose levels, whereas females exhibited transient effects. Muscle cortisol levels remained unchanged by Met in both sexes. These findings indicate that chronic Met exposure induces sex-specific changes in glucose mobilization in adult zebrafish, with minimal immediate effects on stress axis gene expression during early embryonic development. Further research in our lab will investigate the long-term and multigenerational impacts of Met, considering its metabolic and potential endocrine-disrupting effects in fish.

Developing site specific quality management objectives for a stormwater management pond based on biological monitoring and a statistical analysis of historical monitoring data (PL)

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This project involved the development of site-specific water quality management objectives for a stormwater management pond that collects runoff from an industrial manufacturing facility with a log storage yard. When the pond is at capacity, water is released to a drainage ditch that eventually drains to a creek. The closest distance along the flow path from the pond to the creek is approximately 1200 m. Water quality monitoring of the pond has indicated the presence of parameters at concentrations greater than surface water quality guidelines protective of freshwater aquatic life. These parameters are consistent with indicators of log yard runoff such as decaying plant material and compounds that occur naturally in trees. An *in situ* aquatic habitat assessment indicated that the flow path from the pond to the creek is well vegetated and lacks a defined channel in many places. Based on the parameters that were detected in the pond, it was hypothesized water quality would be improved via settling, dispersion, and filtration along the flow path. This hypothesis was evaluated based on surface water sampling and chemical analysis along the flow path as well as a quantitative benthic invertebrate community assessment in the creek. These investigations provided evidence that historical and recent releases from the Pond have not significantly affected downstream aquatic habitat relative to the upstream background conditions. Therefore, it was possible to develop site-specific quality management objectives based on a statistical representation of historical runoff monitoring data at the site.

Diesel fuel weathering and subsequent aquatic toxicity within a freshwater mesocosm (PL)

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Within Canada, more diesel has been spilled in the last six years than any other fuel or oil type. This is of no surprise considering that diesel is the most commonly used fuel for trucks and trains, and is a major energy source in northern regions. However, despite its extensive use across terrestrial landscapes, few studies have characterized the environmental risks to aquatic systems with natural forms of weathering. To address this knowledge gap, we conducted controlled spills of diesel (10 L) within a mesocosm spill-tank using 1,200 L of North Saskatchewan River water at 15 °C with wave-action. Water-column samples were collected weekly to measure changes in the chemical profile of petroleum constituents and the subsequent toxicity to the embryologic development of fathead minnows (*Pimephales promelas*).

Significant mortality was observed throughout the study, with 100 % embryo mortality in the 10 % dilution treatment of the spill-tank for up to 28 days post-spill. Similar experimental spills of diluted bitumen, light conventional crude oil and Bunker C fuel oil demonstrated 100% mortality to embryos only one day post-oil spill and only within the undiluted mesocosm water, demonstrating the significantly greater lethality induced by diesel fuel despite weathering. Increased prevalence of developmental malformations and cardiac deficiencies were observed within the lowest dilution (0.1 % of the spill-tank water) throughout the 35 days of weathering. This study demonstrates the high level of hazardous risk that diesel spills place on freshwater environments despite weathering for up to 35 days.

Altered Ecosystems: The Role of Pesticides in Ant-Aphid Dynamics (PL)

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There is a research gap regarding the role of ants (Hymenoptera: Formicidae) in agricultural systems, particularly in understanding how pesticide exposure affects their behaviour and interactions with other organisms. The mutualistic relationship between ants and aphids can play a significant role in agricultural crop growth. To control the detrimental impact of aphids, pesticides are commonly used. However, these pesticides could impact the beneficial services that ants provide. Understanding the balance between pest control, beneficial services offered by ants, and pesticide use is important for future agricultural land management. This study investigates this balance by testing the impact of pesticide exposure on ant-aphid interactions, aiming to elucidate the mechanisms by which chemical treatments alter these dynamics, and quantifying the services/disservices that these organisms provide. I introduced the common agricultural ant species *Lasius neoniger* and common aphid species *Myzus persicae* onto pepper plants in a controlled environment, subjected and/or absent of various pesticide stressors. Throughout the experiment, survival, behaviour, interspecies interactions, plant growth, fruit set, and seed count were recorded. The results are currently pending. Understanding the nuanced effects of pesticides on ant-aphid dynamics is crucial for developing more sustainable agricultural practices and preserving ecosystem integrity. This research underscores the need for integrated pest management approaches that consider the complex interactions within ecosystems, promoting resilience against chemical disturbances.

Variable effects of forestry on mercury biomagnification pre-and post- harvest in boreal headwater stream food webs (PL)

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Forestry can affect mercury (Hg) dynamics in Boreal stream food webs by influencing the availability and methylation of Hg and by altering dietary exposure to methylmercury (MeHg) through changes in basal resources. In headwater streams, this study compared MeHg biomagnification 1) temporally (pre-, during, and post-harvest) and 2) spatially, across harvested and non-harvested regions. Streams were sampled for basal food sources (aquatic and terrestrial), invertebrate primary and secondary consumers, and top predators (fish). To determine differences in MeHg biomagnification rates and consumer diets across years and landscapes, Hg concentrations ([Hg]) and carbon and nitrogen stable isotope ratios were measured. Temporally, we observed increases in [Hg] in fish and some predatory invertebrates

post-harvest at an extensively harvested stream but saw few differences where standard best management practices were employed. Spatially, we detected significantly higher consumer [Hg] in harvested landscapes, but no difference in MeHg biomagnification rates. On-going analyses comparing the consumer diets within stream food webs pre- and post- harvest will help elucidate effects of forest harvesting on Hg uptake. Ultimately, this work will provide new and impactful information about mercury cycling in impacted Boreal streams and may assist in the development of forestry guidelines that will minimize Hg risk to aquatic systems.

Toxicity of Granular Bayluscide® to Adult Washboard Mussels (*Megalonaias nervosa*)

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Since their invasion a century ago, sea lampreys have posed a threat to the Great Lakes because of their economic and ecological repercussions. Various control measures have been implemented to limit their abundance, with lampricides becoming one of the most effective treatments. There is growing concern about the risk posed to non-target organisms like mussels as many reside in areas overlapping application sites. To determine susceptibility of mussels to this toxin, adult washboard mussels (*Megalonaias nervosa*) were exposed to varying concentrations of Bayluscide® (ranging from 0.0002 – 0.332g) for 7 days followed by a 7-day recovery period to estimate mortality. In addition, scope for growth was determined for mussels exposed to an environmentally relevant dose (0.166g) of Bayluscide® for 24 hours. To do so, their filtering ability, rate of oxygen consumption, and food absorption efficiency were measured following exposure and calculated based on Widdow et al. (2006). No mortalities were observed in the lower treatments, however, some mussels experienced narcosis during environmentally relevant exposures and mortality was observed at doses 2x the application rate. There was a difference between the scope for growth of control mussels and those exposed to Bayluscide®, which can be partially attributed to the decrease in oxygen consumption in the treated mussels. Our results suggest that washboard mussels are sensitive to Bayluscide® and that the potential implications on mussel species needs to be considered when applying this chemical.

RNA-Seq analysis of human lung cells (BEAS-2B) exposed to retene (PL)

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Retene (RET), a major polycyclic aromatic hydrocarbon released during forest fires, is not considered a priority contaminant under current environmental legislation. Despite some studies on its toxicity, little is known about its molecular mechanisms in human lung models. The main objective of this work was to characterize the transcriptomic responses in a human lung cell (BEAS-2B) exposed to RET. RNA sequencing (cutoff ≥1.5 or ≤−1.5) revealed 447 up-regulated and 586 down-regulated genes after 24 hours of RET exposure, and 600 up-regulated and 510 down-regulated genes after 72 hours of RET exposure. Our analysis identified RET-responsive noncoding RNAs, including lncRNAs, miRNAs, and antisense RNA sequences, as well as coding RNAs (mRNAs). At 24 hours, RET dysregulated many genes involved in RNA ribonucleoprotein biogenesis and cellular and primary metabolic processes, suggesting that BEAS-2B cells are preparing for potentially increased toxicity. By 72 hours of RET exposure, dysregulated genes primarily involved in positive regulation of cellular processes (such as cell

proliferation, growth, adhesion, and activation) and cellular organization were observed. Genes related to cellular proliferation and growth (e.g., *FGF9*, *MAPK4*, and *RAB39A*) and cellular organization (e.g., *TUBB8*, *ACTN3*, and *LAMC3*) were notably altered, indicating RET's potential influence on cellular proliferation and migration. Ongoing bioinformatic analyses and experimental validations aim to establish a comprehensive link between these molecular changes and known toxic outcomes associated with RET exposure. This study provides new insights into the mechanisms underlying RET toxicity in human lung cells, particularly relevant due to its high atmospheric concentration.

Identifying neurotoxic effects of chemical warfare agents and their transformation products in the zebrafish model (*Danio rerio*) (PO)

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After large production in World Wars I and II, chemical warfare agents (CWA) were disposed of into seas worldwide, for example, an estimated 50,000 tons in the Baltic Sea. The metal canisters and casings have corroded to the extent that their toxic chemical contents are leaking into the environment, bringing into question the effects of long-term exposure to CWA contaminated water and sediment. There is little scientific literature or research on the effects of the CWAs and their transformation products on aquatic organisms. These chemicals include, though are not limited to, organo-arsinic compounds diphenylchloroarsine, phenarsazine, and triphenylarsine. Their metabolites include, among others, diphenylarsinic acid (DPAA) and diphenylthioarsinic acid; phenarsazinic acid; triphenylarsine oxide (TPAO) and triphenylarsine sulfide (TPAS). The aim of the present study is to evaluate sublethal neurotoxic effects of those CWAs and their degradation products. For this purpose, zebrafish (*Danio rerio*) are used as representative model species for pelagic fish. *D. rerio* embryos were exposed to DPAA, TPAO and TPAS at sublethal concentrations derived in pre-experiments (≤EC10: 2.5mg/L, 5mg/L and 10 mg/L) up to 120 hours post fertilization (hpf) and analyzed for biomarkers of neurotoxicity and oxidative stress and for behavioural alterations. Acetylcholinesterase activity is used as a biomarker for neurotoxic effects, and catalase, glutathione reductase and glutathione-S-transferase activity are used as biomarkers of oxidative stress. Behavioural endpoints including spontaneous tail coiling at 24 (hpf), touch evoked response at 72-96 hpf, and light-dark transition test at 120 hpf are also included as additional indicators for neurotoxic and physiological stress. This research provides new insights into the potential neurotoxic consequences of CWAs from the historical dumping events on aquatic environments and supports future risk assessment.

Ecotoxicity assessment of colored smokes and their dye ingredients (PO)

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Colored smokes have been used for decades as signaling tools and more recently for daytime fireworks. They are usually obtained from cooler-burning formula constituted of potassium chloride as oxidizer, sugar or sulfur as fuel, sodium bicarbonate as coolant and one or several organic dyes selected amongst derivatives of anthraquinone (*e.g.*, Disperse red 9, Solvent violet 47, Disperse violet 1, Solvent green 3)

or quinoline (Solvent yellow 33). Despite significant efforts invested to study the potential toxicity of these dyes to humans, very little is known about their environmental risk. In this study, four C8 colored smoke formulations (Red, Violet, Green, Yellow) currently used by the Canadian Army were thoroughly characterized. Aqueous solubility and hydrolysis/photolysis kinetics were measured for all formulations and their dye ingredients. The ecotoxicity of the individual dyes and the whole formulations was investigated by conducting aquatic tests using marine bacteria (*Aliivibrio fischeri*) and freshwater green algae (*Pseudokirchneriella subcapitata*) as well as terrestrial tests using earthworms (*Eisenia andrei*) and plants (perennial ryegrass *Lolium perenne*). Comparison of ecotoxicity of formulations and pure ingredients was used to identify the source of toxicity and more specifically to distinguish effects caused by the dyes *vs.* those caused by chlorate ions. This study provides insight on the environmental impact of unspent colored smoke formulations and the dyes they contain. Future work should focus on the characterization and environmental risk assessment of all products resulting from the cold combustion of these formulations.

Development and application of water quality thresholds to support aquatic effects monitoring and water management programs (PO)

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Water quality is paramount to sustaining aquatic ecosystem health and ultimately, biodiversity, in western Canada. Baseline aquatic studies, environmental assessments and permitting activities for developing industrial projects (e.g., *Mines Act* for mines in British Columbia, *Environmental Protection and Enhancement Act* for oil & gas facilities in Alberta) focus heavily on aquatic monitoring and management, to ensure that during operations and closure, effluents discharged into ambient waters will not adversely impact aquatic biota. Regulatory aquatic life guidelines, based on ecotoxicity test data from the literature, are inherently conservative to ensure that ambient waterbodies across the province are protected. The degree of conservatism varies by jurisdiction, and therefore guidelines vary even when based on the same underlying data. While these guidelines are not directly relevant for assessing risk, they can be useful for selecting contaminants requiring assessment, and as a starting point for developing permit limits or other benchmarks. Physico-chemical parameters that modify the toxicity of industrial effluents and downstream waters (e.g., pH, hardness, dissolved organic carbon), are now used to derive risk-based, site-specific thresholds; these draw upon a synthesis of toxicity studies, mathematical models, and empirical field data. We will provide an overview of various water quality thresholds that are used to evaluate monitoring data and support adaptive management in aquatic environments in western Canada. Case studies will highlight recent challenges and opportunities associated with applying these thresholds, in particular, when trying to integrate traditional ecological knowledge and “western” science paradigms.

Development of an enantioselective LC-MS/MS method for the separation and quantification of o-desmethylvenlafaxine and tramadol in fish tissue (PO)

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Venlafaxine (VEN) and its metabolite *o*-desmethylvenlafaxine (ODV) are serotonin and norepinephrine reuptake inhibitors (SNRIs) that have antidepressant properties. These small organic molecules have many structural isomers, each of which can lead to issues in their identification by liquid chromatography tandem mass spectrometry (LC-MS/MS). One such isomer of ODV that has been identified as a possible issue is Tramadol (TRA), which is an opioid analgesic and SNRI prescribed to relieve pain, that is also found in wastewaters. These compounds have been detected in surface water partly due to their incomplete removal by wastewater treatment plants. Recent studies indicate there is enantioselective bioaccumulation of these chiral compounds in aquatic organisms (e.g., fish). To measure concentrations of these compounds, chiral LC-MS/MS methods are typically used. With some current methods, *R*-TRA and *S*-ODV co-elute, leading to incorrect quantification. A new chiral LC-MS/MS method was developed capable of separating TRA from ODV in wastewaters. This allows for the accurate quantification of enantiomers and determination of differences in the enantiomeric bioaccumulation of these contaminants in fish.

Integrated Chemical Mixtures Project (PO)

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Recent amendments to the *Canadian Environmental Protection Act, 1999* (CEPA) recognize, for the first time in federal law, that every individual in Canada has a right to a healthy environment. In December 2023, in support of the right to a healthy environment implementation, Environment and Climate Change Canada (ECCC) launched the Integrated Chemical Mixtures Project (ICMP). Over four years, the ICMP will conduct detailed studies on chemical mixtures in Brantford and Sarnia, Ontario. The objective of the project is to develop a proof of concept for a new approach for chemical mixtures based on real-world exposure to mixtures, via research and monitoring activities including targeted, non-targeted and effects-based analyses. To carry out this project, coordinators, researchers, risk assessors and risk managers from ECCC, as well as university researchers, representatives from Indigenous communities, the Ontario provincial government and local stakeholders were mobilized. More than twenty sub-projects have now been initiated. These projects are intended to be integrated and collaborative, reflecting the concerns of local communities. Samples across environmental compartments, including air, water, sediment, soil, and wildlife will be collected to better understand the composition of chemical mixtures, study their effects and identify their sources. The ICMP will expand the knowledge base to protect the right to a healthy environment; notably, to generate knowledge on real-world exposure to, and effects from, chemical mixtures in the environment.

Perfluoroalkyl Acids in crayfish, snails and water upstream and downstream of airports and wastewater treatment plants in Ontario (PO)

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Per- and polyfluoroalkyl acids (PFAAs) are synthetic surfactants extensively used in various industrial and consumer products. Although the production or use of many PFAAs have been restricted, due to their environmental persistence many of these compounds remain prevalent in ecosystems. This study examines the concentrations and bioaccumulation factors (BAFs) of PFAAs in crayfish and aquatic snails upstream and downstream of airports and wastewater treatment plants (WWTPs) in Southern Ontario, Canada. Sampling of both biota and water was conducted from late Summer to early Fall, from 2018 - 2023. C4-C18 perfluorinated carboxylic acids (PFCAs) and C4-C10 perfluorinated sulfonic acids (PFSAs) were measured using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS). Concentrations of PFAAs in biota (crayfish: 0.3 to 92.3 ng/g; snails <MDL to 14.6 ng/g; [wet weight whole body homogenate]) and water (11.6 to 426.2 ng/L) were very site specific, but generally sum PFAAs concentrations were higher downstream of airports, particularly in Hamilton with a notable increase in sum PFSAs. The influence of WWTPs on PFAA concentrations were minimal compared to airports. In situ BAFs increased with PFAA chain length up to C11 for both snails ($\log_{10}$ BAF = -1.27 to 1.33 L/kg wet weight) and crayfish ($\log_{10}$ BAF = 1.13 to 3.78), with crayfish having higher BAFs than snails for given PFAAs. The findings highlight ongoing PFAAs emissions, underscoring the need for continued monitoring and mitigation strategies.

A proposed mechanism to explain how niclosamide synergistically interacts with TFM to increase lampricide toxicity in sea lamprey (*Petromyzon marinus*) (PO)

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Applications of the lampricide TFM (3-trifluoromethyl-4'-nitrophenol) to Great Lakes tributaries have been highly successful at controlling invasive sea lamprey (Petromyzon marinus) populations. Sea lamprey are more sensitive to TFM than non-target fishes due to their limited ability to detoxify TFM. When combined with another lampricide, niclosamide (0.5 – 2.0 %), a well-known molluscicide, the TFM concentration needed to maintain its effectiveness is reduced by up to 40 %. Both lampricides exert their toxicity by disrupting cellular mitochondrial ATP production. However, the mechanism(s) of the interactions between TFM and niclosamide (Nic) is not completely resolved. To evaluate how TFM and niclosamide interact, the concentration additivity or toxic unit (TU) model was used, where 1 TU is defined as the 12-h LC50 for each chemical. Accordingly, if sea lamprey were exposed to 0.5 TU of each lampricide, then the response in TUs would equal 1 if interactions were strictly additive, <1 if less than additive (antagonistic) or >1 if greater than additive (synergistic). When larval sea lamprey were exposed to a range of TFM-Nic combinations, observed mortality was >1 TU, suggesting the interactions were synergistic. Using LC-MS/MS, TFM was then quantified in the liver and muscle of lamprey exposed to the same concentration of TFM in the presence or absence of niclosamide (1 %). There was much greater TFM accumulation in muscle when niclosamide was present compared to the TFM-only treatment. We

conclude that impaired TFM detoxification in the liver by niclosamide explains why these two lampricides interact in a synergistic manner.

Investigation of air and thermal flows, and energy efficiency between a conventional (CE) and enhanced entrance (EE) design (PO)

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Entrances historically retained their conventionality and were used to designate passages between two separate temperature environments. As such, the use of atmospheric pressure to impede the intermix and energy exchange remains a promising but not well explored avenue in the research. This project reviews the conceptual basis and performance of the EE. A small scale model of the closed, CE and EE was constructed to examine forty-eight energy exchange conditions, among them energy consumption, velocity vectors, and temperature change. Universal kriging interpolation algorithms were created from more than 27 million samples. Results indicate the EE did retain thermal energy and toxins compared with the CE which developed sloped isotherm lines and air flow that enabled and maintained thermal exhaust and a single layer density current. Conversely, the EE developed horizontal isotherm lines and a two layer density current to recirculate and retain thermal energy and toxins. With a 0.2 Pascal pressure difference and a stabilized two layered density current, the energy consumption compared with a CE was reduced by 64% to 88%. Additionally, the energy consumption compared with a closed entrance remained steady as it increased by 33% to 37%. Instead of relying on the atmosphere as a location for disposal, the proposed technology scores high both in efficiency and eco-friendliness, reducing thermal energy loss and toxins released into the environment at their “point of source”.

Elemental composition of foraged foods in Sudbury-Manitoulin District, Ontario (PO)

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Foraging, driven by the perceived nutritional benefits of wild foods and ever increasing grocery prices, is a common practice among locals in Northern Ontario. Some of the most popular wild foods collected for either personal consumption or commercial marketing are ostrich fern fiddleheads (*Matteuccia struthiopteris*), lowbush blueberries (*Vaccinium angustifolium*), and various species of mushrooms. Fiddleheads are harvested in spring before unfurling into ferns, mushrooms are gathered throughout the year, with peak blueberry collection season being in July. After consultation with local foragers, a series of sites were described and sampled for both soils and food from across the Sudbury-Manitoulin District for the fiddlehead sub-study. The collected fiddleheads and soils are being dried, ground, and digested with strong acids using an automated digestion-dilution system for analysis of the digest solutions by ICP-MS. The soil samples are being characterized for routine agronomic parameters, as well as extracted with a lithium nitrate solution to provide an estimate of the relative bioavailability of key nutrient elements. The results will be used to inform local foragers of the elemental composition of their foraged foods, and also to describe the natural and regional variability throughout the Sudbury-Manitoulin District.

The Past, Present, and Future of Wastewater Effluent Ecotoxicity

Assessing the neurotoxic effects of benzalkonium chlorides (BACs) in early-life stage zebrafish (*Danio rerio*) (PL)

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The prevalent use of sanitizers and disinfectants during the worldwide COVID-19 pandemic has led to the increased occurrence of antimicrobials in municipal effluents and receiving surface waters. Benzalkonium chlorides (BACs) are quaternary ammonium compounds that is widely used as an active ingredient in products used for sanitation. Currently, there is a lack of toxicological data concerning the effects of these emerging antimicrobials, especially with regard to their potential neurotoxicity. In this research, the neurotoxic effect of BACs was investigated using a combination of behavioural assays and molecular analysis in early-life stage zebrafish (*Danio rerio*). An initial fish embryo toxicity (FET) test was conducted to determine the effective concentration (EC10) at which 10% of the population showed adverse effects. Behavioural endpoints of interest included stimulus-induced response, anxiety-like movements and social interactions that were measured using an automated behavioural tracking system. Additionally, mRNA from zebrafish larvae was extracted and analyzed for transcriptomic changes. The integration of behavioural measurements and molecular data will provide a better understanding of how BACs can exert its potential neurotoxic effects using a well-studied fish model for neurotoxicity. This research highlights the importance of the utilization of other toxicological endpoints, including behaviour, to fully assess the risk of emerging antimicrobial compounds of concern, such as BAC. The data from this study will be used to compare with a relevant freshwater fish species of ecological relevance in North America, rainbow trout (*Oncorhynchus mykiss*), and fill in data gaps associated with the hazard potential of these chemicals to native fishes.

Evaluating the effects of benzalkonium chlorides (BAC) exposure on early-life stage and juvenile rainbow trout (*Oncorhynchus mykiss*) (PL)

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In recent years, the use of antimicrobial-containing disinfectants and personal care products, such as benzalkonium chlorides (BACs), has significantly increased. Discharge of BACs into surface waters via municipal wastewater leads to pseudo-persistence in aquatic environments and concerns about their health on non-target organisms. Limited information is available on the toxicity of BACs to aquatic organisms, particularly on fish – key indicators of aquatic ecosystem health. This study assessed the apical and sublethal impacts of BAC exposure on the early-life and juvenile life stages of rainbow trout (RBT, Oncorhynchus mykiss), a salmonid species of ecological, commercial, and recreational importance in North America. Newly hatched RBT embryos were exposed to BACs (0.31 – 320 µg/L) and water control until 28 days post-hatch (dph). Survival, incidence of abnormalities, and time to swim up were recorded, and morphometrics and gut microbiome changes were assessed at the end of the exposure. After 96 hours of exposure, sub-samples were analyzed for transcriptional responses in whole-body larvae using RBT-specific EcoToxChips. BAC exposure had no significant impact on survival, developmental abnormalities, morphometrics or time to swim up. Differentially expressed genes

quantified using the EcoToxChip included those encoding for proteins that regulate lipid biosynthesis, protein storage, oxygen and fatty acid transport. Juvenile-aged RBT (6 months old) were exposed for 96 hours to BACs (2 – 1,250 µg/L) and water control. Exposure for 96 hours to BACs resulted in no significant impact on survival or morphometrics. Results address knowledge gaps on this ecologically relevant salmonid species and the potentially toxic effects on fish.

Comparative sensitivity of early-life stages of three Canadian freshwater fish species to antimicrobial compounds (PL)

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The widespread use and occurrence of triclosan (TCS), a legacy antimicrobial compound, has led to concerns about its potential toxicity and environmental impact. Subsequent restrictions on TCS use, along with the COVID-19 pandemic, have resulted in increased use of alternative antimicrobials like chloroxylenol (PCMX). Although PCMX has been measured in wastewater and freshwater systems, its potential toxicity to aquatic organisms, particularly fish, remains understudied. This study assessed the developmental impacts of TCS and PCMX on three ecologically-relevant Canadian fish species: rainbow trout (RBT, *Oncorhynchus mykiss*), lake trout (LKT, *Salvelinus namaycush*), and white sucker (WS, *Catostomus commersonii*). Early-life stage (ELS) exposures were conducted where newly-hatched fish were exposed to increasing nominal concentrations (solvent control, 0.38 to 400 µg/L) of TCS or PCMX. Embryos were sub-sampled 4 days post-hatch (dph) to assess early transcriptional responses and remaining fish were exposed through 28 dph for RBT and WS and 50 dph for LKT (2 weeks after swim-up). Mortality, deformities (spinal curvature, edema, and jaw deformities), swim-up time, length, and weight were monitored throughout the study. Calculated LC50s for TCS were 187.7, 61.1, and 125.3 µg/L, and for PCMX were 265, 54.5, and 148.9 µg/L for RBT, LKT, and WS, respectively. Both TCS and PCMX exposure had increased the incidence of deformities in RBT and LKT but not in WS. LKT was the most sensitive species to antimicrobial exposure when considering lethality and developmental abnormalities. Exposure time for LKT (50 days) was longer than RBT or WS (28 days), thus accumulation and longer exposure time may have resulted in lake trout being more vulnerable to adverse effects. These findings support TCS as a toxicant to fish and suggest that PCMX may pose similar risks. Characterizing developmental impacts on ecologically important species is crucial for informing effective risk assessments and protective measures against antimicrobial pollution in aquatic environments.

Multi-generational effects of naphthenic acids isolated from oil sands process-affected water effluent on *Daphnia magna* (PL)

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Oil sands process-affected water (OSPW) is a wastewater generated from the extraction of bitumen during surface mining activities in northern Alberta oil sands. OSPW is a highly complex chemical mixture comprised of both inorganic and organic constituents, and the organic O_2 naphthenic acids (NAs) have been identified as one of the primary toxic components in OSPW. While all OSPW is currently held in large tailings containments, characterizing the toxicity of these mixtures is critical for remediation, monitoring, and regulatory initiatives, and there is little understanding of the chronic and sub-lethal

effects these mixtures might have on biota. The acute sensitivity of the ecologically relevant freshwater cladoceran *Daphnia magna* to NAs isolated from an active OSPW sample was determined by an LC50, found to be 40.3 mg/L (95% CI 24.2-57.5). A chronic multigenerational exposure is underway using sub-lethal NAs concentrations to determine *D. magna* responses of the F0 generation though to the F3 generation. Survival, growth and reproduction (i.e., neonate production) will be assessed for all generations, and oxygen consumption rates will also be considered at the end of each generation to provide insight into metabolism. These findings will illustrate the ability of *D. magna* to contend with NAs and reveal any long-term effects, as well as potential species resilience. This research will help inform remediation strategies, priorities, and policy pertaining to tailings pond management while providing insight into potential ecosystem recovery.

Assessment of cytotoxicity and endocrine disruption potential of water samples from the Frank Lake wetland complex (PL)

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The Frank Lake Wetland Complex near High River, Alberta, Canada, began receiving pretreated wastewater from a nearby meat processing plant and the town of High River after restoration work by the Canadian Lakes and Rivers Alliance in 1989. To assess the long-term and potential impacts of these wastewaters on the Frank Lake wetland system, an investigation was conducted by collecting water samples at Frank Lake to measure cytotoxicity and endocrine disruption. We exposed RTgill-W1 fish cells to seven concentrated water samples collected from different locations and times to evaluate cytotoxicity. Cell viability was compared across different concentrations of the water samples. For with cell viability exceeding 80%, we will use estradiol (E2) and dihydrotestosterone (DHT) as standard curve for human cell lines T47D-Kbluc and MDA-kb2. These cell lines will be exposed to different concentrations of the water samples to predict the impact of endocrine-disrupting chemicals on the human estrogen (ER) and androgen receptor (AR) transactivation. Our current experiment shows that the water sample extracts from Frank Lake did not cause significant cytotoxicity in fish gill cells. However, the long-term and potential effects on ER/AR transactivation require further experimental validation. These assays could help relevant stakeholders understand the effects of xenobiotics on fish and mammals in the surrounding areas and make improvement based on our results.

Fate of pharmaceuticals in aquatic-riparian food webs at sites receiving municipal wastewater discharges (PL)

John Fast[1], Karen Kidd[1]
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Wastewater treatment plant (WWTP) effluents contain complex mixtures of pharmaceuticals due to their incomplete removal or transformation during treatment. This results in detection of multiple classes of pharmaceuticals in receiving waters downstream of WWTPs. Aquatic insects inhabiting receiving waters may bioaccumulate these compounds and transfer them to riparian predators as they consume emergent adults. This study quantifies the concentrations of pharmaceuticals in aquatic insects and riparian spiders to assess the fate of aquatic contaminants in downstream ecosystems impacted by WWTP effluents. Several aquatic larval and terrestrial adult insects representing different feeding groups, and nearshore spiders, were collected in the summer of 2022 upstream and downstream of four WWTPs

along the Grand River. Chemical analysis is ongoing to determine pharmaceutical concentrations in whole body invertebrates. A modified QuEChERS method with matching internal standards is being used to compensate for interferences across multiple taxa and life stages. 20 Pharmaceutical targets and select metabolites, which may impose as much risk to aquatic life as the parent compound, were selected for analysis. Classes of targets include antidepressants, anticonvulsants, antibiotics, and anti-inflammatories commonly detected in surface waters. Pharmaceutical data will be paired with stable isotope analysis of relative trophic position ($\delta^{15}N$) and food source ($\delta^{13}C$). Based on previous research, larval invertebrates downstream are predicted to contain more pharmaceuticals compared to those present upstream of WWTPs. Bioconcentration due to mass losses during metamorphosis are predicted to result in increased pharmaceuticals in emergent adults. Consumption of emergent adults by riparian spiders may result in similar aquatic contaminant profiles in terrestrial predators. This data will add to our understanding regarding the fate of pharmaceuticals in aquatic food webs and the risks posed to riparian predators consuming emergent insects along the boundaries of aquatic ecosystems.

Combining innovative survey techniques to investigate the correlation between waterbirds and other biotic factors on Escherichia coli and total suspended solids in sewage treatment centre polishing ponds (PL)

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With the global loss of natural wetlands, waterbirds have become increasingly reliant on alternative and artificial habitats, including wastewater polishing ponds. The polishing ponds at the West End Sewage Treatment Plant (WESTP) in Winnipeg, Manitoba is frequented by thousands of waterbirds during fall migration. The WESTP releases treated wastewater into a multi-celled former lagoon system for cooling and passive UV treatment prior to discharge as treated effluent to the Assiniboine River. A study was launched in the fall of 2023 to investigate how waterbirds and other wildlife may be contributing to occasional exceedances of total suspended solids (TSS) and *Escherichia coli* (*E. coli*) in the effluent discharge. The abundance and distribution of waterbirds at the WESTP was documented using a combination of ground-based visual surveys, drone surveys photographing birds on the treatment cells, and a time-lapse camera. The presence of fish (e.g., common carp [*Cyprinus carpio*]) was assessed using environmental DNA (eDNA) metabarcoding analysis, and multi-parameter water quality sampling provided data to track changes in TSS, *E. coli*, and chlorophyll-a. Waterbird surveys at the WESTP resulted in the detection of over 25,000 birds from 38 species. Eight fish taxa were detected in eDNA samples collected from the WESTP. Water quality monitoring showed that TSS and *E. coli* increased from mid-summer to fall, corresponding with the fall migration period for waterbirds. While waterbirds are a probable source of *E. coli,* statistical analysis did not reveal a direct correlation between bird abundance and TSS or *E. coli* at the WESTP. TSS exceedances correlated with high concentrations of chlorophyll-α at the effluent discharge location suggesting that algae are likely a major contributor of TSS exceedances. Higher levels of chlorophyll-α and *E. coli* at the effluent discharge location may be influenced by the physical characteristics of the lagoon cells as effluent moves through smaller, algae- and sediment-rich lagoon cells before discharge.

A global review to examine study design considerations for detecting impacts of municipal effluents on fish reproduction (PL)

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The main objective of the current review was to determine the potential application of the Canadian Environmental Effects Monitoring approach in studying municipal effluent impacts on fish reproduction with focus on whole organism endpoints and the potential influence of reproductive life strategy on interpretation of data collected outside of the species-specific recommended time of sampling. 139 studies were reviewed (containing 175 cases of fish species) published between 1999-2023. The review included collections of 74 fish species from 33 families, with >70 endpoints examined, and >90% of studies reported some reproductive impact. A variety of challenges were seen, including small sample sizes, lack of appropriate reference sites, pooling of sexes, mixing immature and mature fish, and inadequate timing of sampling. The review showed that 64% of species were sampled within the recommended time window. Only three endpoints were sampled in more than 50% of studies; gonadosomatic index (GSI) was reported to be statistically different in 66% of cases, gonadal histology was different in 62% of cases, and condition factor (K) was different in 47% of cases. While apical endpoints such as GSI, liversomatic index, and K are critical to understanding the ecological relevance of changes, two of these endpoints were reported in <33% of studies. The pattern of responses in whole organism characteristics reflected eutrophication, toxicity, and endocrine disruption in approximately equal proportions. Studies would benefit from better timing of sampling, that corresponds to reproductive life strategy of species of interest, larger sample sizes, and a greater inclusion of whole organism endpoints in reproductive responses studies.

Lessons learned from whole-lake additions of a synthetic estrogen and its broader impact (PL)

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Municipal wastewaters contain complex mixtures of chemicals, including natural estrogens and the synthetic estrogen used in the birth control pill (17α-ethynylestradiol; EE2), to which fishes living downstream are continuously exposed. Three decades of research has shown that estrogens and their mimics in these wastewaters are affecting the endocrine system and sexual development of fishes worldwide. A whole lake experiment was done at the IISD-Experimental Lakes Area in northwestern Ontario from 1999-2010 to understand whether EE2 (~ 5 ng/L) directly affects fish populations and their supporting food web. EE2 led to high levels of vitellogenin, the presence of eggs, and delayed sperm cell development in male fishes and the near extinction of fathead minnow from the lake due to its reproductive failure. When the additions of EE2 stopped, mimicking improved treatment of municipal wastewaters, the fish recovered completely. However, the reduced abundances of small-bodied fishes resulted in indirect effects on other trophic levels including a decline in the lake's top predator, lake trout, and an increase in several invertebrate taxa likely due to reduced predation pressure. This talk will highlight key results from the study and how/whether it and other studies have influenced the regulation of estrogens in municipal wastewater effluents worldwide.

Reflecting on the environmental issues and progress in the Grand River, Ontario (PL)

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Over the last century the Grand River has been rehabilitated from an open sewer to a relatively healthy watershed providing numerous environmental services and benefits to the local community. As one of the most rapidly growing regions in Canada, with more than a million people, and one of the most intensive agricultural areas in Ontario, the river and its ecosystems continue to be impacted by past activities (e.g., urbanization, wastewater, agricultural runoff, dams, habitat loss), as well as emerging threats (e.g., chemicals of concern). Studies have shown that endocrine disrupting substances, such as natural and synthetic hormones, may cause reproductive disfunction in fish. Early studies in the Grand River showed that a sentinel species, rainbow darter (*Etheostoma caeruleum*) displayed biological changes that included altered gene expression, sex steroids, secondary sex characteristics, and expression of high rates of intersex. However, over the last two decades the Region of Waterloo has upgraded its main wastewater treatment plants to reduce nutrient pollution and prevent eutrophication and resulting oxygen depletion downstream. Studies conducted over two decades while these upgrades occurred, demonstrated that these infrastructure investments have greatly reduced the downstream impacts, with most indicators of fish health returning to near reference conditions. However, many contaminants remain elevated, even in effluents from advanced treatment facilities, and the influence of these mixtures remains a concern. In addition, the combination of several other stressors in the watershed may represent potential for cumulative effects which continue to be difficult to assess or remediate.

Teat to Teeth: Mobilizing Food Sovereignty and Indigenous Nations in Environmental Effects Monitoring (PL)

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Canada monitors both paper and mining effluents through the Environmental Effects Monitoring (EEM) program. EEM strives to provide robust scientific methods to ensure minimal or no impacts of paper or mining effluents on receiving waters; however, this has resulted in a lack of consultation with local communities, especially Indigenous communities, thereby removing their perspectives and monitoring from EEM practices. In this theoretical paper, I propose that both food sovereignty and slow violence/toxic space frameworks should be mobilized in EEM practices to ensure that all parties are considered and consulted. Using Manoomin (wild rice), Lake Sturgeon, and Breast milk as case studies, I will discuss the importance of intersectionality, consultation, and identity in monitoring and research regarding defining impacts and what constitutes an affected environment. In extension to staple foods, monitoring should also integrate communities' members who live near or on-site since they are themselves members of the environment. Interviews, archival work, and qualitative should be utilized in EEM in tandem with theoretical frameworks to ensure that all parties are satisfied with thorough monitoring efforts.

Non-targeted chemical examination of water and sediment extracts collected from an industrial and municipal effluent-driven wetland complex in southern Alberta, Canada (PO)

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The scarcity and deteriorating quality of freshwater are growing, widespread issues being intensified by rapid urbanization and industrial development. Recently, efforts towards investigating the ability of wetlands to remediate a wealth of contaminants within municipal wastewater are underway due to their low costs and minimal impact on the environment however, further work is required to assess these effects on industrial effluent. The unique opportunity to monitor the capability of a natural wetland to remediate contaminants from municipal and industrial sources lies within the Frank Lake wetland complex and Little Bow River in southern Alberta, Canada, which receives partially treated wastewater from both the municipality of High River and a Cargill beef processing plant. Non-targeted analyses *via* UHPLC-HRMS uncovered 349 and 350 chemicals within water extracts from Frank Lake across 2022 and 2023, and another 350 compounds within the sediments from both Frank Lake and Little Bow River. The use categories representing the greatest fractions of chemicals detected include endogenous metabolites, therapeutics and prescription drugs, industrial chemicals, and extractables/leachables. Spatiotemporal trends expose little differences in total peak areas of chemicals within water extracts across spring, summer, and fall within a single year of sampling however, annual differences suggest a near halving of total chemical abundance from 2022 – 2023. Continued effort is underway to evaluate the toxicological potential of each unique chemical found within media from Frank Lake and Little Bow River. Future work will involve characterizing the chemical inventory of whole fish collected from the Frank Lake area as well.

The effects of benzalkonium chloride on freshwater zooplankton in a mesocosm experiment (PO)

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Benzalkonium chloride (BAC) is a potent antimicrobial commonly used as the main active ingredient in non-alcohol-based disinfectants and sanitizers. As such, BAC use has increased drastically in industrial, clinical, and household settings since the onset of COVID-19. Such ubiquitous usage and its recalcitrance have led to the continuous discharge of this compound into surface waters primarily via wastewater treatment plant effluent, exposing downstream aquatic biota. Little is known about the effects of chronic BAC exposure on aquatic biota, particularly lower-trophic-level organisms such as zooplankton. In the summer of 2023, a six-week mesocosm study was conducted at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) to assess the effects of chronic BAC exposure on zooplankton abundance (#/L), Inverse Simpson's Index of diversity, and reproductive rates (# of eggs/female) at five environmentally relevant concentrations (nominal 20, 112, 632, 3556, and 20 000 ng/L). Zooplankton samples were collected and preserved at one-week intervals and were identified to species and life stage using a stereoscope. Water quality and BAC samples were also collected weekly. Preliminary analyses of water quality data show clear decreases in dissolved oxygen in the mesocosm treated with the highest concentration of BAC (20 000 ng/L), indicating changes in ecosystem metabolism. Preliminary analysis of zooplankton samples indicate that communities are dominated by

the cladoceran *Bosmina*. Analyses of BAC effects on zooplankton endpoints are ongoing. This work will aid in understanding the broader, ecosystem level effects of BACs and will help to inform future decisions relating to Canada's freshwater resources.

Assessing the reproductive status of fathead minnows (*Pimephales promelas*) exposed to Frank Lake, AB, Canada effluent as a bio-indicator of ecosystem health (PO)

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Municipal and industrial wastewaters are complex chemical cocktails, including personal-care products, pharmaceuticals, and pesticides, many of which are endocrine disrupting chemicals. Frank Lake (FL) is a multi-basin wetland in southern Alberta, Canada, that was restored to stable water levels in 1990 with the addition of a combined effluent from the town of High River and the Cargill beef processing. At high precipitation levels, water from FL drains into the Little Bow River that ultimately drains into the Twin Valley Reservoir, Alberta's newest reservoir. The biological effects that input of largely uncharacterized chemicals might be causing in the wetland, river, and multi-use reservoir are unknown. The study objective is to assess the reproductive physiology of fathead minnows exposed to the combined effluent from High River and Cargill. Standard 96-hr and 21-day assays are being performed to assess reproductive toxicity. Fecundity and fertilization success are being measured to indicate reproductive performance. Plasma concentrations of sex steroids and vitellogenin, expression of genes along the hypothalamus-pituitary-gonad-liver axis, histological assessment of gonads, and a novel oocyte maturation assay are being used to determine mechanisms of any reproductive impairment in male or female fish. To complement laboratory assays, molecular and biochemical mechanisms that regulate reproduction are being assessed in male and female fathead minnows captured form Frank Lake and from a reference lake that does not receive effluent. This research will expand the understanding of industrial and municipal effluent impact on aquatic ecosystems, including health of exposed biota, and inform future management decisions regarding Frank Lake.

Investigating the Behavioral Response of Freshwater Mussels to Venlafaxine Exposure (PO)

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The presence of many types of pharmaceuticals in aquatic ecosystems has raised concerns about their potential impact on the organisms that thrive there. Venlafaxine, a commonly prescribed antidepressant, is among the emerging contaminants found in aquatic environments. This study examines the behavioral response of freshwater mussels to venlafaxine exposure, aiming to elucidate potential sublethal effects on this ecologically important species. Mussels were exposed to environmentally relevant concentrations of venlafaxine in controlled laboratory conditions. The mussels tested were recorded via GoPros above their aquaria, set on a timelapse series for 8 hours each day. Behavioral endpoints include valve opening, ability to bury, and foot presence over time. Preliminary results suggest significant alterations in mussel behavior following exposure to venlafaxine, with potential implications for their ecological interactions and population dynamics. Understanding the behavioral responses of mussels to pharmaceutical contaminants like venlafaxine is crucial for assessing the broader ecological consequences. Mussels that are incapable of burying themselves or retracting their foot back into their shells leave them at risk of being predated on and thus declining in mass within their aquatic ecosystem.

Pollution's Power Play: The Effects of Temperature and Contaminants on Aquatic Species

Venlafaxine and elevated temperatures impact liver mitochondrial function in Nile tilapia (PL)

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Aquatic environments are inundated with anthropogenic stressors including climate change associated heat waves, and pharmaceuticals released from wastewater treatment plant effluent. Fish living in these environments are consequently exposed to a mixture of stressors which could have additive or synergistic effects to their metabolism. Previous work in zebrafish has demonstrated that venlafaxine (an SNRI antidepressant) impacts brain mitochondrial oxidative phosphorylation (OXPHOS) *in vitro*, and that venlafaxine has an interactive effect with elevated temperatures impacting routine metabolic rate. Thus, we investigated the interactive effects of contaminants and warming on liver mitochondrial function in Nile tilapia (*Oreochromis niloticus*). We performed high-resolution respirometry and enzyme assays on liver mitochondrial isolates, with acute *in vitro* exposure to both elevated temperatures (25 [control], 35, 40, and 45 °C), and environmentally relevant concentrations of venlafaxine (0.001 to 10 µg/L). We found that elevated temperatures and venlafaxine impacted mitochondrial OXPHOS respiration through Complexes I&II (entry point complexes for electrons into OXPHOS) and Complex II alone, and that there was a significant interaction of stressors impacting Complex IV-linked respiration (Complex IV is the terminal step of the electron transfer system). Interestingly, these effects were not mirrored in enzymatic assays for Complex II (succinate dehydrogenase) and Complex IV (cytochrome c oxidase) as warming and venlafaxine only affected succinate dehydrogenase, but not cytochrome c oxidase activity. Overall, our results suggest that these stressors have interactive effects on Nile tilapia liver mitochondrial function, and that enzyme activity measured alone may be less sensitive than respiration of intact mitochondria.

The effect of temperature on the nocturnal behaviour of yellow perch (*Perca flavescens*) (PL)

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Global water temperatures are anticipated to rise due to anthropogenic activities, affecting the development of aquatic species. The yellow perch (*Perca flavescens*) is of great economic and cultural importance to Ontario and has experienced significant population declines in recent years. Previous work in our lab has demonstrated that increases in incubation temperature can lead to changes in light sensitivity, primarily through modulation of eye development in yellow perch. Whether this can lead to changes in behavioural responses to light, however, has yet to be tested. We tested the hypothesis that increases in temperature during embryonic rearing could lead to disruption of circadian rhythms. We recorded swimming through a 24-hour period to assess circadian responses of yellow perch larvae reared at 12, 15, and 18°C. The results suggest that yellow perch are a nocturnal species, moving more in nocturnal over diurnal periods, by 1-day post-hatch. 18°C fish appear to be more active in the nocturnal period, possibly an adaptive change in responses to alterations in light sensitivity. We measured the performance of yellow perch at 30-days post-hatch in a feeding trial. In diurnal windows, yellow perch reared in colder temperatures appear to eat more than fish reared in warmer temperatures. However, when recorded during a nocturnal window, fish reared in warmer temperatures appear to outperform

colder conspecifics. Together, these results suggest that in response to increased incubation temperature, yellow perch larvae have altered circadian rhythms in movement and foraging, with an increase in nocturnal periods at the cost of diurnal periods.

Higher temperatures increase juvenile and adult mummichog (*Fundulus heteroclitus*) growth: Examining the role of the GH-IGF1 pathway under combined exposure to elevated temperature and ammonium chloride (NH_4Cl) or 17α-ethinylestradiol (EE_2)

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Ammonia and 17α-ethinylestradiol (EE_2), a synthetic estrogen, are well-studied stressors influencing fish growth. Increased temperatures accelerate juvenile fish and gonadal growth. The mechanism(s) by which these stressors disrupt fish growth remains unknown. Flow-through exposures were conducted using mummichog (*Fundulus heteroclitus*), an estuarine teleost, with weights taken every three days to measure growth. The expression of liver insulin-like growth factor 1 (*igf1*) and receptors (*igf1ra* and *igf1rb*) were assessed. Experiment 1 utilized three-month-old mummichog exposed to control (salt water), 250 mg/L ammonium chloride (NH_4Cl), or 250 ng/L EE_2 at 20 and 25°C for 21 days. EE_2 did not affect weights; 25°C increased and NH_4Cl decreased weight. Liver *igf1ra* and *igf1rb* increased in EE_2-exposed mummichog at 20°C and NH_4Cl-exposed mummichog at 25°C compared to controls. Experiment 2 utilized adult post-spawning female mummichog exposed to Experiment 1 conditions. EE_2-exposed mummichog at 25°C weighed more than all groups except the 25°C control. Mummichog at 25°C exhibited higher gonadosomatic indices (% gonad weight relative to body weight) than mummichog at 20°C. Liver *igf1* and *igf1rb* increased in EE_2-exposed mummichog at 25°C compared to control. Higher temperatures increased weight in both mummichog life stages and increased ovarian size, indicating that temperature influences the reproductive state of adults. NH_4Cl decreased juvenile mummichog growth, suggesting that juveniles are more vulnerable to ammonia compared to adults. EE_2 did not affect mummichog growth at both life stages, but at 25°C, adult weight increased due to enlarged ovaries. Liver molecular endpoints vary; further studies are needed to determine GH-IGF1 pathway involvement.

Modulation of mitochondrial bioenergetics and reactive oxygen species production by binary mixtures of copper, cadmium, and zinc in permeabilized fish cardiac fibers (PL)

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The mitochondria have emerged as key intracellular target sites and mediators of metals toxicity. Conventional methods for assessing the outcomes of toxicant exposure to mitochondria entail isolation of the organelles which not only removes the moderating effects of the intracellular environment but may alter mitochondrial functional integrity. We investigated the effects of metals (copper, Cu; cadmium, Cd; zinc, Zn) individually and as binary mixtures on mitochondrial bioenergetics and reactive oxygen species (ROS, as hydrogen peroxide, H_2O_2) emission using permeabilized rainbow trout (*Oncorhynchus mykiss*) cardiac fibers in which the intracellular environment and integrity of mitochondrial membranes are preserved. Permeabilized cardiac fibers were energized with glutamate + malate without and with added adenosine diphosphate (ADP) to impose state 2 and state 3 bioenergetic states, respectively, and exposed to Cu, Cd, and Zn singly and in binary mixtures. Respiration and H_2O_2 emission rates were

measured simultaneously using Oroboros O2k fluorespirometer for unambiguous association of bioenergetic state and ROS production. Cardiac fibers were highly sensitive to Zn with the state 3 respiration EC_{50} being more than an order of magnitude lower compared with Cu or Cd. Cu and Cd greatly stimulated state 2 respiration but markedly inhibited state 3 respiration whereas Zn was inhibitory irrespective of the respiratory state. Interestingly, Cd antagonized the inhibitory effect of Cu on state 3 respiration as well as the stimulatory effects of both Cu and Zn on state 2 respiration. Cu singly evoked biphasic H_2O_2 emission patterns in both bioenergetic states. The Cu-Cd mixture exhibited antagonistic interactions on H_2O_2 emission in which Cd reduced the effect of Cu irrespective of the bioenergetic state. In contrast, the Cu-Zn mixture acted synergistically to increase H_2O_2 emission while the Cd-Zn mixture acted additively (state 3) or had no added effect (state 2) on H_2O_2 emission relative to the respective metals singly. Overall, our study demonstrates that contrary to common belief, the essential trace metals, Cu and Zn, have greater inhibitory and stimulatory effects on respiration and H_2O_2 emission in cardiac fibers, respectively, compared with the non-essential metal, Cd. Moreover, it appears that Cd can mitigate oxidative distress in cardiomyocytes caused by Cu or Zn by reducing ROS production.

You're hot then you're cold: Diel thermal fluctuations during development alter microRNA expression and performance in zebrafish (PL)

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*presented by Jaycelyn Caklec[1]

Fish are exposed to diel thermal fluctuations in their natural environments, and with the increase in frequency of heat waves, the range of these fluctuations is expected to increase dramatically. Recently, it has been posited that epigenetic modulators like microRNAs (miRNAs) could buffer fish against such rapid changes as they act on a more rapid timeframe than genetic adaptation. To investigate this, we exposed zebrafish (Danio rerio) embryos to either constant control (CTRL; 28°C) or fluctuating (FLUX; 23-33°C) conditions until 30 days post fertilisation (dpf). The expression of 7 miRNAs was quantified using qPCR during the larval (5 dpf) and juvenile (30 dpf) stages, and performance metrics such as survival and oxygen consumption rate (MO2) were measured at 30 dpf. To understand whether their ontogenetic history affected their response to elevated temperatures, the remaining juveniles from both treatments were reared under CTRL conditions until adulthood (6 months post fertilization; mpf) and measured thermal tolerance along with miRNA expression in the brain. While survival did not differ significantly, FLUX fish had a lower MO2 and higher expression of miR-181a. Similarly, while there were no differences in thermal tolerance between the groups at 6 mpf, miR-181a was elevated in the brains of adult FLUX fish at baseline and the thermal maximum point (CTmax). This study highlights that epigenetic changes could drive plastic responses to thermal variability, and that ecologically realistic conditions need to be incorporated into studies to accurately predict how fish will respond to the multiple stressors caused by climate change.

Climate impacts on cadmium concentrations in marine bird species from the Canadian Arctic (PL)

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Understanding the cumulative impacts of environmental pollution and climate change is important to the long-term conservation of marine birds in the Canadian Arctic. Cadmium (Cd) is a trace element of toxicological concern that has been monitored in marine birds in Arctic Canada since 1975. Despite nearly 50 years of monitoring, climate impacts on Cd accumulation in these species was not well understood. We addressed this information gap using hepatic Cd concentrations collected from eight seabird species from 12 Arctic breeding colonies between 1975 and 2018. We examined temporal, spatial, and interspecific variation in hepatic Cd levels. We also evaluated whether interannual variations in Cd were driven by broad-scale teleconnection patterns and variations in sea ice. Hepatic Cd concentrations ranged from 1.6 to 124 µg/g dry weight across species, and were highest in thick-billed murres (*Uria lomvia*) and king eiders (*Somateria spectabilis*), and lowest in black guillemots (*Cepphus grylle*), black-legged kittiwakes (*Rissa tridactyla*), and long-tailed ducks (*Clangula hyemalis*). All sites with multiple years of data showed interannual fluctuations in Cd, which were correlated with the North Atlantic Oscillation (NAO) index and with the previous year's June sea ice coverage, where marine birds exhibited higher Cd concentrations in positive NAO years and following years with lower sea ice coverage. Climate change is likely to shift the NAO to being more negative and to reduce sea ice coverage, and our results thus identify various ways by which climate change could alter Cd concentrations in marine birds in the Canadian Arctic.

Metformin does not impact larvae adapting to food by mouth and appears to not act as a glucose modulator in zebrafish (PO)

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Metformin, a potent glucose modulator in humans, is commonly prescribed to treat type-2 diabetes globally. Due to its high usage level in the global population, metformin is found in wastewater and aquatic habitats receiving effluent. Despite this, little is known about the effects of this compound on aquatic organisms living in these waterways, such as fish, particularly at early and sensitive life stages. We have previously shown that continuous exposure of zebrafish to metformin throughout development (3-120 hours post-fertilization, hpf) leads to a modest increase in mortality, but no changes in energetics or behaviour. However, these embryo-larvae were supported by yolk reserves, with the prior study ending before the transition to exogenous food. Here, we tested the hypothesis that metformin would alter the adaptation to food by mouth in zebrafish larvae. We exposed freshly fertilized zebrafish embryos (~2 hpf) to metformin at either typical (3.4 µg/L) or extreme (34 µg/L) environmentally relevant levels until 7 days post-hatch (DPF). Larvae were assessed for general swimming at 6 DPH, before either being offered exogenous food or remaining without food. At 7 DPF, fish were assessed for foraging performance. Fed state and metformin did not influence foraging performance. Fish were sampled at 6 and 7 DPF, and measures of glucose, glycogen, lactate, and ATP levels will be compared. To assess whether metformin could act as a glucose modulator in fish, we additionally performed a glucose tolerance test with adult males and females, intraperitoneally injecting the fish with saline, metformin,

glucose, or a combination of glucose and metformin. Metformin did not modulate the glucose response of adult zebrafish. Taken together, these results imply that metformin exposure, even at current environmental extremes, does not affect the adaptation to food by mouth in zebrafish.

Life on the Coast: Assessing the Effects of Environmental Contaminants on Coastal Organisms

From toxicology to ecology: a case study in adapting research plans following declines in clam populations impacted by an oil spill (PL)

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Coastal ecosystems are faced with a suite of stressors, from invasive species to contaminant impacts that continue to confound our understanding of anthropogenic impacts and ecological change. We need new, adaptive strategies to investigate drivers of ecological change that move beyond considerations of single stressor impacts. I will provide a high-level overview of our investigation into the declines in intertidal clam density and poor recovery trajectories following the Nathan E. Stewart oil spill in Haíłzaqv (Heiltsuk) First Nation territory on the central coast of British Columbia. The investigation was co-developed with the Heiltsuk Integrated Resource Management Department, a process which drove the initial research focus and its subsequent evolution. Using an investigative approach, we first ruled out ongoing impacts from the oil spill by: (1) undertaking an assessment of environmental hydrocarbon concentrations; and examining effects on (2) the reproductive capacity and scope for growth of adult clams (*Ruditapes philippinarum*) as well as (3) juvenile health. Focus then shifted to a new threat, the invasive European green crab (*Carcinus maenas*), which is wreaking havoc on bivalve populations along the coast. Field experiments provide clear evidence that green crab predation can limit juvenile recruitment, and this represents a concerning factor in green crab-invaded areas. As green crab spread, it represents an additional stressor for clam populations faced with ongoing and future contaminant loads and climate change impacts. It is vital that research and management understand these interacting stressors to support clam bed conservation and restoration efforts.

The effects of metal exposure on the first larval stage of the invasive green crab (*Carcinus maenas*) (PL)

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Intertidal animals face daily fluctuations in environmental parameters (e.g. salinity, temperature), exacerbated by climate change, and are increasingly subjected to pollution due to growing human populations. One of the most successful intertidal crustaceans is the green crab (*Carcinus maenas*); *C. maenas* is globally distributed but is considered invasive throughout Canada. Their success is attributed to their capacity to survive in an array of environmental conditions; however, the tolerance of their larvae is poorly characterized. Considering the spread of *C. maenas* is attributed to this stage, determining this will facilitate focused mitigation efforts while acting as a model for other marine

decapods. We sought to understand the acute lethality and tolerance of stage 1 *C. maenas* larvae to copper (Cu), cadmium (Cd), nickel (Ni), cobalt (Co), and lithium (Li), many trace metals involved in green technology. Dose-response curves were generated by exposing larvae for 48 hours to a given concentration series and monitored for lethality and immobilization. Co, Li, and Ni had effects at concentrations that exceeded environmental concentrations. Cu and Cd toxicity, however, approached environmental relevance with median concentrations that resulted in 50% mortality (LC50) of 144 µg/L [95% CI: 120 – 178] and 648 µg/L [546 – 790], respectively. Immobilization EC50 values were determined as 80 µg/L [66 – 100] and 538 µg/L [435 – 693], respectively. The applicability of *C. maenas* toxicity results to native crustaceans and standard test species has yet to be elucidated, however, this provides the foundation and facilitates the investigation into mechanisms and sublethal effects in larval decapods.

Evaluating the potential interactive effects of copper and phenanthrene on ion regulation in mummichog (*Fundulus heteroclitus*) (PL)

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Metals and polycyclic aromatic hydrocarbons (PAHs) are common pollutants in coastal ecosystems, but their combined effects on aquatic organisms are not well understood. Although some studies suggest that these pollutants might have more-than-additive effects in specific scenarios, current water quality guidelines are based on the individual toxicity of metals and PAHs, highlighting a significant knowledge gap. This study conducted 48-h toxicity tests in freshwater and seawater to evaluate the individual effects of copper (Cu) and phenanthrene (PHE) on iono- and osmoregulation in mummichog (*Fundulus heteroclitus).* Cu experiments conducted in seawater (nominal concentrations: 0. 0.5, 1.0, 2.0, 3.0 mg/L) and freshwater (nominal concentration: 0, 0.025, 0.1, 0.2, 0.3 mg/L) demonstrated that tissue sodium and chloride concentrations increased, while muscle moisture content decreased. In addition, ammonia excretion rates were also inhibited by Cu in both freshwater and seawater. EC_{50} values, determined using a three-parameter log-logistic model, were 0.56 mg/L Cu for tissue sodium and 0.51 mg/L Cu for tissue chloride, while muscle moisture and ammonia excretion did not fit this model. PHE was tested exclusively in seawater (nominal concentrations: 0, 0.25, 0.5, 0.75 mg/L) using two dosing methods: passive dosing and single spiking with 0.01% dimethyl sulfoxide (DMSO) as a co-solvent. PHE exposures did not result in a significant change in any of the parameters. These preliminary findings suggest that more-than-additive ionoregulatory toxicity is unlikely, given the absence of PHE-induced effects on these parameters. Subsequent studies will investigate whether PHE can amplify the iono-distruptive effects of Cu in both freshwater and seawater.

The use of mesocosms to assess impacts from an anthropogenic stressor (PL)

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Following the 2010 Pulp and Paper Environmental Effects Monitoring Technical Guidance Document, alternative monitoring methods for benthic invertebrate community (BIC) monitoring studies are appropriate where there is the presence of hazardous conditions, unsuitable habitat for sampling, or the presence of confounding factors. This study sought to provide a feasible alternative monitoring method through the use of indoor mesocosms. The objective of this study was to validate that the test system

can hold and maintain a BIC over the course of 45 days and that an effect can be induced on the BIC as measured by the BIC indices. The establishment of 16 mesocosms (151-L plastic black ovals) involved the collection of marine sediments (~1200-L) and their associated BIC using the Huntsman *R/V Fundy Spray* with grab sampling using a Van Veen sampler (volume of ~28 L and penetration depth of ~22 cm). The grab samples were coarsely sieved to remove macrofauna and then distributed amongst all 16 mesocosms (~75 L per cosm) within 24 hrs of collection. The mesocosms were allowed to acclimate under flow-though conditions for 12 days prior to the introduction of the stressor (i.e., fertilizer). A concentrated stock solution of fertilizer was then continuously added to 8 mesocosms over the course of 45-days to mimic eutrophication. The mesocosms were monitored daily, with sediment core samples (triplicate) collected from each mesocosm at days 0, 21, and 45. The sediments were sieved, and the biota sorted, identified, and enumerated. Differences in the BIC metrics were assessed through multivariate analyses.

The cumulative effects of marine shipping: impacts of vessel discharges on the South Coast of BC (PL)

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Vessels such as container ships, ferries, cruise ships and pleasure crafts discharge various contaminants to marine waters. It is estimated that at least 600 contaminants are released to the environment via the various waste produced by ships. This work built on the report prepared by the World Wildlife Fund, where they evaluated the volumes of waste discharge in Canadian waters for four waste streams (bilge water, scrubber washwater, sewage, and greywater) from all vessels equipped with an Automatic Identification System (AIS). Our project further evaluated the concentrations and risks associated with the release of ten major contaminants from these four waste streams for the whole South Coast of BC. The contribution of small, non-AIS vessels (e.g., pleasure crafts) was evaluated using the ratio of non-AIS to AIS vessels in each region using the Global Fishing Watch dataset. The predicted environmental concentration (PEC) of the ten selected contaminants were modelled for each square kilometre of the South Coast using the MAMPEC model. The PECs were compared to their respective marine water quality guidelines to calculate a risk characterization ratio (RCR). The cumulative risk in all regions was calculated using the sums of the individual contaminant's RCR. The results showed that the largest risk was found in major ports and shipping lanes, and was posed by the discharge of sewage, greywater, and associated contaminants in coastal areas. However, the risk was highly dependent on the wastewater treatment system used on large vessels, and on the vessels' compliance with Canadian regulations.

Assessing the impacts of individual aromatic compounds on Pacific purple sea urchin behaviour and fertilization success (PO)

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Marine diesel oil (MDO) is a ubiquitous fuel used by marine traffic on all Canadian coasts. Despite its common use, its environmental risks are difficult to predict due to its variation in composition. The

Haíłzaqv (Heiltsuk) First Nation has identified the need for better understanding of MDO due to its presence in vessels in their territory (and the broader coast), to inform stewardship and mitigation of potential spills. In MDO, polycyclic aromatic compounds (PACs) are attributed as the primary toxic ingredients. Testing the effects of individual PACs can allow for modelling of impacts of petroleum on a given species. The species of interest in this study, the Pacific purple sea urchin (*Strongylocentrotus purpuratus*), has ecological and cultural significance to the Haíłzaqv First Nation. In collaboration with and at the request of the Nation, this study is assessing acute toxicity of 5 individual PACs on *S. purpuratus* by investigating the effects on behaviour and fertilization. The PACs used cover a range of lipophilicities, described by octanol-water partition coefficient values ($\log K_{OW}$; 2.9-4.5). To assess behaviour, adult urchins were exposed to known PAC concentrations over 48 hours; righting response was used to quantify the effect on urchin vigour. To assess fertilization success, urchin gametes were exposed to known PAC concentrations for 40 minutes; presence of fertilization membrane around eggs was used to quantify the effect on reproductive viability. Findings of behavioural tests, and preliminary findings of fertilization tests will be presented, along with predictions on how PACs might affect the broader marine environment.

Advancing Wildlife Ecotoxicology Through Traditional and Emerging Approaches

Leveraging the strengths of community science data to investigate drivers of productivity declines in Common Loons (PL)

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Community science programs have the potential to provide more data and greater spatial coverage compared to more traditional monitoring efforts, at lower cost and management effort. Although community science is broadly used in avian ecology, thus far, its use in ecotoxicology is limited. Here, we show the value of using community science data to identify drivers of temporal changes in Common Loon (*Gavia immer*) productivity. We analyzed 38 years of reproductive data from over 1500 lakes using data from the Canadian Lakes Loon Survey, a community science loon monitoring program managed by Birds Canada that has run annually in Ontario since 1981. We defined underlying baseline patterns of loon reproductive success in Ontario. We also assessed the influence of 14 factors on loon productivity. Overall, we estimated a declining trend in Common Loon reproductive success of −0.10 six-week-old young per pair per year in Ontario between 1981 and 2018. We identified low pH and associated higher mercury as factors linked to loon productivity declines. We also demonstrated that lake area, longitude, and April temperatures can predict the number of six-week-old young per pair per year. We hypothesize that climate change-induced stress, acting through multiple interacting pathways involving mercury, acidity, fish abundance, lake size, and geographic location, may account for declining loon productivity. These results will be important for focusing future research and conservation efforts to help understand and mitigate threats to Common Loon populations.

Spatially mapping plastic-related contaminants in breeding herring gull colonies across the St. Lawrence system (PL)

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To accurately identify contamination hotspots, it is essential to consider spatial differences in contaminant concentrations. Studies have revealed geographical variations in POPs and other plastic-related contaminants, reflecting differences in regional diets, wintering areas, and pollution sources. Monitoring seabird eggs can provide valuable insights into these geographic patterns of contaminant loads. Herring gulls have become sentinel species for biomonitoring efforts, specifically for plastic pollution, where the extent of contamination exposure in Herring gulls may vary depending on colony location and dietary choice. Birds, like Herring gulls, that forage near urbanized areas where terrestrial foods are accessible, may accumulate higher plastic-related contaminants. Therefore, investigating spatial trends of plastic-related contaminants in the St. Lawrence is essential for understanding contamination status, distribution, fate, and associated risks throughout the ecosystem. Here, we provide findings on the variability in breeding seabird UV Absorbants (UVAs; Benzotriazole UV Stabilizers and UV Filters) concentrations based on their geographic distributions and proximity to contamination sources across the St. Lawrence. During 2022-2023, we collected Herring gull eggs in 10 areas along the St. Lawrence, to determine the average contaminant loads (n = 61). We found that herring gulls were effective biomonitors of UVAs where maternal transfers were indeed occurring, where all target contaminants of both classes were frequently detected. UV-328, UV-329, HMS and EHS were the main additives at each colony location. We found a significant decreasing trend moving farther from Montreal of contaminant concentration for both UV-328 and UV-329. These results reveal important insights into the ubiquity of these contaminants for the first time in the St-Lawrence.

Oil exposure patterns and diet in seabirds following a diesel spill in Nunatsiavut, Canada (PL)

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Oil development and increased shipping in Arctic marine ecosystems are expected to increase wildlife environmental exposure to oil pollution, with a potential for health, reproductive, and population effects. While most studies to date on oil pollution have focused on large acute spills and in-vitro research, studies into sub-lethal oil exposure in wildlife are limited, including avian monitoring. More research is needed to establish long-term exposure patterns and effects on avian health after an oil spill, especially within the context of diet. The goal of this Indigenous-led research program was to examine the foraging-based routes of oil exposure in seabirds breeding at a 3000L diesel spill site (Postville, Nunatsiavut) and a non-spill site (Nain, Nunatsiavut) over a 3-year post-spill period (2020-2022). The focal species for this project, common eider (*Somateria mollissima*), black guillemot (*Cepphus grylle*), and great black-backed gull (*Larus marinus*), are of cultural significance in Nunatsiavut as the eggs are harvested for consumption. New results show a significant increase in oil found in guillemot eggs at the spill site during the year of the spill (2020), and the following year (2021). Eider and gull eggs had

significantly less oil compared to guillemots, related to diet and breeding strategy. Oil levels in all eggs then declined in 2022. Notably, high oil levels found in these eggs informed human health risk assessments, in collaboration with Health Canada, that were communicated by the Nunatsiavut Government to beneficiaries in June 2023. The results of this research have been an essential part of impact assessments and oil spill response protocols for these species and regions, which have the potential to be applied to other regions within Canada and across the pan-Arctic.

Effects of sulfolane exposure on early life stage wood frogs (*Lithobates sylvanicus*) (PL)

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Sulfolane is a chemical used in many industrial processes, notably in the refinement (or "sweetening") of sour natural gas. In this context, sulfolane is used to help remove sulfur from natural gas before the gas is sold as fuel. Sulfolane has been released into the environment, including into ground water at industrial sites in Alberta, and is an emerging contaminant of concern due to its high solubility in water. Ecotoxicology experts in western Canada have recommended the impacts of sulfolane on amphibians be characterized in order to inform ecological risk assessments (ERAs) at contaminated sites. This investigation aims to determine the effects of acute sulfolane exposure on the behaviour and vital rates of developing wood frogs (*Lithobates sylvaticus*), a species commonly found across North America. Six wood frog egg masses were collected from a natural area with limited human disturbance and were assigned to one of two experiments. Both experiments consisted of a single 48h exposure to one of five sulfolane concentrations (0.1, 1, 10, 100, or 1000 mg/L) or control, one exposure occurring during early embryonic development, and the other during early larval development. After exposure, all experimental animals were raised until the late larval stage (Gosner 42, emergence of forelimbs). A subset of tadpoles at Gosner stage 25 (free swimming stage) were placed in a novel environment and their locomotor behaviour was tracked using automated software. In addition to locomotion, we report several apical endpoints (mortality, hatch rate, body metrics, and developmental rate). These data are needed to support ERAs of sulfolane for amphibians and develop environmental quality guidelines.

Northern leopard frog (*Rana pipiens*) apical and metabolomic responses to plastic and rubber additives (PL)

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Two common chemical additives used in products, such as plastics and rubber, include the benzotriazole ultraviolet stabilizer UV329 (CAS:3147-75-9) and the synthetic phenolic antioxidant 2,4-DTBP (CAS: 96-76-4). These chemicals are suspected endocrine receptor binders, leach from their natal products into the environment and pose an exposure risk to aquatic organisms, such as amphibians. Therefore, we evaluated the sex-steroid and thyroid disrupting potential of UV329 and 2,4-DTBP up to 100 µg/L on northern leopard frog (*Rana pipiens*) embryos and larvae using acute (96-h) and sub-chronic (30-d) aquatic exposures. We found an initial reduction in body length of tadpoles after 96 h at 10 µg/L UV329, but this effect on body size was not evident after 30 d of exposure up to 100 µg/L UV329 or 2,4-DTBP. There were also no significant effects on development or sex ratio for either chemical. We found contrasting concentration-dependent effects on the hepatosomatic index (HSI) where the HSI was

decreased at 20 µg/L UV329 but increased at 100 µg/L UV329 with no effects of 2,4-DTBP on HSI. However, shifts in liver metabolomic profiles were evident with sub-chronic exposures to 2,4-DTBP. Here lipid metabolites, specifically acylcarnitines and glycerophospholipids concentrations were elevated at 50 µg/L. In summary, we found that up to 100 µg/L of UV329 or 2,4-DTBP is not affecting sexual differentiation or thyroid-mediated development, but 2,4-DTBP may be interfering with metabolic pathways. Further work is required to determine if UV329 is also affecting metabolomic profiles and if these shifts in metabolites have fitness-relevant consequences.

Sex-specific gene expression responses to methylmercury exposure in wildlife rainbow trout (*Oncorhynchus mykiss*) in Chilean watersheds (PL)

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Understanding methylmercury (MeHg) toxicity has advanced significantly, revealing that MeHg bioaccumulation in fish alters reproductive hormone synthesis, and affects energy metabolism, oxidative stress, immune response, and lipid metabolism. However, molecular responses to MeHg in fish have not been evaluated in Chile. This study investigates MeHg's impact on gene expression in rainbow trout (*Oncorhynchus mykiss*), an ideal bioindicator due to its high position in the aquatic food web, lipid-rich meat favorable for MeHg bioaccumulation, and widespread distribution in Chilean watersheds. We selected three sites with varying MeHg levels in trout muscle (low: 0.005-0.008 µg g-1; middle: 0.034-0.136 µg g-1; high: 0.168-0.310 µg g-1). Male and female trout from each site were collected, and their livers were analyzed by qPCR for six genes and Next Generation Sequencing (NGS) for gene discovery related to MeHg exposure. qPCR results indicated that high MeHg exposure in female trout altered the expression of genes encoding zona pellucida, estrogen receptor beta, and metallothionein. In male trout, genes encoding estrogen receptor alpha and beta, and metallothionein were affected. NGS data showed 826 out of 14,016 genes were differentially expressed in middle and high MeHg-exposed female trout, compared to 529 out of 13,484 genes in similarly exposed male trout. This suggests sex-specific gene expression responses to MeHg, with more overexpressed genes in females and more underexpressed genes in males. Enriched pathway analyses confirm that increased MeHg exposure disrupts metabolic and energy generation pathways in rainbow trout, emphasizing the need to consider sex differences in environmental toxicity studies.

High-throughput screening of chemical libraries to understand chemical metabolism in fish (PL)

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Chemical metabolism remains understudied in wildlife such as fish. While major gene families are present in fish genomes, evolutionary differences across taxa suggest that functions differ. Yet, these

functions determine the toxicokinetics and tissue concentrations of contaminants. A key superfamily mediating chemical metabolism are the cytochrome P450 enzymes, particularly in CYP1, CYP2 and CYP3 families. There are clear orthologs of mammalian CYP1A genes in fish, but not the CYP2 or CYP3 genes. CYP1A, CYP2Y3 and CYP3A65 are highly expressed in the liver of adult zebrafish, suggesting major roles in chemical metabolism. Yet CYP2Y3 is an orphan gene with no known function. Our approach to determining CYP function is to select genes based on expression in organs important for toxicokinetics. Genes are heterologously expressed for functional studies using chemical libraries to identify substrates and define enzyme function in high throughput screening. We focused on zebrafish CYP1s, CYP2Y3, CYP3A65, and CYP3C1 genes with a large (4000 compound) commercial library of bioactive molecules including off-patent pharmaceuticals or the Phase 2 ToxCast chemical library (1920 compounds) including pesticides, endocrine related compounds, anti-microbials, food additives, green chemistry alternatives, failed drugs, flame retardants, and toxicology reference compounds. We have successfully identified substrates of zebrafish enzymes and for the best studied CYP, CYP1A, identified substrates included expected polycyclic aromatic hydrocarbons. High throughput screening provides an opportunity to understand chemical metabolism and in conjunction with transcriptomics, can establish the key genes controlling toxicokinetics.

The Chemical Defensome of Zebrafish (PL)

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Toxicokinetics is the movement and fate of foreign chemicals in the body, and is dictated by chemical uptake, distribution, metabolism, and excretion. In response to xenobiotics animals respond by upregulating genes critical to detoxifying and eliminating foreign substances. In fish, we know little about the major chemical defense genes, their regulation, and the tissue specific patterns of gene expression. For example, chemical defense is expected to be largely coordinated through major organs, such as the gill, intestines, liver, and the kidney. However, previous studies have largely focused on chemical defense genes located within the liver. In this study, we tested the hypothesis that fish exhibit tissue specific expression of chemical defensome genes. Using existing transcriptomics data, we examined the basal expression of chemical defensome gene families in the intestine and gills of unexposed male zebrafish. Although both the gills and intestines play critical roles in chemical uptake, our functional analysis suggests that the intestine is primed to respond and metabolize chemicals while the gills is primed for response to other abiotic stressors such as temperature. Further, while investigating the basal expression of defense genes across the major organs for toxicokinetics, I will explore sex specific differences in chemical defensome genes. Determining the gene expression patterns of chemical defensome genes in gill and intestine provide key information on chemical uptake from water soluble and dietary xenobiotics into fish and ultimately will help us identify the genes controlling toxicokinetics.

Inter- and intra-species sensitivity of whole organism and liver transcriptomics in fish to tertiary treated municipal wastewater effluents (PL)

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Transcriptomics is being used as a complementary tool to whole organism responses (condition factor, liver somatic and gonadosomatic index) used in Canadian Environmental Effects Monitoring programs to help evaluate appropriate physiological endpoints that could be considered as part of municipal wastewater effluents (MWWE) monitoring. Species show different sensitivity to exposure to constituents in complex mixtures in MWWEs that depends on their size, mobility, lifespan, habitat preference, reproductive stage etc. To determine inter-species sensitivity of liver gene expression (using ribonucleic acid sequencing) and whole organism responses to MWWE, three fish species (longnose dace (*Rhinichthys cataractae*), trout perch (*Percopsis omiscomaycus*) and spoonhead sculpin (*Cottus ricei*)) were caged in experimental raceways at Advancing Canadian Water Assets (Calgary, AB), containing either 5% effluent or Bow River water for 28 days. Longnose dace were also exposed to 15% MWWE and compared to the 5% exposure. The results showed that species responded differently and there was a gradual recovery in the whole organism responses by the end of the 28-day exposure. Contrary, gene expression showed an increase in significantly different genes compared to day 0, and a significant enrichment in pathways associated with the immune responses and potential pathogen challenging. Trout perch whole organism endpoints showed the highest sensitivity, followed by longnose dace and spoonhead sculpin. Interestingly intra-species sensitivity of 15% MWWE had comparable effects on the longnose dace to those seen in the 5% exposure. Results from this study could have implications for fish species selection for MWWE monitoring and point to common gene expression changes across multiple species.

Investigating the lethal and sublethal effects of road salt contamination on the development and physiology of coho salmon (*Oncorhynchus kisutch*) (PL)

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In the Vancouver Lower Mainland, Pacific salmon spawn in freshwater streams, some of which traverse highly urbanized areas. Even in a region with a relatively mild climate, each year, road salt is heavily dispensed, with the potential for contamination of local waterways. Road salting coincides with critical developmental stages for salmonids, as many species spawn between October and February. Collaboratively with citizen scientists, government, and three academic institutions, we have assessed the extent of road salt contamination and the impact that environmentally relevant exposures have on the development and physiology of Pacific salmon. Building on a preliminary experiment on early life stages of rainbow trout (*Oncorhynchus mykiss*), we aimed to identify the lethal and sublethal effects of a 24-h NaCl pulse on coho salmon (*Oncorhynchus kisutch*) at six environmentally relevant salt concentrations and three developmental time points: fertilization, <1-h post-fertilization, and 50% hatch. Lethal effects were observed depending on the concentration and the developmental time point of the

salt exposure. Our research aims to raise public awareness among those who distribute road salt and to advocate for stricter regulations of its use.

Interspecies variation in fish species sensitivity to PAHs: effects of alkylation and development of predictive tools (PL)

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment. PAHs can be alkylated, and alkyl-PAHs are more abundant in certain environments. PAHs can exert toxicity to fishes by activating the aryl-hydrocarbon receptor (AhR). Although there is evidence that alkylation could increase potency of PAHs, this is not well characterized. To characterize how alkylation affects potency of PAHs, the potency for activation of the zebrafish (*Danio rerio*) AhR by benz[a]anthracene and three alkyl homologues was quantified using a standardized cell-based assay. Alkylation had position-dependent effects on potency. Specifically, 8-methylbenz[a]anthracene, was 6.9-fold more potent than benz[a]anthracene, 4-methylbenz[a]anthracene was 1.4-fold more potent, and 7,12-dimethylbenz[a]anthracene was 0.8-fold as potent. Next, potency for early life-stage mortality was quantified by injecting zebrafish embryos with each PAH to determine whether the cell-based assay was representative of *in vivo* exposure. Relative potencies *in vivo* were like those *in vitro*. To assess interspecies differences in sensitivity, the cell-based assay was performed for phylogenetically diverse species. Alkylation had position-dependent effects on species sensitivity with sensitivity ranging up to 561-fold. To determine whether differences in sensitivity to AhR activation correspond with differences in sensitivity to early life-stage mortality, fathead minnow (*Pimephales promelas*) and brook trout (*Salvelinus fontinalis*) embryos were microinjected with PAHs. There is a significant relationship between sensitivity to AhR activation and sensitivity to early life-stage mortality ($p = 0.007$, $R2 = 0.86$). Due to the abundance of PAHs in the environment and differences in species sensitivity, *in vitro* tools to efficiently assess toxicities could be essential for ecological risk assessments.

Application of Avian In Vitro Substrate Depletion Assays to Study Biotransformation of Organic Chemicals (PO)

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Avian species play a critical role in aquatic and terrestrial ecosystems, and historically have seen critical population level impacts by the bioaccumulation of organic pollutants. Bioaccumulation is typically assessed in fish using B-metrics such as bioconcentration or bioaccumulation factors (BCF/BAF) depending on aqueous versus dietary exposure routes, however such methods are challenging to apply to air breathing organisms. These metrics are often determined using costly *in vivo* testing in fish and rats, with little focus on birds outside of field studies. Recently, it has been suggested that a toxicokinetic approach that views these metrics as the product of competing rates of whole-body uptake and elimination may be refined by incorporating tissue-specific biotransformation rates derived from substrate depletion assays using isolated hepatocytes or liver S9 subcellular fractions. In fish, these approaches have been standardized within the Organisation for Economic Co-operation and

Development (OECD) as Test Guideline 319, with the potential for adaptation into other species. The present study seeks to optimize, evaluate, and apply these assays to multiple relevant bird species using a diverse suite of benchmark compounds. Initial studies have focused on mallard duck (*Anas* platyrhynchos) isolated hepatocytes to determine the intrinsic clearance of pyrene (2.95 mL/h/10^6 cells) and 4-*n*-nonylphenol (6.36 mL/h/10^6 cells), demonstrating the adaptability of *in vitro* substrate depletion methods to quantify hepatic biotransformation in birds. Further research will expand into legacy organic pollutants including organochlorines, polychlorinated biphenyls, and brominated flame retardants, as well as emerging contaminants of wildlife concern such as anticoagulant rodenticides, azole fungicides, and neonicotinoids. The goal of this study is to generate toxicokinetic data for use in tiered bioaccumulation assessment strategies, as well as lay the foundation for future ring trial or similar applications to inform wildlife risk assessment.

Road salt exposure during early development has similar impacts on rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*) (PO)

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The application of road salt has the potential for contamination of local waterways, but the extent and nature of this issue has not been carefully assessed in British Columbia's Lower Mainland. Through collaboration with citizen scientists, we have documented spikes in sodium chloride levels in multiple freshwater streams in the region, sometimes surpassing British Columbia's provincial guidelines by more than 14-fold. With this information, we performed laboratory tests exploring the impacts of road salt on developing rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*). We investigated the lethal and sublethal effects of a 24-h NaCl pulse at six environmentally relevant salt concentrations during sensitive developmental time points (e.g. fertilization, <1-h post-fertilization, eyed stage, and hatch). The lethal effects observed were comparable in rainbow trout embryos and coho salmon embryos, highlighting the severity of potential harm posed by road salt contamination to various species. Our objective is to gain a comprehensive understanding of the impact of road salt contamination on nearby aquatic ecosystems, aiming to raise public awareness and advocate for stricter regulations on its distribution.