Proceedings of the 48th Annual Canadian Ecotoxicity Workshop: October 2 - 5, 2022, Winnipeg, Manitoba

Editors
Carrie Rickwood, Julie Anderson, Mark Hanson, José Luis (Pepe) Rodríguez-Gil, Sarah Marteinson, and Jennifer Miller

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Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

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2024

PROCEEDINGS OF THE 48TH ANNUAL CANADIAN ECOTOXICITY WORKSHOP: OCTOBER 2 - 5, 2022, WINNIPEG, MANITOBA

Editors

Carrie Rickwood[2], Julie Anderson[3], Mark Hanson[4], José Luis (Pepe) Rodríguez-Gil[5], Sarah Marteinson[1], and Jennifer Miller[6]

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PL = Platform presentation; PO = Poster presentation, VPL = Virtual platform presentation, VPO = Virtual poster presentation

Abstract

Rickwood, C., Anderson, J., Hanson, M., Rodríguez-Gil, J.L., Marteinson, S., and Miller, J. (Editors). 2024. Proceedings of the 48th Annual Canadian Ecotoxicity Workshop: October 2 - 5, 2022, Winnipeg, Manitoba. Can. Tech. Rep. Fish. Aquat. Sci. 3642: xxviii + 152 p. https://doi.org/10.60825/1n06-0d73

For 41 years, the annual Aquatic Toxicity Workshop (ATW) was held in various locations across Canada. In 2015, the ATW was rebranded as the annual Canadian Ecotoxicity Workshop (CEW) to reflect the broad scope of environmental interests held by workshop participants.

The 48th annual CEW was held at the Fort Garry Hotel in Winnipeg, Manitoba, in 2022, as the pandemic restrictions were lifted to allow greater levels of in-person attendance than in 2021. This workshop was the second hybrid CEW event held in the organization's history and included 143 platform presentations (both virtual and in-person) and 66 virtual poster presentations. Total participation was 317 attendees (241 in-person and 76 virtual attendees).

This workshop was one of a continuing series of annual workshops in Canada on ecological toxicology, covering topics including mechanistic toxicology, environmental effects monitoring, behavioural toxicology, environmental radioactivity, Indigenous voices in ecotoxicology, the development of non-lethal and non-invasive techniques, and toxicity assessments for emerging contaminants. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments, consulting, and academia. Additionally, CEW provides an annual focus on the principles, current problems, and approaches in ecotoxicology. These workshops are administered by a Board of Directors and organized by local organizing committees annually. The Proceedings are published with the support of Fisheries and Oceans Canada.

Résumé

Rickwood, C., Anderson, J., Hanson, M., Rodríguez-Gil, J.L., Marteinson, S., and Miller, J. (Editors). 2024. Proceedings of the 48th Annual Canadian Ecotoxicity Workshop: October 2 - 5, 2022, Winnipeg, Manitoba. Can. Tech. Rep. Fish. Aquat. Sci. 3642: xxviii + 152 p. https://doi.org/10.60825/1n06-0d73

Pendant 41 ans, l'atelier annuel sur la toxicité aquatique (ATW) s'est tenu à divers endroits au Canada. En 2015, l'ATW a été renommé l'atelier annuel sur l'écotoxicité au Canada (CEW) afin de mieux refléter le large éventail d'intérêts environnementaux des participants.

La 48e édition de CEW s'est tenue à l'hôtel Fort Garry de Winnipeg (Manitoba) en 2022, lorsque les restrictions liées à la pandémie ont été levées pour permettre une participation en personne plus élevée qu'en 2021. Cet atelier était le deuxième événement hybride dans l'histoire de l'organisation et comprenait 143 présentations de plateformes (virtuelles et en personne) et 66 présentations d'affiches virtuelles. Le nombre total de participants était de 317 (241 en personne et 76 virtuels).

Cet atelier faisait partie d'une série continue d'ateliers annuels au Canada sur la toxicologie écologique, couvrant des sujets tels que la toxicologie mécaniste, la surveillance des effets environnementaux, la toxicologie comportementale, la radioactivité environnementale, les voix autóctone en écotoxicologie, le développement de techniques non létales et non invasives, et l'évaluation de la toxicité des contaminants émergents. Ces ateliers mettent l'accent sur un échange informel d'idées et de connaissances sur les sujets abordés entre les personnes intéressées issues de l'industrie, des gouvernements, du secteur du conseil et du milieu universitaire. En outre, CEW met chaque année l'accent sur les principes, les problèmes actuels et les approches en matière d'écotoxicologie. Ces ateliers sont administrés par un conseil d'administration et organisés chaque année par des comités d'organisation locaux. Les actes sont publiés avec le soutien de Pêches et Océans Canada.

Editors' comments

This volume contains papers, abstracts, or extended abstracts of all presentations at the workshop. An author index is also included. The papers and abstracts were subject to limited review by the editors but were not subjected to full formal or external review. In most cases, the papers are published as presented and therefore are of various lengths and formats. Comments on any aspects of individual contributions should be directed to the authors. Any statements or views presented here are entirely those of the speakers and are neither condoned nor rejected by the editors. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The editors would like to thank Remy Tadonleke for his assistance in preparing these proceedings.

Remarques des éditeurs

Ce compte rendu renferme le texte intégral ou le résumé de toutes les communications présentées aux ateliers. Un index des auteurs est aussi inclus. Les communications et les résumés ont été revus sommairement par les éditeurs, mais ils n'ont pas fait l'objet d'une revue exhaustive en bonne et due forme ou d'une revue indépendante. La longueur et la forme des communications varient parce que ces dernières sont pour la plupart publiées intégralement. Les lecteurs sont priés de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont entièrement celles des conférenciers; elles ne sont ni approuvées, ni rejetées par les éditeurs. La mention de marques de commerce ou de produits commercialisés ne constitue ni une approbation, ni une recommandation d'emploi.

Les rédacteurs voudraient remercier Remy Tadonleke pour son aide dans la préparation de ces comptes rendus.

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

Effects of 1,2,5,6-tetrabromocyclooctane (TBCO) on oocyte maturation and reproductive performance of Japanese medaka (*Oryzias latipes*)

Yamin Raza[1] , Chloe Devoy[1], Paul Jones[2], Jon Doering[1,3], Steve Wiseman[1]

[1]Université de Lethbridge, [2]University of Saskatchewan, [3]Louisiana State University

Brominated flame retardants (BFRs) are chemicals added to flammable products to increase their fire resistance. They can leach into aquatic environments where they have been shown to bioaccumulate, biomagnify, and induce toxicity in aquatic organisms. 1,2,5,6- tetrabromocyclooctane (TBCO) is an emerging BFR that's usage is predicted to soon increase. Although little is known regarding the toxic effects of TBCO, it has been shown to impair reproduction in Japanese medaka (*Oryzias latipes*), but the molecular mechanism of this effect is not clear. It has been reported that TBCO impairs oocyte maturation in zebrafish (*Danio rerio*), but this has not been investigated in Japanese medaka, primarily because an oocyte maturation assay has not been developed for this species. This study explored inhibition of oocyte maturation as a potential mechanism by which TBCO impairs reproductive performance of Japanese medaka. To do this, an assay that assesses oocyte maturation disruption by anthropogenic chemicals was developed. Maturation assays were conducted using *in vitro* and *in vivo* exposure techniques. To assess effects of *in vitro* exposure, stage IX oocytes were excised from sexually mature female Japanese medaka and exposed to 0, 2, 20, and 200 $\mu g \cdot L^{-1}$ of TBCO, followed by exposure to maturation-inducing hormone (MIH). To assess effects of TBCO on oocyte maturation following *in vivo* exposure, sexually mature fish were fed either a control, 100 $\mu g \cdot g^{-1}$, or 1000 $\mu g \cdot g^{-1}$ concentration of TBCO-spiked fish food for 21 days. Fecundity was measured daily. Following the exposure, stage IX oocytes were excised to assess maturation in response to MIH. The *in vitro* exposure showed a significant decrease in maturation between the control oocytes and oocytes exposed to TBCO at 20$\mu g \cdot L^{-1}$. *In vivo*, fecundity decrease significantly in TBCO-exposed female fish, and there was a significant decrease in MIH-stimulated maturation of oocytes from females given 1000$ug \cdot g^{-1}$ concentration of TBCO. This study was the first to assess oocyte maturation disruption in Japanese medaka using an assay. The results suggest that decreased reproductive performance in Japanese medaka exposed to TBCO is partly due to inhibition of oocyte maturation. This finding helps explain the larger-scale impacts of TBCO on fish reproduction.

A new toxicity test with the arctic algae *Nitzschia frigida*

Rebecca Eldridge[1], Mark Hanson[1], Benjamin de Jourdan[2]

[1]University of Manitoba, [2]Huntsman Marine Science Centre

There is a pressing need to understand the impact of emerging contaminants on Arctic ecosystems; however, most standard toxicity tests are for temperate species. The lack of Arctic-specific tests may result in an underestimation of harm to Arctic organisms and contribute to significant uncertainty in risk assessments. As well, of the Arctic species tests that exist there has been no assessment of their reliability and relevance for use in environmental risk assessment. To help address these concerns, this thesis consisted of two phases: first, we conducted a critical review on the current state of Arctic ecotoxicology where reported effects were summarized, methodological reliability and endpoint relevance were assessed, and future testing needs were identified. We developed an objective scoring system and evaluated a total of 48 individual studies capturing 39 tested compounds, 73 unique Arctic test species, and 95 distinct endpoints published from 1975 to 2021. Our analysis shows that of 253 test substance and species combinations scored, 207 (82%) failed to meet at least one critical study criterion that contributes to data reliability for use in risk assessment. Significant data gaps were identified related to standardized toxicity testing with Arctic species, diversity of compounds tested with these organisms, and the inclusion of ecologically relevant sublethal and chronic endpoints. In the second phase of the project, we developed a laboratory bioassay for the Arctic diatom *Nitzschia frigida*. We optimized culturing conditions by testing various environmental conditions to determine which combination resulted in the most growth within a 14-day period. Under the resulting optimized conditions, we exposed *N. frigida* to copper, zinc, and 1-methylnaphthalene, with concurrent exposures of the standard, temperate species *Skeletonema costatum* in order to compare sensitivity. The test was consistent and repeatable based on control performance and EC_{50} results across three trials. *N. frigida* demonstrated greater sensitivity than *S. costatum* to the two metals, but not to 1-methylnaphthalene. Overall, this thesis has identified a need for ongoing improvement in test conduct and reporting in the scientific literature to support effective risk assessments, and has provided new tools to inform environmental risk assessments in Arctic regions.

Occurrence, Fate, and Effects of Tire and Road Wear Particles and Related Contaminants

Non-targeted screening of organic contaminants in the Yamaska River: Evidence of occurrence of tire additives (VPL)

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[1]Université de Sherbrooke, [2]Environnement et changement climatique Canada, [3]Université de Sherbrooke

Non-targeted analysis methods are of interest to screen for the presence of multiple organic contaminants in environmental samples and thus could be employed to improve and guide quantitative studies of emerging contaminants such as tire additives based on targeted methods of analysis. During a sampling campaign in the summer of 2019, water samples were collected upstream and downstream of the cities along the Yamaska river in southeastern Québec and the samples were submitted to extraction by solid phase extraction and analysis by liquid chromatography-quadrupole-orbitrap mass spectrometry using a data-dependent acquisition. Data files were processed with a non-targeted screening workflow based on computational analysis of mass spectra, comparison to experimental spectral libraries as well as molecular networks and clusters. Results showed that among the 438 compounds detected, three tire-related contaminants were detected in all points, including diphenylguanidine (DPG) and ditolylguanidine (DTG), both vulcanization accelerators. The antioxidant 2,2,4-trimethyl-1,2-dihydroquinoline (TMQ) was also observed in all points as well as a potential transformation product, 2,2,4-trimethyl-1,2-dihydroquinolin-6-ol. The presence of DPG, DTG and TMQ in the samples was confirmed with reference standards. While running the developed workflow still requires experienced operators and employs multiple tools, these results demonstrate that non-targeted analysis methods are powerful tools that are ready to assist water quality monitoring programs in identifying contaminants of emerging concern in the aquatic environment.

Hepatic biotransformation of N-(1,3-dimethylbutyl)-N′-phenyl--phenylenediamine-quinone (6PPD-Q) in rainbow trout, an acutely sensitive species (PL)

David Montgomery[1], Xiaowen Ji[1], Matthew Schultz[1], Alper James Alcaraz[1], Summer Selinger[1], Steve Wiseman[2], Markus Hecker[1], Markus Brinkmann[1]

[1]University of Saskatchewan, [2]University of Lethbridge

6PPD-Q is an abiotic transformation product of 6PPD, a tire anti-degradant. 6PPD-Q is deposited onto roads and then dispensed into waterways through stormwater and

snowmelt. 6PPD-Q is acutely toxic (24 hours) to coho salmon, brook trout and rainbow trout but tolerated by many other fishes including white sturgeon and Artic char. However, little is known about its mechanisms of toxicity and whether the culprit is the parent chemical or a metabolite. Therefore, it is important to determine if 6PPD-Q is biotransformed to potentially explain its highly species-specific toxicity. In this study, an isolated liver perfusion assay was used to quantify the biotransformation of 6PPD-Q and determine associated toxicokinetic parameters in rainbow trout. This assay measured the hepatic extraction fraction (E) used to calculate clearance (Cl) of 6PPD-Q. An active transport inhibitor, Cyclosporin A (CsA), was introduced midway throughout the assay to assess active transport from plasma into hepatocytes. Furthermore, to determine the specific metabolites formed *in vivo*, bile samples from rainbow trout acutely exposed (<96 hours) to 6PPD-Q were analyzed utilizing liquid chromatography-mass spectrometry (LCMS). The results revealed a high E (>0.70), no difference in E or Cl values with CsA administration, and discovery of an oxy-glucuronide metabolite with a hydroxy-fragment in rainbow trout bile. Further research will focus on tolerant species bile analysis in addition to *in vitro* hepatocyte and gill biotransformation assays.

Acute toxicity of tire rubber-derived chemicals, 6PPD-quinone and HMMM, to various life stages of Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) (PL)

Danielle Philibert[1], Ryan Stanton[2], Christine Tang[2], Tillmann Benfey[3], Michael Pirrung[2], Benjamin de Jourdan[1]

[1]Huntsman Marine Science Centre, [2]University of California Riverside, [3]University of New Brunswick

Recent studies on the toxicity of stormwater runoff from urban areas have indicated that N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) may be responsible for the mass mortality of coho salmon (*Oncorhynchus kisutch*). In the wake of this discovery, 6PPD-quinone has been measured in freshwater waterways around urban centers, along with other tire wear leachate compounds like hexamethoxymethylmelamine (HMMM). Very little is known about the potential toxicity of HMMM, and of the data available it appears that 6PPD-quinone toxicity can range considerably depending on the species being exposed. In this study we determined the toxicity of 6PPD-quinone and HMMM to Atlantic salmon (*Salmo salar*) fry, pre-smolt juveniles, and post-smolt adults, and brook trout (*Salvelinus fontinalis*) fry and fingerlings. Twenty-four-hour exposures were conducted using synthesized 6PPD-quinone and commercially sourced HMMM in both freshwater and seawater life stages. Water samples were taken to measure the concentration of 6PPD-quinone and HMMM using GC-MS. At concentrations up to 1.2 $mg \cdot L^{-1}$, HMMM caused no effects. For Atlantic salmon, 6PPD-quinone was most toxic to the fry, and no lethality was observed at the concentrations tested in our pre- and post-smolt salmonids. Following exposure, blood samples were collected from the pre- and post-smolts, and the only changes observed was an increase in

hematocrit in the pre-smolt salmon after exposure to 6PPD-quinone. Brook trout fry were more sensitive than Atlantic salmon fry to 6PPD-quinone exposure with E/LC_{50}s determined at 1, 4, 6, and 24 hours of exposure. This study highlights the importance of testing the toxicity of 6PPD-quinone on a range of different life stages of fishes.

Acute cardiorespiratory effects of 6PPD-quinone to juvenile salmonids (PL)

Summer Selinger[1], Alper James Alcaraz[1], David Montgomery[1], Xiaowen Ji[1], Steve Wiseman[2], Markus Hecker[1], Lynn Weber[1], David Janz[1], Markus Brinkmann[1]

[1]University of Saskatchewan, [2]University of Lethbridge

N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) is a transformation product of the most widely used rubber tire antioxidant, 6PPD. Commonly found in road-way runoff, this compound has been reported to cause acute lethality at roughly 1 μg·L^{-1} in a variety of salmonid species including coho salmon, rainbow trout, and brook trout. However, additional studies have shown other salmonid species such as Arctic char and bull trout to be insensitive, even at significantly greater concentrations (20 μg·L^{-1}). Sensitive species show distinctive symptoms including gasping, spiraling, increased ventilation, and loss of equilibrium, suggesting a possible impact on cardiorespiratory physiology. This study aims to further investigate mechanisms of 6PPD-quinone toxicity to salmonids of differing sensitivity, specifically underlying cardiovascular and metabolic responses of acute exposure to 6PPD-quinone in juvenile rainbow trout and Arctic char. Fish will be exposed to 1 μg·L^{-1} or 10 μg·L^{-1} 6PPD-quinone in respirometry chambers for 48 hours to assess temporal changes in oxygen consumption compared to unexposed controls. Following exposure, ultrahigh resolution B-mode and Doppler ultrasound will be used to characterize cardiac function by analyzing changes in ejection velocity, stroke volume, ventricular and atrial contractile rates, and cardiac output. Furthermore, electrocardiography will be used to evaluate changes in the heart's electrical activity, and blood gas analyses will be completed to determine a variety of parameters such as glucose and methemoglobin. Data will be analyzed to investigate potential linkages between 6PPD-quinone exposure and integrated cardiorespiratory responses in native salmonid species.

Toxicity of 6PPD-quinone to early life stage rainbow trout (PL)

Catherine Roberts[1], Alper James Alcaraz[1], Evan Kohlman[1], Niteesh Jain[1], Markus Hecker[1], Markus Brinkmann[1]

[1]University of Saskatchewan

N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has recently been implicated in urban runoff mortality syndrome in coho salmon, leaching from tire wear particles into roadway runoff. Sensitivity to this toxicant is variable across fish

species, but previous studies place the LC_{50} for rainbow trout at 1.00µg·L^{-1}. To assess early life stage sensitivity, rainbow trout larvae were exposed for 28 days to six different concentrations of 6PPD-quinone, beginning at hatch. Larvae were maintained under semi-static conditions, with a 70% water renewal every 24 hours. Significant mortality occurred in the high concentrations, starting approximately 7 days post exposure. A subsequent 96-hour acute study with 6-week-old fry exhibited mortality in the highest concentration within 4 hours of exposure. Confirmation of exposure concentrations is ongoing. Subsequent analysis of samples will focus on histological and transcriptome changes between concentrations and age of exposure.

Effects of 6PPD-quinone on vulnerable fishes: Results of early life stage toxicity tests for river and copper redhorse (PL)

Hugo Marchand[1], Markus Brinkman[2], Nathalie Vachon[3], Emily Boulanger[1], Jessica Head[1]

[1]McGill University, [2]University of Saskatchewan, [3]Ministère des Forêts, de la Faune et des Parcs du Québec

Environmental concentrations of the tire rubber-derived chemical, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone), have been shown to be acutely toxic to several salmonid species including coho salmon, brook trout, and rainbow trout. However, mounting evidence suggests that not all species of fish are equally sensitive to this contaminant. Here, we assess the toxicity of 6PPD-quinone in two vulnerable fishes that are native to Canada: the copper redhorse (*Moxostoma hubbsi*), which is an endangered species that is endemic to Québec, and the river redhorse (*Moxostoma carinatum*), which is more broadly distributed throughout eastern Canada and the United States. Newly fertilized river and copper redhorse embryos were obtained from the provincial (Ministère des Forêts, de la Faune et des Parcs) copper redhorse artificial breeding program. Embryos were incubated at 19–21°C in clean water until hatching. Movement and aeration were maintained by pumping oxygen into incubation vessels. After hatching, larvae were exposed to control, solvent control, or 80, 40, 20, 10, 5 or 2.5 µg·L^{-1} of 6PPD-quinone with 75% daily renewal. Mortality and deformities were monitored daily until 2 weeks post-swim-up (21 days post-hatching). Preliminary results suggest no effects in either species on deformities, mortality, or time to swim-up. Overall, these two species do not appear to be as sensitive as salmonids. These results contribute to our understanding of variability in species sensitivity to 6PPD-quinone, and support risk assessment for this chemical in two vulnerable fishes.

Assessing the health effects of 6PPD-quinone on newly feeding juvenile Chinook and coho salmon (PL)

Bonnie Lo[1,2], Vicki Marlatt[1], Sofya Reiger[2], Carys Galilee[2], Tanya Brown[2]

[1]Simon Fraser University, [2]Fisheries and Oceans Canada

An ozonation product of the tire rubber antioxidant 6PPD, N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone) has been identified as a serious threat to the health of coho salmon. Recent studies have reported a wide range of sensitivity to 6PPD-quinone among several fish species (e.g., rainbow trout, brook trout, Arctic char, zebra fish), with coho being by far, the most sensitive. Chinook salmon are among the salmon species whose sensitivity to 6PPD-quinone has not been established. In Canada and the United States, many populations of Chinook have been classified as either endangered or threatened under relevant federal endangered species laws. Juveniles from many populations (including 19 in the Fraser River, British Columbia), migrate through urban-impacted waterways prior to entering the ocean, thus risking exposure to 6PPD-quinone during an early, sensitive life stage. Current 6PPD-quinone toxicity testing on juvenile salmonids has focused on the 1+ year range, therefore a data gap exists for younger fish. In this study, newly feeding (approximate 3 weeks) juvenile Chinook and coho were exposed to five concentrations of 6PPD-quinone for 24 hours. Mortality was assessed and LC_{50} values were calculated for each species. Results indicate that newly feeding juvenile coho are more sensitive to 6PPD-quinone than 1+ year old fish. Also, the exposures demonstrate that juvenile Chinook, are an order of magnitude less sensitive to 6PPD-quinone than coho. Results from this research will be used to inform the risk of this prevalent urban contaminant to juvenile Chinook and coho, and aid in the conservation, recovery, and management efforts of these much-valued species.

Understanding toxicity of tire-wear contamination in three freshwater species: From chemical mixture to nanoparticles (VPL)

Eva Roubeau Dumont[1], Jun-Ray Macairan[1], Laura Hernandez[1], Xiaoyu Gao[1], Jingyun Zheng[1], Anca Baesu[1], Stacey Robinson[2], Stephane Bayen[1], Subhasis Ghoshal[1], Nathalie Tufenkji[1]

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In recent years, tire-wear particle contamination has raised global awareness due to its worldwide ubiquity and its harmful effects on various species. Because of the complexity of this contaminant, involving a mixture of dissolved chemicals and rubber particles, mechanisms behind the toxicity remain to be understood, as some component types may be more harmful than others depending on species' sensitivity. Our work aimed to shed light on the toxicity of the different component types of tire-wear leachate on three freshwater model species: *Chlorella vulgaris*, *Lemna minor*, and *Daphnia magna*. Through sequential filtrations, tire-wear particle leachate was divided into three fractions: the first

fraction contained all components (dissolved chemicals, i.e., organics and trace metals, and particles <0.22 μm), the second fraction contained the dissolved chemicals (metals and organic chemicals), and the third fraction contained nanoparticles <0.22 μm only. The toxicity of each fraction was assessed in single-species assays using modified standard OECD protocols, monitoring acute and sublethal effects depending on each species' sensitivity. Exposures revealed very high sensitivity of *D. magna* for all three fractions, with LC_{50}s below 1.5%. *C. vulgaris* was not sensitive to any fraction, whereas *L. minor* showed a decrease in growth and photosynthesis efficiency only for fractions containing dissolved chemicals. Our results suggest that both chemicals and nanoparticles may have concerning effects on filter-feeding species, whereas photosynthetic organisms are more sensitive to the dissolved chemicals. Overall, our results raise concerning questions about potential long-term effects, such as population weakening and species interactions.

Metabolomics provides insights into potential sublethal effects of 6PPD-quinone on the fathead minnow (*Pimephales promelas*) (PL)

Katherine Anderson-Bain[1], Justin Miller[1], Catherine Roberts[2], Alper James Alcaraz[2], Dave Janz[2], Lynn Weber[2], Markus Hecker[2], Markus Brinkmann[2], Tony Montina[1], Steve Wiseman[1]

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N-(1,3-dimethylbutyl)-N′-phenyl-p-phenylenediamine-quinone (6PPD-quinone), an oxidation product of the tire-rubber antioxidant, 6PPD, is an emerging contaminant of concern. 6PPD-quinone is acutely lethal to coho salmon, brook trout, and rainbow trout, but does not cause acute lethality of other fishes, including Arctic charr and white sturgeon. The current study used H1-NMR metabolomics to investigate effects of 6PPD-quinone on the metabolome of fathead minnows, a species that we show is insensitive to 6PPD-quinone acute lethality, to gain insight into potential sublethal effects of this contaminant. Sexually mature minnows were exposed for 96 hours to 6PPD-quinone at nominal concentrations of 0, 0.2, 2, or 20 $\mu g \cdot L^{-1}$, and gills and livers were harvested for analysis. Pathway topology analysis identified potential disturbances in 13 biochemical pathways in the gills and 16 biochemical pathways in the livers. In gills, compared to the control, abundances of 17 metabolites were different in low and medium exposures, and abundances of 16 metabolites were different in the high exposure. In livers, abundances of 20 metabolites in the low exposure, 19 metabolites in the medium exposure, and 30 metabolites in the high exposure were different from the control. Tissue specific changes in abundances of metabolites related to one-carbon metabolism, the methionine cycle, and DNA methylation were identified. Notable metabolites include S-adenosylhomocysteine (gills: low=-6.0%, medium=-21.0%, high=-24.5%; livers: low=+32.0%, medium=+26.7%, high=+33.7%), choline (gills: low=-2.7%, medium=-17.8%, high=-23.7%; livers: low=+33.0%, medium=+28.5% high=+37.2%), methionine (gills: low=+8.5%, medium=+10.4%, high=+1.2%; livers: low=+29.0%, medium=+24.1%, high=+29.9%), and homocysteine (livers: low=+35.6%, medium=+35.4%, high=+39.5%). Reasons for tissue-specific responses to 6PPD-quinone and functional impacts of these responses are being explored.

Indigenous Voices in Ecotoxicology

Maintaining mino-bimaadiziwin in research (VPL)

Monica Garvie (Waasabiikwe)[1]

[1]*Queen's University*

Navigating the academy and the scientific method, as an Indigenous person, poses many challenges. Not only must we achieve the western standards of successful research and scholarship in institutions which systemically disadvantage and devalue our ways of knowing and being, but we must do so while remaining culturally intact. To achieve these often-conflicting needs, scientific research must be practiced in a different way than the current western methods. Many Indigenous scholars maintain a culturally relevant relationship with their research despite not disclosing such actions to their peers, supervisors, or funding agencies. Here I discuss my developing experience as an Anishinaabe scientist and some of the frameworks I utilize to maintain good relations with the land, communities (both Indigenous and scientific), my research data, myself, my culture, and all of creation. I will also provide examples from my current research on Crawford Lake (in Ajetance Treaty, No. 19 Territory), a candidate for the Anthropocene GSSP, and the Upper Kenogami Watershed (in Treaty #9 Territory) which has been impacted by gold mining, logging, and an inter-basin water diversion built in 1939. My research spans two different nations (Wendat and Anishinaabeg), and I will discuss how I maintain (and grow into) mino-bimaadiziwin while maintaining protocols appropriate to the nations with which I work, as well as scientific/paleolimnological methods.

How to address the persistence of colonialism in environmental monitoring (PL)

Daniel Kanu[1], Kianna Durston[1]

[1]*Lake Winnipeg Indigenous Collective*

Indigenous Peoples have lived in close relation with the lands and waters surrounding Lake Winnipeg since time immemorial. Generation after generation of Indigenous Peoples survived and thrived by the formation of complex Indigenous Knowledge systems. However, once the newly formed Canadian confederation began to assert authority over the land, Indigenous People were forced into reserves and residential schools to make way for the exploitation of resources. Over the next century, pollution from agricultural, urban, and industrial development have been particularly harmful to Indigenous Peoples. Community-led environmental monitoring is one potentially important tool for communities to identify, address, and adapt to harmful environmental contaminants. The trade-off is that many of the processes, assumptions, and funding mechanisms underlying monitoring continue to reinforce colonialism and in turn subverts Indigenous Knowledge,

jurisdiction, and rights. We examine the critical elements of colonialism within environmental monitoring and explore alternatives for Indigenous-led programs and their partners.

Indigenous-led oil spill emergency response development and avian impact assessment in Nunatsiavut (PL)

Reyd Smith[1], Frédéric Dwyer-Samuel[2], Rodd Laing[2], Regina Wells[3], George Gear[2], Samantha Jacque[2], Joseph Bennett[1], Jennifer Provencher[3]

[1]Carleton University, [2]Nunatsiavut Government, [3]Environment and Climate Change Canada

In June 2020, over 3000 L of diesel oil was released into a fjord near Postville, Nunatsiavut, Labrador which is known critical seabird breeding habitat. Following the spill, Nunatsiavut Government (NG) officials contacted ECCC (Environment and Climate Change Canada) to collaborate on an oil spill emergency response protocol. NG and ECCC then co-developed a plan to sample seabirds via community-based collections at the spill site (Postville), and a reference site ~200 km north (Nain). NG conservation officers and community members carried out egg collections in June–July 2020 to examine any immediate impacts on laying seabirds, as well as in 2021–2022 to examine inter-annual trends. Collections focused on three species selected by NG that are commonly found across Nunatsiavut and consumed by people in the region: pigeons (black guillemots - *Cepphus grylle*), sadlers (black-backed gulls - *Larus marinus*), and ducks (common eiders - *Somateria mollissima*). Hunters were also engaged by NG during the fall harvest of 2020–2021 to collect migratory, post-breeding seabirds for examination of oil-related contaminants. Planning and implementation of sample collection was directed by NG. As analytical data has become available, ECCC and Carleton University staff prepare summaries of the data, and the project team (ECCC, Carleton, NG) meet to review the data. NG then directs the next steps in the research process. Nunatsiavut governance and autonomy over the planning, execution, data, and communication of results (in collaboration with ECCC) is an essential part of our program. Overall, we present our collaborative and inclusive research project as an example of Indigenous-led conservation.

Contaminated sites and Indigenous communities in Canada and the United States: A scoping review (PL)

Katherine Chong[1], Niladri Basu[1]

[1]McGill University

The Kanien'kehà:ka Community of Kanesatake currently leads the ECHIP (Environmental Contaminants and Health Impact Project) funded under the First Nations Environmental Contaminants Program (FNECP), aiming to investigate contamination from

a nearby urban waste site. Such a situation is not uncommon as federal contaminated sites are disproportionately located near Indigenous lands, and the management of these sites is challenged by epistemological differences, regulatory barriers, and minimal research to date. To help increase understanding of the topic, and provide our community partners with actionable knowledge, this scoping review mapped information on contaminated sites and Indigenous Peoples in Canada and the USA, utilizing three streams of data: a systematic literature search (Keywords: "contaminated sites", "Indigenous communities"); grey literature; and federal databases (US EPA Superfund and Canadian Federal Contaminated Sites Inventory). Our search yielded 49 peer-reviewed and 19 grey articles, and 8114 federal records, allowing us to summarize the state-of-the-science on the topic. The results revealed the contamination of the lands of 815 distinct Indigenous tribes and nations and the presence of 440 different contaminants found at 4976 sites. The results also highlighted the need for holism, collaboration, efficiency, and Indigenous leadership in site assessment, management, and research, including community-specific risk assessments that understand Indigenous conceptualizations of health. This scoping activity provides foundational information for the ECHIP team to build on and a basis for next steps in addressing local contaminated sites. We note that study authors are non-Indigenous ECHIP collaborators based in McGill's Centre for Indigenous Nutrition and Environment.

Building relationships continues with community engagement at world's freshwater laboratory (PL)

Dilber Yunus[1]

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Building relationships with Indigenous communities continues at world's freshwater laboratory – IISD-Experimental Lakes Area (IISD-ELA), with a focus on community engagement. IISD-ELA is a freshwater research facility located in the traditional Anishinaabe territory of Treaty #3 in northwestern Ontario. For years, it has been working to expand its portfolio in public communication and prioritizing Indigenous engagement. Located in Treaty #3 traditional land, IISD-ELA has seized upon a unique opportunity to work more closely with Indigenous communities to look at how the two ways of knowing can work together and benefit each other. During this session, Dilber Yunus, IISD-ELA's outreach officer, will explain how IISD-ELA has partnered with Indigenous groups through various projects in areas of community engagement in environmental dialogues, lake monitoring, and youth program to promote knowledge sharing and transfer. She will also explore what IISD-ELA has learned along the way, including the importance of engaging community members in dialogues, understanding communities' needs, and incorporating the preservation and revitalization of Indigenous culture and language into collaboration efforts. Finally, she will explain what the lessons IISD-ELA has learned could mean for other scientific facilities who want to engage with Indigenous communities in a meaningful way.

Bigstone Cree moose and water monitoring (VPL)

Janelle Baker[1], Helen Noskiye[2]

[1]Athabasca University, [2]Bigstone Cree Nation

This paper and short ethnographic film demonstrate the sakawiyiniwak (Northern Bush Cree) ethnobiological knowledge of naming and butchering the cultural keystone species mooswah, or moose (*Alces alces*). Bigstone Cree Nation Elders promote the linguistic continuation of sakaw nehiyawewin (northern Bush Cree language) naming of moose anatomy and the association of this knowledge with good and respectful moose butchering protocols and behaviour. We explain the significance of traditional butchering practices and demonstrate them in a short film of Helen Noskiye and her brothers butchering a moose in 2016. We have partnered with a team of scientists and Bigstone Cree Nation environmental monitors for research in which we use moose sampling kits to test them for indicators of health and contaminants, along with water sampling for microbiological analysis and toxicology related to moose and human health. We describe the community observations in changes in moose health and movements that informs this monitoring.

Ecological Effects of Agricultural, Wastewater, and Industrial Contaminants in Canada

Ecotoxicity of two perfluoroalkyl carboxylic acids (PFCAs) and two perfluoroalkyl sulfonic acids (PFSAs) on northern leopard frogs (*Rana pipiens*) (PL)

Stacey Robinson[1], Sarah Young[1], Jillian Rohonczy[2], David Carpenter[1], Amila De Silva[1], Cassandra Brinovcar[1], Adrienne Bartlett[1], Ève Gilroy[1]

[1]Environment and Climate Change Canada, [2]Carleton University

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds found in a wide array of consumer products. The persistence of former PFAS compounds in the environment and their adverse health effects have led to increased use of replacement PFAS compounds, such as perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Our objective was to determine the sub-lethal ecotoxicity of two PFCAs and two PFSAs on northern leopard frog (NLF; *Rana pipiens*) metamorphosis and gonad development. Using chronic laboratory aquatic exposures, we exposed tadpoles to perfluorobutanoic acid (PFBA; CAS: 375-22-4), perfluorohexanoic acid (PFHxA; CAS: 307-24-4), perfluorobutane sulfonic acid (PFBS; CAS: 375-73-5), or perfluorohexane sulfonic acid (PFHxS; CAS: 355-46-4) individually at concentrations ranging from 0 to 1000 $\mu g \cdot L^{-1}$. For the two PFCAs, we found high survival; control and treatment survival ≥95%. We also found no significant differences on tadpole snout-to-vent length, hepatosomatic index, or Gosner stage of development. However, tadpoles exposed to PFBA at 0.1 and 1 $\mu g \cdot L^{-1}$ and PFHxA at 0.1, 10, and 1000 $\mu g \cdot L^{-1}$ had significantly increased body mass. In addition, compared to a high proportion of female tadpoles in the controls (77.6 ± 0.2%), we found a significant decrease in the proportion of females at 1 and 100 $\mu g \cdot L^{-1}$ of PFBA, where the ratio of female to males approached 50:50. Currently, our results suggest low toxicity of PFBA and PFHxA on NLF during their aquatic life stages; however, effects of the two PFSAs are pending. Further work will assess the stress response of tadpoles after chronic exposure.

In vitro characterization of the emerging perfluoroalkyl substance replacement, perfluoroethylcyclohexane sulphonate (PFECHS) (VPL)

Hannah Mahoney[1], Markus Brinkmann[1], John Giesy[1,2,3]

[1]University of Saskatchewan, [2]Michigan State University, [3]Baylor University

The widespread application of poly- and per-fluoroalkyl substances (PFAS) has resulted in some substances being ubiquitous in environmental matrices, and their resistance to degradation have allowed them to accumulate in wildlife and humans with associated toxic

effects. While certain substances of concern have been phased-out or banned, new substances continue to be produced. One such substance is perfluoroethylcyclohexane sulphonate (PFECHS), an analogue of perfluoroctanesulphonic acid (PFOS) which has recently been detected in multiple environmental media around the globe. However, there is little information on the toxic potency of PFECHS and other cyclic-PFAS in general. Therefore, this research aimed to characterize PFECHS and elucidate its effects in the aquatic environment using *in vitro* techniques. Liver cell lines RTL-W1 and ZFL-4 were used to predict the exposure response of PFECHS in rainbow trout, and further analyses focused on membrane effects were completed with rainbow trout leukocytes. PFECHS does not result in apical adverse effects at environmentally relevant concentrations apart from decreasing plasma membrane polarity. While molecular alterations were also observed at exposure concentrations lower than those that induce apical effects, chemical analyses of the exposure media have further supported PFECHS as not as acutely potent, nor as potentially bioaccumulative as legacy congeners. Determining the toxic potency of PFECHS is an important step in determining the safety of potential PFAS replacements, and such research will help better inform the viability of replacements as a strategy for PFAS management in the future.

Benzotriazole ultraviolet stabilizers (UV-P, UV-9, UV-090) induce AhR-mediated toxicity in fishes (PL)

Hunter Johnson[1], Justin Dubiel[1], Zhe Lu[2], Jon Doering[1,3], Steve Wiseman[1]

[1]University of Lethbridge, [2]Université du Québec à Rimouski, [3]Louisiana State University

Benzotriazole ultraviolet stabilizers (BUVSs) help counter degradation and discoloration of plastic materials caused by UV radiation. Due to improper disposal of plastics, BUVSs are ubiquitous in aquatic environments and biota, causing concern for the health of fishes and other aquatic wildlife. Of the limited toxicity data for BUVSs, studies have suggested that certain BUVSs might dysregulate the aryl hydrocarbon receptor (AhR) causing early life stage toxicity in fishes. Therefore, there is need for a more comprehensive analysis of the effects caused by exposure to BUVSs and risks posed by these chemicals. The present study exposed zebrafish (*Danio rerio*) embryos to serial doses of three priority BUVSs, namely 2-(benzotriazol-2-yl)-4-methylphenol (UV-P), 2-(benzotriazol-2-yl)-4-methyl-6-prop-2-enyl-phenol (UV-9), or 2-[3-(2H-benzotriazol-2-yl)-4-hydroxyphenyl]ethyl methacrylate (UV-090), by use of microinjection. Toxicity of each BUVS was assessed by recording malformations and mortality. Embryos exposed to BUVSs experienced mortality in a dose-dependent manner, with the most potent chemical tested being UV-P, that had a median lethal dose (LD_{50}) of 4772 $ng \cdot g^{-1}$ egg which is less potent than TCDD, the prototypical AhR agonist. The potency of AhR activation by each BUVS was determined with a luciferase reporter gene (LRG) assay using COS-7 cells transfected with the AhR of zebrafish. Consistent with results of the embryotoxicity assay, UV-P was the most potent AhR agonist with a median effect concentration (EC_{50}) of 1580 nM. Results

confirm that UV-P, UV-9, and UV-090 cause toxicity via activation of the AhR. Studies to assess sensitivity of native species of fishes to these BUVSs are ongoing and will be presented.

Temporal trends of mercury and organohalogenated contaminants in a bioindicator of the Gulf of the St. Lawrence, the northern gannet (PL)

Raphael Lavoie[1], Louise Champoux[1]

[1]*Environment and Climate Change Canada*

The St. Lawrence is a vast and complex ecosystem where biological productivity is high. It is subjected to many stressors including acidification, hypoxia, ship traffic, fisheries, invasive species, warming of the sea surface temperature, and pollution. The northern gannet was selected as a sentinel species to monitor the state of the Gulf of the St. Lawrence four decades ago after organochlorine contaminants were found to impact reproductive output and eventually, population size. In the last decade, their reproductive output was once more challenged, but by reductions in prey distribution and abundance. This study aims to determine the latest trends of contaminants in eggs of gannets between 1969 and 2019. Eggs were collected every five years to measure concentrations of mercury, legacy organochlorine contaminants (e.g., DDT and PCBs), and brominated flame retardants. In addition, stable isotopes were measured to indicate possible diet shifts. Most contaminants decreased in the last four decades, but recently the rate of decline decelerated, with contaminant concentrations increasing in some cases. Foraging ecology tracers such as stable isotopes revealed trophic shifts that partially explained concentrations of contaminants. Recent studies showed that climate change affected prey availability and shifted diet composition, which could in turn affect trophic transfer of contaminants up to gannets.

Environmental exposure to NSAIDs and potential contribution to eggshell thinning in birds (PL)

Shane de Solla[1], Laura King[1], Ève Gilroy[1]

[1]*Environment and Climate Change Canada*

Abnormally thin eggshells can reduce avian reproductive success, and have caused rapid population declines. The best-known examples of this phenomenon are the widespread population crashes in birds, mostly raptors, fish eating birds, and scavengers, caused by the pesticide DDT and its isomers in the 1960s. A variety of other chemicals have been reported to cause eggshell thinning. Non-steroidal anti-inflammatory drugs (NSAIDs), which are extensively and increasingly used in human and veterinary medicine, may be one particularly concerning group that demonstrates an ability to impair eggshell development, based both upon laboratory studies and on their known mechanism of action. In this

review, we outline environmental and wildlife exposure to NSAIDs, describe the process of eggshell formation, and discuss the pathways that NSAIDs affect. We gather a list of pharmaceuticals and other compounds demonstrated to affect eggshell thickness, and highlight their main mechanisms of action. Dosing studies empirically demonstrated that NSAIDs inhibit cyclooxygenases, which then suppress prostaglandin synthesis and reduce the calcium available for the mineralization of eggshell. Using the US EPA's CompTox Chemicals Dashboard, we show that NSAIDs can strongly inhibit cyclooxygenases. NSAIDs have been observed both in the putative diet of scavenging birds, and we report examples of NSAIDs detected in eggs or tissues of wild and captive Old-World vultures. We suggest that NSAIDs in the environment represent a hazard that could impair reproduction of wild birds.

Incorporation of new approach methodologies into species sensitivity distributions for ecological risk assessment (PL)

Rebecca Dalton[1], Dan Villeneuve[2]

[1]Environment and Climate Change Canada, [2]United States Environmental Protection Agency

New approach methodologies (NAMs) have the potential to modernize ecological risk assessment and require uptake into regulatory frameworks. This case study aims to provide a data-driven "gateway" for the use of NAMs in regulatory ecological risk assessment by incorporating NAM data into an approach that is already familiar to many risk assessors. Species sensitivity distributions (SSDs) can be used to estimate chemical exposure concentrations expected to have minimal to negligible effects on ecosystems (i.e., predicted no effects concentrations (PNECs)). This case study will compare PNECs derived from SSDs that include 1) only traditional endpoints, 2) only NAM endpoints and 3) both traditional and NAM endpoints. Through automation of extraction and filtering of data from the US EPA's ECOTOX knowledgebase, SSDs will be derived for over 50 chemicals representing a diversity of modes of action. Results from a pilot study found that the addition of NAMs into SSDs resulted in lower PNECs and an increased sample size compared to SSDs using only traditional data for two substances (bisphenol A and triclosan). For a third substance (tetrabromobisphenol A), there were insufficient data to generate an SSD with traditional data only and the incorporation of NAM data allowed for the derivation of a PNEC using the SSD approach. The potential benefits and impacts of incorporating NAM data into SSDs in regulatory ecological risk assessment contexts will be discussed.

Derivation of a site-specific guideline for the protection of aquatic life for unionized ammonia in the City of Calgary reach of the Bow River (PL)

Melanie Gallant[1], Dave Morgan[2], Vince Palace[3], Andrews Takyi[4], James Elphick[1]

[1]Nautilus Environmental, [2]Stantec Consulting Ltd., [3]International Institute for Sustainable Development-Experimental Lakes Area, [4]The City of Calgary

Wastewater treatment plant effluent discharged within the City of Calgary reach of the Bow River was identified as a potential risk to aquatic life due to exceedances of the long-term exposure water quality guideline for un-ionized ammonia (0.016 $mg \cdot L^{-1}$ as NH_3-N). This guideline was derived in 2001 from toxicity tests conducted in laboratory waters with a variety of species; however, only 6 of the 13 species are known to occur in the relevant section of the Bow River. Furthermore, toxicity of ammonia can be influenced by the water quality characteristics of a particular site. Therefore, chronic toxicity tests were undertaken following the resident species approach using site-relevant species (or appropriate surrogates) exposed to ammonia in Bow River water. The 12 species tested included: green algae (*Pseudokirchneriella subcapitata*), common duckweed (*Lemna minor*), water fleas (*Ceriodaphnia dubia* and *Daphnia magna*), mayfly (*Neocloeon triangulifer*), midge (*Chironomus dilutus*), amphipod (*Hyalella azteca*), fingernail clam (*Sphaerium* sp.), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), mountain whitefish (*Prosopium williamsoni*), and fathead minnow (*Pimephales promelas*). These data exceed the minimum requirements for derivation of a type A guideline. The species sensitivity distribution for chronic toxicity of un-ionized ammonia in Bow River water resulted in a hazard concentration for the fifth percentile of species (HC5) of 0.033 $mg \cdot L^{-1}$ as NH_3-N. The study provides an example of the effective use of the Resident Species Approach to deriving site specific water quality guidelines and illustrates the importance of considering locally relevant species and the influence of site water characteristics on toxicity.

Increased use of sanitizers and disinfectants during the COVID-19 pandemic: Identification of antimicrobial chemicals and considerations for aquatic environmental contamination and ecological effects (PL)

Sarah Marteinson[1], Michael Lawrence[2] , ZofiaTaranu[3], Kerri Kosziwka[4], Jessica Taylor[4], Alexandria Green[1], Amanda Winegardner[1], Trina Rytwinski[4], Jessica Reid[4], Cory Dubetz[1], Judith Leblanc[1], Michal Galus[1], Steven Cooke[4]

[1]Fisheries and Oceans Canada, [2]University of Manitoba, [3]Environment and Climate Change Canada, [4]Carleton University

During the COVID-19 pandemic there has been an increased need for sanitizing hands and disinfecting surfaces to reduce transmission of the SARS-CoV-2 virus. Products used for these purposes contain biocidal active ingredients, or antimicrobials, which have the potential to enter aquatic environments and may be emerging contaminants. Our

objectives were to: 1) identify current antimicrobial active ingredients, 2) quantify increased use of antimicrobial-containing products in Canada, 3) determine which antimicrobials may be candidate aquatic contaminants, and 4) prioritize antimicrobials for further ecotoxicological research. We consulted multiple sources of publicly available data; antimicrobials were identified using lists of registered sanitizers and disinfectants (Health Canada) and use trends were evaluated using import mass and grocery store retail sales data (Statistics Canada). Candidate aquatic contaminants were determined based on their likelihood to reach aquatic systems and for these we reviewed Canadian environmental concentrations, and indicators of persistence, bioaccumulation, and toxicity to aquatic biota. We identified 32 candidate antimicrobials which were dominated by quaternary ammonium compounds (QACs, particularly benzalkonium chloride), phenols, acids, then salts. Economic data showed increased imports and sales of sanitizing and disinfecting products in response to the COVID-19 pandemic. Considerable data gaps were evident for most candidate antimicrobials including aquatic environmental concentrations, fate and persistence indicators, toxicity to aquatic biota, and related water safety benchmarks, suggesting they would be considered as emerging contaminants if they are entering aquatic ecosystems. We recommend empirical research begin focusing on environmental monitoring of candidate antimicrobials and assessing the toxicity of QACs.

Time-resolved metal exposure using fish otoliths: A case study of a natural tundra wastewater wetland in Baker Lake, Nunavut, Canada (PL)

Bronte McPhedran[1], Mark Hanson[2], Heidi Swanson[1]

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Variability in metal exposure over the lifetime of a fish cannot be elucidated using metabolically active tissues, namely muscle or liver. However, the exposure history of select metals may be reconstructed using metabolically inert tissues, such as otoliths (i.e., calcium structures in the inner ear of teleost fish). Otoliths continuously accrete, allowing for time-resolved analyses of environments that lack baseline data or are challenging to sample, as is the case in many Arctic regions. Wastewater in the Arctic is primarily treated via natural tundra wetlands that rely on biogeochemical processes and dilution for effective treatment. Although favoured due to minimal maintenance and infrastructure, performance is constrained by extreme climatic conditions and increasing human activity. We aim to understand past and present metal accumulation in fish downstream of the wastewater treatment lagoon in Baker Lake, Nunavut by 1) investigating metabolically inert fish otoliths as a monitoring tool for metal exposure, and 2) characterizing routes of metal exposure in different fish tissues from upstream (n=1), wastewater-affected (n=3), and reference (n=2) lakes to help inform future system upgrades. Large- and small-bodied fish, benthic macro-invertebrates, zooplankton, sediment, and water were sampled in the summer of 2019 and 2021. Results to date depict a distinct separation of otolith marginal edge metal concentrations among sites and relationships with ecosystem variables that

may indicate exposure source. This study will help advance otolith microchemistry as an accessory research and monitoring technique in wastewater and Arctic environments.

Wastewater discharges alter microbial community composition in surface waters of the Canadian prairies (PL)

Milena Esser[1], Cameron Hoggart[1], Helen Baulch[1], Jonathan Challis[1], Yuwei Xie[1], John Giesy[1,2,3], Markus Hecker[1], Markus Brinkmann[1]

[1]University of Saskatchewan, [2]Baylor University, [3]Michigan State University

As one of the most common anthropogenic impacts on freshwater systems, wastewater poses a particular threat to natural microbial communities in receiving waters. These effluents are a significant source not only of pharmaceuticals and personal care products, but also of antimicrobial agents and other microorganisms that threaten natural microbial communities in freshwater ecosystems. However, the effects of wastewater treatment plant (WWTP) effluents on these communities and their functioning remain largely unexplored. Using a combined approach of analytical chemistry and DNA metabarcoding, we investigated the effects of five different wastewater treatment plants in southern Saskatchewan, Canada, on prokaryotic and eukaryotic communities in their respective receiving waters. The effluents were found to be significant sources of nutrients and anthropogenic pollutants. High nutrient loads and the presence of pollutants correlated with a decline in microbial diversity. Several taxa occurred in greater relative abundance in the wastewater-influenced river segments, indicating anthropogenic pollution and eutrophication, while other taxa, such as sulfur bacteria, declined, indicating changes in functional biodiversity and possible negative effects on ecosystem services. In addition, downstream of the WWTP with the highest pollutant input, a large increase in cyanotoxins was detected, coinciding with a significant shift in cyanobacterial community composition in the biofilms. Overall, our data suggest a causal relationship between anthropogenic pollution and changes in microbial communities that may reflect a decline in ecosystem health.

The identification of novel antimicrobial peptides from amphibia to reduce the impact of the poultry industry on the antibiotic resistance threat (PL)

Lorissa Corrie[1], Hossein Ebrahimikondori[2], Vanessa Thompson[3], Bita Raeisi[1], Darcy Sutherland[2,3,4], Anat Yanai[2], René Warren[2], Inanc Birol[2,4], Caren Helbing[1]

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The World Health Organization has identified antibiotic resistance as “one of the biggest threats to global health, food security, and development today”. This is exacerbated by the environmental release of antibiotics and their bi-products, resistant organisms, and

antibiotic resistant genes as contaminants. These enter the environment through human and animal waste, agricultural run-off, antibiotic production, improper disposal of antibiotics and their containers, and the application and use of manure fertilizer and reclaimed water. To mitigate the threat of resistance, it is crucial to implement tighter antibiotic restrictions and discover antibiotic alternatives such as antimicrobial peptides (AMPs). These small cationic peptides act as a part of the innate immune system of virtually all living organisms through directly killing microbes and modulating the host immune system. Herein, we identified 12 novel AMP candidates from amphibian genomic resources using rAMPage, an innovative bioinformatic identification pipeline. These exhibited growth inhibition and bactericidal properties on select pathogens (≤32 $\mu g \cdot mL^{-1}$) and low hemolytic activity (>128 $\mu g \cdot mL^{-1}$). Their ability to induce protective cytokine activity in embryos and macrophage cells were also examined. The data indicate that several of the AMP candidates possess desirable characteristics that can further be applied in the context of pathogen-infected poultry. While our work is currently focused on the poultry industry, the present work has far-reaching applications as facilitating the identification of AMPs for use in agriculture and health settings, which directly contributes to the reduction of adverse ecological effects of antibiotics.

Using hepatic gene expression assays in English sole (*Parophrys vetulus*) to investigate the effects of Metro Vancouver wastewater effluents in fish (VPL)

Karan Parekh[1], Vicki Marlatt[1]

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This study investigated the effects of Metro Vancouver's wastewater treatment plant (WWTP) effluents on English sole (*Parophrys vetulus*) hepatic gene expression using novel qPCR (quantitative polymerase chain reaction) assays to compliment Metro Vancouver's 2017 Burrard Inlet Ambient Monitoring Program. Seven locations of varying distance to the WWTPs were included. Twelve genes involved in xenobiotic defense (CYP1A, HSP70), thyroid function (DIO1), lipid and glucose metabolism (FABP1, FASN, GLUT2, PPARδ, PPARγ), protein synthesis (18S rRNA, RPS4X), and reproduction (ERα, VTG) revealed several differences between these impacted sites. We report the induction of biomarkers of reproductive endocrine axis abnormalities (i.e., elevated VTG and ERα in males and females, advanced ovarian development) were most prevalent nearest the primary treatment Lion's Gate WWTP, but the gene expression analyses indicate widespread exposure to estrogenic contaminants throughout the Burrard Inlet. Several genes involved in lipid and glucose metabolism and one enzyme involved in thyroid hormone metabolism were also associated with exposure to primary WWTP effluent. We hypothesize the former is at least partly attributed to the lipid regulating pharmaceuticals known to be present near sewage discharge sites. While the latter may be due to several chemicals present in sewage effluents and other industrial effluents (i.e., alkylphenols, dioxins and furans, metals, PAHs, PBDEs, PCBs, pesticides, and PPCPs), several of which were present in

English sole tissue. This study demonstrates the high potential of molecular biomarkers of urban contaminant exposure in wild caught English sole to assess a wider range of adverse health effects when combined with conventional whole organism health indicators.

Chronic venlafaxine exposure at environmental concentrations does not appear to pose a significant risk to freshwater ecosystems (PL)

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Venlafaxine is a prevalent antidepressant drug that has been detected at >2.0 $\mu g \cdot L^{-1}$ in freshwater ecosystems. Toxicological effects have been studied in laboratory settings and sublethal responses in fish have been reported, such as reduced swimming activity and altered feeding behaviour. However, it has not been investigated in a whole ecosystem context where direct and indirect effects of chronic exposure could pose a risk to the long-term balance of an ecosystem. To assess the potential ecological risk of chronic venlafaxine exposure, limnocorrals (n=10, 2 m diameter, 1.5 m deep) were deployed in-lake at the IISD-Experimental Lakes Area, spiked weekly with venlafaxine (4 to 100 000 $ng \cdot L^{-1}$) for 8 weeks and monitored for a suite of biotic and abiotic responses. Limnocorrals contained native plankton and invertebrates (zooplankton, emergent insects, macrobenthos) and were each stocked with finescale dace (*Chrosomus neogaeus*), a native small-bodied littoral fish. We characterized the behavioural responses of fish and responses (direct and indirect) of plankton and invertebrate communities to chronic venlafaxine exposure. For most endpoints, few differences were observed at environmentally relevant concentrations. The concentrations tested were deemed not directly lethal to the aquatic biota monitored. In the highest treatment of venlafaxine, water quality changes and shifts were observed in invertebrate community structure. Anecdotal evidence suggests bold behaviour in fish, which may have indirect effects on ecosystem structure. This study integrates the whole ecosystem response to a commonly detected contaminant and found that venlafaxine at environmentally relevant concentrations does not appear to pose a significant ecological risk to aquatic ecosystems.

Effects of clothianidin, thiamethoxam, and neonicotinoid mixtures on early life stages of sockeye salmon (*Oncorhynchus nerka*) (PL)

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This is the first study to report the acute and chronic effects of environmentally relevant concentrations of clothianidin and thiamethoxam (ranging from 0.15–150 $\mu g \cdot L^{-1}$ [acute only]), and a mixture of clothianidin, imidacloprid, and thiamethoxam neonicotinoid

pesticides (ranging from 0.045–450 μg·L^{-1}) on one wild stock of British Columbia sockeye salmon. No effects were observed on growth, development, and a targeted suite of 10 genes involved in reproduction, growth, stress response, nervous and immune system function after chronic neonicotinoid mixture exposures. However, acute thiamethoxam exposure during fertilization showed reproductive toxicity via a 25% reduction in fertilization success, and subsequent teratogenic effects via abnormal length, weight, and condition factor in swim-up fry in all thiamethoxam and some of the neonicotinoid mixture concentrations tested. These findings could have potential implications for wild salmon populations as pulse exposures are environmentally relevant, especially given the amount agriculture and rainfall in the lower Fraser River in British Columbia, Canada.

Effects of early life stage exposure of zebrafish (*Danio rerio*) to river water contaminated by agricultural activities (PL)

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Fish worldwide spawn in waters that are contaminated with complex mixtures of environmental chemicals. This may negatively affect the health of offspring, particularly if exposure occurs at a sensitive early life stage (ELS). We evaluated how ELS exposure to pesticide-contaminated river water affected zebrafish as larvae and adults. Water samples were collected from an agricultural river (Richelieu River, Québec, Canada) during the spawning season of an endangered species of fish (June 2019). Two surface water samples were collected at two known spawning areas (Chambly and Saint-Ours) following heavy rain events when pesticide concentrations peak. Based on targeted pesticide analysis, the Saint-Ours sampling site, which is downstream of tributaries fed by agricultural drainage, had higher pesticide concentrations than the Chambly site. Zebrafish embryos (3 replicates of 100 embryos each) were exposed to river water (Chambly or Saint-Ours) or laboratory water (control group) until 120 hours post-fertilization. Thereafter, zebrafish were raised to maturity in clean water. Exposure to river water delayed zebrafish hatching compared to controls but had no effect on larval survival or deformities. For adults that were exposed as embryos, the number of eggs produced was not different among treatment groups. However, fertilization success for the fish exposed to Chambly and Saint-Ours river water was lower than the control group (65, 72, and 81% respectively). Overall, effects were more pronounced in the fish exposed to water from the more contaminated site. These results increase our knowledge on the potential long-term effects of realistic mixtures to ELS fish.

Does agricultural activity influence brain size in a wild stream fish? (PL)

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Increased agricultural activity is required to feed a growing world population, which results in increasing land modification and use of agrochemicals. Agricultural runoff in aquatic ecosystems includes pesticides that are neurotoxic to fish at high doses through oxidative stress and fertilizers that can influence environmental dissolved oxygen levels through eutrophication processes, which can also lead to oxidative stress in aquatic organisms. Because oxidative damage can cause cell death and decrease neurogenesis, it could potentially contribute to decreases in brain size. This study examined off-target effects of agricultural activity on a stream fish, with an emphasis on potential neurotoxic effects associated with agricultural runoff. Creek chub (*Semotilus atromaculatus*) were sampled from 14 stream sites in southern Ontario adjacent to lands spanning a gradient of agricultural activity. Relative brain size was assessed against liver antioxidant gene expression (gpx1a, nfe2l2a, and sod1) and several stream site characteristics to evaluate factors that contribute to variation in mean brain size across sites. Our results suggest that variation in relative brain size was primarily driven by summer average temperature and aquatic invertebrate population composition. These variables explained 73% of the variation in mean relative brain size across sites. Thus, stream site characteristics, not directly related to agricultural runoff and oxidative stress, had the greatest impact on fish brain size in these southern Ontario streams, suggesting that the concentration of agrochemicals there did not reach levels that cause neurotoxic oxidative stress in fish. Funded by NSERC through the Canada First Research Excellence Fund and Discovery Grant programs.

Evaluation of biomagnification of the neurotoxin β-methylamino-L-alanine (BMAA) and its isomers in a Lake Erie food web (PL)

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Harmful algal blooms (HABs) release toxic compounds in water and are increasing in frequency worldwide due to eutrophication. HABs in Lake Erie are monitored extensively but one HAB toxin, the neurotoxin β-methylamino-L-alanine (BMAA), has not been investigated. Our goal was to evaluate the presence of BMAA and its isomers, AEG (N-(2-aminoethyl) glycine), DAB (2,4-diaminobutyric acid), and BAMA (β-amino-N-methyl alanine), in Lake Erie organisms and to evaluate the possibility of biomagnification. We sampled zooplankton, mussels, forage fish, yellow perch, and walleye near Point Pelee,

Ontario, and quantified their trophic position using nitrogen stable isotope signatures. BMAA and its isomers were quantified using validated methods for liquid chromatography with tandem mass spectrometry in whole invertebrate samples and fish brain tissue. We detected all four isomers among samples, but BMAA was detected the least frequently. The most abundant isomers varied greatly between groups of organisms. Sum concentrations of BMAA and its isomers increased with trophic position in adult walleye brains, but not in other organisms. BMAA and its isomers are present in organisms of Lake Erie and our investigation highlights important uncertainties related to the ecotoxicology of these compounds. First, considering that BMAA isomers also exhibit toxic effects, focus on BMAA at the expense of its isomers may be misguided. Second, variability in isomer abundance among organisms suggest species-specific metabolism or exposure to these compounds. Third, toxin biomagnification may be species-specific. These findings open new research avenues on the effects of HABs in Lake Erie. Funded by NSERC through the Canada First Research Excellence Fund and Discovery Grant programs.

Influence of watershed features on stream water quality along a gradient of agricultural activity (VPL)

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Streams are nested, hierarchical structures wherein the larger scale characteristics constrain the smaller components, determining instream ecology. The objective of this study was to assess the influence of different landscape and instream characteristics on water quality by assessing watershed characteristics, water chemistry, and benthic community structure across a gradient of agricultural intensity in the Grand River watershed in southern Ontario. Twenty-one sites across the central watershed were sampled in October 2020 and June 2021. A micro-basin polygon was generated for each site wherein land drainage for that site was mapped and analyzed for spatial heterogeneity (i.e., land use, riparian buffer extent) using ArcGIS®. Structural metrics such as the Shannon diversity index, Ephemeroptera, Plecoptera, Trichoptera index, and species richness showed distinct benthic macroinvertebrate communities across sites, with poorer values being correlated with greater agricultural land cover percentages, higher tile drainage presence, and lower riparian buffer extent. Results of the Canonical Correspondence Analysis corroborated these results, indicating significant differences between microbasins across the watershed based on underlying natural and anthropogenic factors. Lower pollution tolerance values were found to be associated with micro-basins that had lower agricultural extent, less artificial tile drainage, and greater riparian buffer presence, suggesting that land use and buffer extent play a key role in determining stream health. This study will enhance our understanding of the characteristics that most significantly influence water quality and help watershed managers provide more accurate recommendations for upstream catchments to preserve stream health.

Toxicity of salt to the early life stages of freshwater mussels is altered by hardness (PL)

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This study investigated how water hardness affects salt toxicity in two freshwater mussel species, *Lampsilis fasciola* and *Lampsilis siliquoidea*. Using standard toxicity testing methods the acute (24- and 48-hour) toxicity of sodium chloride to glochidia (i.e., freshwater mussel larvae) was examined in reconstituted waters of varying hardness. Reconstituted waters were created according to the USEPA (2002) recipes where varying prescribed amounts of $NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4$, and KCl are added to distilled water. One exception was that the amount of potassium chloride added to create the hard and very hard waters was reduced from the indicated amounts (hard, 8 $mg \cdot L^{-1}$; very hard, 16 $mg \cdot L^{-1}$) to the moderately-hard water amount (i.e., 4 $mg \cdot L^{-1}$), as potassium was suspected to be responsible for the observed reductions in control viability in the harder waters. Hardness of the seven dilution waters employed ranged from 7 $mg \cdot L^{-1}$ $CaCO_3$ (very soft water diluted 50:50 with distilled water) to 290 $mg \cdot L^{-1}$ $CaCO_3$ (very hard water). Chloride concentrations ranged from 5 to 5000 mg $Cl^- \cdot L^{-1}$ depending on water hardness. Glochidia viability (ability to close valves) was used as a surrogate for survival to derive effect concentrations (EC_{50}s). In both mussel species, increasing water hardness resulted in a sharp reduction in salt sensitivity up to a point (~150–200 mg $CaCO_3 \cdot L^{-1}$, depending upon species) after which no further protection (i.e., increase in EC_{50}) was observed. The 48-hour EC_{50}s for *L. fasciola* ranged from 7 mg $Cl^- \cdot L^{-1}$ in very soft (7 mg $CaCO_3 \cdot L^{-1}$) water to 390 mg $Cl^- \cdot L^{-1}$ in hard water (180–190 mg $CaCO_3 \cdot L^{-1}$). The 48-hour EC_{50}s for *L. siliquoidea* ranged from 14 mg $Cl^- \cdot L^{-1}$ in very soft water to 222 mg $Cl^- \cdot L^{-1}$ in hard water. These data demonstrate that increased water hardness can provide some protection against salt toxicity to early life stage freshwater mussels.

Evaluating the toxicity of legacy and emerging antimicrobial compounds to early life stages of rainbow trout (*Oncorhynchus mykiss*) (PO)

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Products with antimicrobial properties can be released with wastewater effluent and enter into freshwater systems, where they pose a risk to the health of aquatic organisms. While the effects of legacy antimicrobial compounds, such as triclosan (TCS), is well-studied, less is known about the toxicity of emerging alternative antimicrobials. This study aimed to evaluate and compare the effects of TCS and two commonly used and high-volume antimicrobials, chloroxylenol (PCMX) and methylisothiazolinone (MIT) on early life stage

rainbow trout (RBT; *Oncorhynchus mykiss*). Embryos of RBT were exposed for 28 days post-hatch (dph) to TCS, PCMX, and MIT separately at nominal concentrations of 0.38–400 $\mu g \cdot L^{-1}$. A solvent control of 0.01% DMSO was included for PCMX and TCS while MIT did not require a solvent. Throughout the 28-day exposure, mortality along with sublethal developmental responses (e.g., presence of edema and spinal curvature, time to swim-up) were recorded and water sampled to confirm exposure concentrations. At study termination, individuals were weighed and length recorded. Samples were taken for histopathological assessment and gut microbiome profiling. Preliminary analyses show a reduction in survivability and increased incidence of developmental abnormalities in TCS and PCMX at the higher exposure concentrations as compared to MIT. Analyses of other endpoints is ongoing and will be presented. It is anticipated that this research will provide information on biological effects of antimicrobials on an ecologically relevant fish species and provide data to inform risk assessment of these compounds in Canadian freshwater systems.

Effects of aquaculture waste on aquatic invertebrate communities within wild rice (*Zizania palustris*) planted mesocosms (PO)

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Production of northern wild rice (*Zizania palustris*) in Canada when paired with aquaculture operations could result in a sustainable alternative to traditional wild rice cultivation. The waste generated from aquaculture operations could be used to fulfill the nutrient requirements of wild rice, but may result in alterations to downstream waterways, potentially causing a reduction in water quality. These suboptimal conditions could secondarily affect the biological components of the aquatic ecosystem. We conducted a study to investigate the impacts on aquatic invertebrate abundance and community structure due to the exposure of varying aquaculture waste quantities in wild rice planted systems. We established outdoor mesocosms (2 m diameter; ~3200 L) with natural wild rice or with a commercial variety. All mesocosms were open to aerial insect colonization, as well as addition of invertebrates from local wetlands, plus introduced fathead minnows (*Pimephales promelas*). The mesocosms were then exposed to a gradient of waste from a local aquaculture operation on a bi-weekly basis. Zooplankton were sampled from activity traps, and benthic invertebrates were sampled at the conclusion of the study via sediment collection. Traditional enumeration methods were then conducted. Environmental DNA (eDNA) sampling was conducted monthly as a supplemental approach to the invertebrate analysis. The results of this study can provide valuable information regarding the suitable quantity of waste that could be applied to large-scale wild rice systems, such that water quality conditions remain non deleterious, as well as the utility of eDNA approaches.

Using mucus as a minimally invasive sampling tool to assess gene and protein expression pathways in fathead minnows exposed to graded nutrient loading (PO)

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The rising demand of nutritious and low-fat foods has corresponded to rapid growth in the wild rice and finfish aquaculture industries. Aquaculture operations generate substantial quantities of wastewater where fish waste (FW) contains a high concentration of nitrogen and phosphorus due to nitrogenous waste sourced from fish excretion and uneaten food. To achieve successful yields, wild rice (*Zizania palustris*) demands high quantities of these nutrients. Therefore, applying FW as fertilizer for wild rice presents a sustainable way of adding nutrients to agricultural practices whilst recycling effluents that would otherwise be destined for a natural receiving environment. However, the addition of excess nutrients to aquatic environments presents significant risks including ammonia and nitrite toxicity at high concentrations. In addition, excess phosphorus from FW can lead to toxic algal blooms leaving a potentially anoxic environment unsuitable for aquatic animals. We assessed the potential ecological risks of fertilizing wild rice with FW by investigating stress pathways in fathead minnows (*Pimephales promelas*) exposed to graded nutrient loadings in 33 wetland mesocosms. We focused on developing minimally invasive techniques to analyze gene and protein expression through fish mucus. We aimed to validate the use of mucus as a non-lethal sampling method for the EcoToxChip, a quantitative polymerase chain reaction (qPCR) array, and for proteomic analysis. This study may reduce the requirement for lethal sampling by validating the use of mucus for genetic and proteomic analysis, and help reveal environmental risks of aquaculture wastewater effluents discharged into aquatic environments.

Phytoremediation of aquaculture wastewater using wetland plants (PO)

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Aquaculture has been widely adopted as a sustainable alternative to traditional fisheries and offers a highly efficient method for the production of aquatic food products. Consequently, aquaculture has undergone rapid growth and is now among the fastest growing food production sectors in the world. Similar to other agricultural activities, aquaculture generates wastewater high in organic matter and nutrients such as nitrogen

and phosphorus. These effluents threaten water quality and pose a risk of eutrophication in receiving waters. Here we provide details of recent studies implementing cattail (*Typha* spp.) and northern wild rice (*Zizania palustris* L.) in the remediation of aquaculture wastewater. Model wetlands (2 m diameter; ~1500 L) were established and a gradient of aquaculture wastewater was applied routinely over several months. Cattail wetlands were robust to changes in water quality and demonstrated the ability to reduce phosphorus concentrations to background levels over time (<0.05 $mg \cdot L^{-1}$ PO_4-P). Two subsequent mesocosm studies were also conducted to calibrate the quantity of aquaculture wastewater that could be effectively remediated by commercial variety wild rice paddies and natural variety wild rice wetlands. Water quality data on nitrogen (total N, total dissolved N, NH_4-N, NO_3-N, and NO_2-N) and phosphorus (total P, total dissolved P, and reactive P) will be presented. These results demonstrate the ability to treat aquaculture wastewater via phytoremediation, improve water quality, and offer a potential fertilizer source for wild rice production in the future.

Contaminants of emerging concern in wastewater treatment plant effluent and recreational water sites (PO)

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Throughout the last 25 years, the term 'contaminants of emerging concern' has morphed into an open-ended ambiguous phrase to include compounds such as pharmaceuticals, personal care products, antibiotic resistant bacteria and genes, pesticides, and hormones. These compounds are increasingly detected at lower limits of detection in various environmental compartments, however large data gaps remain surrounding the types of chemical mixtures present, as well as the potential impact of such environmentally relevant mixtures on organisms. The objective of this study is to develop an analytical method to screen for 25 contaminants of emerging concern and test environmental aquatic compartments where such compounds are prevalent. Samples were collected from a wastewater treatment plant and outdoor swimming pools in the City of Winnipeg, Manitoba, Canada, as well as from recreational beach/lake in St. Malo, Manitoba. The focus of this method is for the simultaneous detection of 25 active pharmaceuticals and personal care products (PPCPs) including eight antibiotics (ampicillin, cephalexin, ciprofloxacin, imipenem, methicillin, sulfamethoxazole, trimethoprim, and tetracycline), eleven types of antimicrobial and disinfecting agents (alkyl trimethyl ammonium halides, benzalkonium chlorides, benzethonium chloride, didecyl dimethyl ammonium chloride, chlorohexidine, chlorohexidine gluconate, triclosan, triclocarban, nonylphenol, and 2-butoxyethanol) and six commonly used indicators for wastewater contamination in freshwater sources. These six indicators are acetaminophen, caffeine, carbamazepine, N,N-diethyl-m-toluamide (DEET), ibuprofen, and pentachlorophenol. Target analytes in effluents and water samples

were extracted using solid-phase extraction (SPE) with Oasis® HLB cartridges and quantitated via ultra-high-performance liquid chromatography with tandem mass spectrometry (UPLC/MS/MS).

Toxicity of oil sands process-affected water in early life stage wood frogs (PO)

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Oil sands process-affected water (OSPW) produced by the Canadian oil sands industry during the bitumen extraction processes is currently stored in large tailings ponds on-site and is comprised of a mixture of various organic and inorganic constituents. The composition and toxicity of OSPW is influenced by its age, source, and location within tailings ponds, and this experiment is part of a multi-year study aimed at evaluating a biomimetic extraction via solid-phase microextraction (BE-SPME) analytical technique, which has recently been developed as a reliable, cost-effective tool for hazard assessment of OSPW. We investigated the potential lethal and sub-lethal effects of a range of dilutions of OSPW (80, 40, 20, 10, 5, 2.5, and 1.25%) in wood frogs (*Lithobates sylvaticus*) native to Alberta from pre-hatching embryonic stages through to metamorphosis. Developmental delays at concentrations of 80% are evident, and the final analyses for growth are underway. In addition to BE-SPME measurements, speciated and total naphthenic acid (NA) concentration analyses of OSPW were conducted. BE-SPME and NA concentration data will be used to represent effect concentrations and derive concentration-effect relationships for OSPW exposure in an environmentally-relevant amphibian model. The results will be beneficial for future hazard assessment of raw and remediated OSPW in the Canadian oil sands.

Effects of contaminated groundwater on zooplankton community dynamics in shallow wetland mesocosms (PO)

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Groundwater contamination from industrial activity can result in complex mixtures that are difficult to characterize both chemically and toxicologically. This contamination can also migrate, putting nearby freshwater ecosystems at risk. To explore the effects and to characterize the potential risk of contaminated groundwater on relevant biological communities, 12 outdoor mesocosms were established with sediment, macrophytes, invertebrates, and fathead minnows (*Pimphales promelas*). Once the model wetlands were established, contaminated groundwater from wells located on an industrial site in the City of Winnipeg was added to the mesocosms in triplicate of control (0%), low (1%), medium

(3%), and high (6%) treatments (concentrations by volume). Here we report the impacts of contaminated groundwater on zooplankton community dynamics over the course of a 37-day exposure period. Mesocosms exposed to the contaminated groundwater demonstrated a significant ($p<0.05$) response in zooplankton community when compared to the control treatment as indicated by principal response curve (PRC). As well, significant ($p<0.05$) declines in diversity indices for treatments relative to control were observed at various timepoints. Mesocosms exposed to groundwater saw a dramatic increase in rotifer abundance, while control mesocosms did not, indicating a shift to poor water quality. The results suggest that contaminated groundwater may adversely impact zooplankton abundance and community dynamics, and put higher level trophic organisms such as fish at indirect risk of effect.

Assessing novel reproductive toxicity test methods for *Hyalella azteca* using imidacloprid (PO)

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Hyalella azteca is a freshwater benthic crustacean used in ecotoxicology because it is ubiquitous in North American freshwater systems and is sensitive to changes in water quality. The standard reproduction toxicity test method for this species outlined by Environment and Climate Change Canada (ECCC) is a 6-week sediment test where reproductive endpoints typically occur in weeks 4 to 6. It is difficult to achieve robust data for reproduction in *H. azteca* because there is naturally a high biological variability associated with reproductive yield, and because effects on reproduction often co-occur with effects on growth. The purpose of this study was to create a novel reproduction toxicity test method for *H. azteca* to increase the reproductive data generated. Four-week, water-only, reproduction tests were initiated using sexually mature (6- to 7-week-old) organisms in a three male : seven female sex-ratio, based on a previous study that determined the optimal sex ratio of *H. azteca* to reduce brood size variability. Standard 6-week reproduction toxicity tests using juvenile amphipods were adapted to water-only conditions. For both methods, amphipods were exposed to sub-lethal concentrations of imidacloprid (0.33, 1, 3, and 9 $\mu g \cdot L^{-1}$, determined from previous studies), and were exposed to weekly static-renewal conditions, at which point survival and reproduction were assessed. Growth was measured at the end of the tests. Comparisons between methods will be focussed on reproduction, but will also include assessments of survival, growth, and amount of resources invested. Results will reveal if the novel reproduction test method will improve toxicological assessments using *H. azteca*.

Environmental DNA (eDNA): Approaches to Assess Biodiversity and Manage Aquatic Ecosystem Health

Monitoring lake sturgeon (*Acipenser fulvescens*) spawning grounds using environmental DNA (VPL)

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Lake sturgeon (*Acipenser fulvescens*) is a freshwater species of great importance to the culture, economy, and livelihood of several Indigenous Peoples in Canada. Unfortunately, most lake sturgeon populations are declining, mainly because of over-harvesting, pollution, and dams. Spawning grounds are particularly affected and are deteriorating due to human activities. Close monitoring of lake sturgeon spawning grounds is thus crucial for the protection of this at-risk species. However, conventional surveying methods such as visual observation, adult capture, and egg counting are usually inaccurate, time-consuming, and invasive. The measurement of environmental DNA (eDNA), i.e., genetic material shed from organisms into their environment, is a promising alternative to monitor fish spawning grounds. This method is often cheaper and faster, and thus allows closer monitoring. The goal of this study was therefore to evaluate the use of eDNA for spatio-temporal monitoring of a lake sturgeon spawning ground. Once a week before and during the spawning period in May 2022, we sampled water from multiple stations at a known spawning ground near Québec City. We analyzed lake sturgeon eDNA using a newly developed and validated species-specific primer/probe set and quantitative polymerase chain reaction (qPCR). According to our preliminary results, eDNA offers valuable information on the spawning ground and spawning process, such as the date of fish arrival, upstream migration progress, and fish relative abundance. The next step of this study will be to refine the quantification of lake sturgeon abundance using eDNA by investigating the relationship between eDNA concentrations and absolute fish abundance.

Enabling oolichan (eulachon: *Thaleichthys pacificus*) health and distribution assessments in the Pacific Northwest using novel molecular tools (PL)

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Protection of threatened aquatic species relies on timely, reliable, and detailed monitoring data, which can be highly challenging for species whose life histories are not

well described. Oolichan (*Thaleichthys pacificus*), an anadromous smelt with a discontinuous spawning range from California to Alaska, has experienced steeply declining populations over the past few decades. Here we assess oolichan populations on the Central Pacific Coast in Haisla First Nations territory near Kitimat, British Columbia, a region that has seen significant recent development, including a new fossil fuel processing plant and increased marine traffic. Using a newly developed and highly sensitive environmental DNA (eDNA) assay, we demonstrate that eDNA in waterways provides earlier detection of inward spawning migration than paired conventional monitoring methods, and can be used to indicate the day of the major spawning event. In addition, we are generating novel genomics resources for oolichan through transcriptomic analysis of larvae and non-lethal fin clips using RNA-seq and quantitative real-time polymerase chain reaction methods to address the need for more effective population health assessment methods. Caudal fin clips have successfully been used to identify and characterize biological responses to exposure to water soluble components of marine oil spills. By comparing specimens from spawning habitats with different levels of ecological impact, combined with rigorous abundance estimates of eDNA sampling methods, we aim to track species population numbers, range, and health to a degree of detail that is impossible without this new generation of cutting edge, non-invasive molecular tools.

Factors limiting or enhancing environmental DNA detections in marine sediments (PL)

Laury-Ann Dumoulin[1,2], Marion Chevrinais[2], Richard Saint-Louis[1], Geneviève Parent[2]

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Natural matrices affect environmental DNA (eDNA) detections. Metal ions and humic acids were previously identified as inhibitors of polymerase chain reactions. Yet, factors affecting the DNA lysis and extraction steps are mostly unknown. We assessed the effect of physicochemical factors present in marine sediments on an eDNA detection protocol, from the DNA lysis to the qPCR (quantitative polymerase chain reaction) detection. We denatured marine sediments, mainly composed of sand and silt, with chemical treatments to remove organic compounds (e.g., DNA, humic acids) and labile metal ions. A specific copy number of synthetic DNA was then added to each matrix aliquot prior to the eDNA detection protocol, consisting of commercial kits for DNA lysis and extraction (PowerLyzer™ PowerSoil®), and probe-based qPCR detection (TaqPath™). We showed that eDNA detections (3 ± 0% to 12 ± 3%) increased proportionally with silt composition (7 to 55%, respectively). We then characterized the effect of metal ions (calcium (Ca^{2+}), iron (Fe^{3+}), manganese (Mn^{2+}), and copper (Cu^{2+})) or humic acids on eDNA detections using a 30% silt matrix. Total inhibition of eDNA detections occurred when calcium, iron or manganese was added at levels observed in surface marine sediment in Canada. Alternatively, eDNA detections were increased with the presence of copper (max. 14 ± 2%) or humic acids (max. 23 ± 3%). Additional tests suggest that humic acids chelate inhibitors

still present in the denatured sediments. The addition of ethylenediaminetetraacetic acid (EDTA) prior to the DNA lysis also improved eDNA detections (max. 18 ± 2%). Our findings highlight new limitations of eDNA detections and provide avenues for optimization of eDNA detection protocols applicable to multiple environmental matrices.

qPCR-based eDNA workflow for humic-rich lake sediments: Combining Indigenous Knowledge and sedimentary ancient DNA (sedaDNA) to reconstruct historical fish records (PL)

Mark Louie Lopez[1], Michael Allison[1], Fin MacDermid[2], Erin Ussery[3], Mark McMaster[3], Ave Dersch[4], Matthew Bonderud[1], Paul Drevnick[5], Caren Helbing[1]

[1]University of Victoria, [2]Cold Lake First Nations, [3]Environment and Climate Change Canada, [4]Moccasin Flower Inc., [5]Alberta Environment and Parks

Lake sediment core samples serve as a natural archive of historical biological information. The use of sedimentary ancient DNA (sedaDNA), a form of environmental DNA (eDNA) shed by aquatic organisms and preserved in sediment layers, has been useful in reconstructing past faunal composition in aquatic communities. However, the old age and humic-rich nature of lake sediments present methodological challenges for accurate detection of target DNA using quantitative polymerase chain reaction (qPCR)-based approaches. Herein, we present a comprehensive qPCR-based eDNA workflow to reconstruct historical fish (pre-development baseline data) records in selected lakes in the oil sands region of northeastern Alberta, which were then validated using Indigenous Knowledge from two First Nations communities. Results show that combining column- and precipitation-based PCR inhibitor clean-up, nucleic acid concentration steps, and the use of endogenous chloroplast DNA as a sample integrity control help avoid false-negative detection of target fish DNA. The present study also highlights the importance of using highly sensitive species-targeted qPCR assays with known limits of detection (LOD) and quantification (LOQ) to increase confidence in the reported results. Lastly, increasing the number of technical replicates for qPCR analysis aids in improving the detection of less abundant fish eDNA preserved in sediment layers. Overall, this study addresses common methodological concerns in processing lake sediment samples for fish eDNA detection, and demonstrates the great potential of combined sedaDNA data and Indigenous Knowledge in reconstructing historical fish records in aquatic communities.

Development of environmental DNA (eDNA) in support of fish community assessments in support of the Oil Sands Monitoring (OSM) program (PL)

Gerald Tetreault[1], Erika Myler[2], Robert Hanner[2], Cassandre Pyne[2], Keegan Hicks[3], Fred Noddin[3], Jasmin Gee[4], Aurora Janson[4], Mark McMaster[1]

[1]Environment and Climate Change Canada, [2]University of Guelph, [3]Alberta Environment and Parks, [4]Hatfield Consultants

Environmental DNA (eDNA) may be captured through collection of bulk environmental samples, and analyzed using DNA barcoding approaches to detect biological species without organism capture or sighting. Fish community biodiversity assessment is possible through eDNA metabarcoding using universal primers and next-generation sequencing technology. However, limitations imposed by the taxonomic coverage of existing universal primer sets and their corresponding databases remains a significant challenge for community detection. In this study, we evaluate the congruence between eDNA-generated fish community identification using metabarcoding (genetic markers: 12S rRNA and COI mitochondrial gene fragments) and fish community assessment collection data as part of the Oil Sands Monitoring (OSM) program using standard electrofishing techniques. Water samples were filtered for eDNA collection on site, prior to fish surveys, to limit site disturbance. During the processing of the electrofishing collections, fin clips were taken from individuals of each species for species-specific primer sequences, to develop a mock community for validation of results from the metabarcoding. The fish community profile generated through metabarcoding will be evaluated against fish survey results to identify false positive and false negative detections, and frequency of congruence between technologies. This study emphasizes the importance of validating eDNA metabarcoding-generated data through parallel experiments using traditional techniques, as well as through historical and local knowledge and experience.

Applying ecotoxicology to respond to the needs of a pandemic: SARS-CoV-2 wastewater surveillance in support public health action (PL)

Mark Servos[1], Hadi Dhiyebi[1], Meghan Fuzzen[1], Carly Sing-Judge[1], Yash Badlani[1], Heather Ikert[1], Nivetha Srikanthan[1], Samina Hayat[1], Nathanael Harper[1], Eli Zeeb[1], Hoang Dang[1], Patrick Breadner[1], Sean McLay[1], Leslie Bragg[1], John Giesy[2]

[1]University of Waterloo, [2]University of Saskatchewan

Early in the first wave of the COVID-19 pandemic it became evident that there was the potential to monitor SARS-CoV-2 viral fragments in wastewater as an alternate way to do community surveillance. Our research group rapidly responded to the needs of the public health agencies by pivoting research from environmental toxicology to assessment of trends of SARS-CoV-2 in wastewater. This was essentially an application of environmental DNA (eDNA) concepts and techniques to measure RNA fragments in a very complex sewage

matrix. After validating methods early in 2020, we worked closely with three municipalities and public health units (York, Peel, and Waterloo), and established public dashboards to disseminate the information. Regular sampling of influent (3 to 6 days per week) conducted at >12 wastewater sites has shown wastewater surveillance to be a useful tool to track community spread, and parallels closely the reported clinical cases. Wastewater surveillance has the advantage that it integrates everyone within the sewershed, including asymptomatic cases, and is independent of how testing is done for individuals over time. The onset of the Omicron variant and changes in clinical testing in Ontario left wastewater as the only reliable method to track infections during the 5th and subsequent waves of the pandemic. Wastewater also emerged as an effective way to rapidly monitor the spread of variants of concern and this information has supported traditional public health data and interpretation. Many Canadian labs have adopted similar methods and quickly established a national wastewater-based epidemiology (WBE) network to monitor for COVID-19.

iTrackDNA: Recent progress on a national environmental DNA large scale applied research project (PL)

Caren Helbing[1], Louis Bernatchez[2], Jérôme Dupras[3], Valerie Langlois[4]

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The analysis of environmental DNA (eDNA) – genetic material shed from organisms into their environment – promises to provide rapid, non-destructive, accurate, and cost-effective biodiversity information. However, inconsistent practices and poor quality eDNA detection tools threaten end-user (regulators, industry, Indigenous Peoples, and NGOs) uptake because of unacceptably high false negatives and false positives that can compromise effective management decision-making. iTrackDNA is a new, multi-year, large scale applied research project that is addressing these concerns with researchers and end-users across Canada and sectors. It will build end-user capacity through innovative, accessible, socially responsible, genomics-based analytical eDNA tools for effective decision-making by: 1) supporting the creation of a targeted eDNA detection national standard; 2) building eDNA kits to detect 100 priority invertebrates, fish, amphibians, birds, reptiles, and mammals in Canadian coastal and inland ecosystems; 3) applying 10 eRNA kits for determining animal biosurveillance, biosanitation, and bioremediation effectiveness; 4) generating decision support software for modeling regional biodiversity changes integrating Indigenous Ecological Knowledge; 5) developing an eDNA training, certification, and inter-lab validation framework for consultants, researchers, regulators, and managers; and 6) producing a guidance document on eDNA-based methods integration into management, policy, and regulations. Recent progress will be presented to highlight activities to build and augment the eDNA community of practice. iTrackDNA has an unprecedented, broad stakeholder commitment to make Canada a global frontrunner on

eDNA standards adoption, policy development, and transformative testing, and to confidently enable eDNA applications in coastal and inland ecological surveys and biosurveillance for mining, forestry, energy, and infrastructure projects.

Invisible fish: Taxonomic blind spots of environmental DNA metabarcoding-based detection of Canadian freshwater fish communities and solutions to improve coverage (PO)

Erika Myler[1], Cassandre Pyne[1], Robert Hanner[1], Gerald Tetreault[2]

[1]University of Guelph, [2]Environment and Climate Change Canada

Environmental DNA (eDNA) metabarcoding is a novel biodiversity assessment technique which involves collection and analysis of DNA fragments from environmental samples for multi-species detection. With increasing adoption of eDNA metabarcoding for fish community assessment on the horizon, it is critical to identify, address, and communicate the capabilities and limitations of this emerging tool. Several genetic markers and bioinformatics pipelines are employed in the eDNA metabarcoding field, which necessitates a broad-scale, cross-methods analysis to identify consistent method-specific and ubiquitous taxonomic blind spots (i.e., fishes that are undetected). These blind spots result from gaps in reference sequence libraries, on which eDNA metabarcoding relies, and/or inefficient binding of 'universal' primers to DNA of certain species, leading to poor sequence amplification and non-detection. Identifying species which are reliably detected with this tool is of equal importance. Here, I compiled and analyzed the lists of Canadian freshwater fishes detected by eDNA metabarcoding across colleagues' projects, in Ontario and Alberta using variable markers and pipelines, to identify taxonomic blind spots. Paired electrofishing or historical species lists were used, where available, to increase confidence in detections and non-detections with eDNA to strengthen evidence of a blind spot or reliable detection. Both method-specific and ubiquitous blind spots and reliably detected species will be discussed, followed by solutions and recommendations for improving taxonomic coverage. The conclusions will highlight current capabilities and limitations and contribute to methods optimization towards the development of eDNA for Canadian freshwater fish surveys. Acknowledgements:Kathleen Nolan, Yoamel Milián García, Robert Young for data contributions.

Dual marker environmental DNA-based detection of fish communities from surface water and sediment in a headwater stream (PO)

Erika Myler[1], Robert Hanner[1], Gerald Tetreault[2]

[1]University of Guelph, [2]Environment and Climate Change Canada

Environmental DNA (eDNA) metabarcoding is a novel biodiversity assessment technique which involves collection and analysis of DNA fragments from environmental samples for multi-species detection. With increasing adoption of eDNA metabarcoding for fish community assessment on the horizon, it is critical to identify, address, and communicate the capabilities and limitations of this emerging tool. While eDNA has typically been collected from surface water for fish surveys, it is known to bind to and persist in sediments for longer periods of time. Therefore, sedimentary eDNA is regarded as a biodiversity time-capsule, relaxing the time constraints for sampling. The efficacy of sedimentary eDNA surveys deserves further study. Here, I evaluate and compare the capabilities and limitations of eDNA metabarcoding for the detection of freshwater fishes using two genetic markers (12S rRNA and COI mitochondrial gene fragments) across three environmental sample types: surface water, suspended and benthic sediment. eDNA samples were collected monthly from six stations (two pools, riffles, and runs) along Marden Creek (Guelph, Ontario, Canada). To ground-truth the metabarcoding results, a self-created Marden mock community generated from sequenced fin clips was used as positive control, and also compared to electrofishing collections. The methods were evaluated based on alpha diversity, and compared to identify method-derived or ubiquitous gaps in taxonomic coverage. Electrofishing detected 27 fish species. Corroboration with and differences from fish communities detected across eDNA methods will be discussed. The conclusions will highlight current capabilities and limitations, and contribute to methods optimization towards the development of eDNA for Canadian freshwater fish surveys. Acknowledgements: The Ignatius Jesuit Centre for permitting and encouraging this study.

Nothing but water: Comparing biases in monitoring fish stocks of Lake Winnipeg using environmental DNA and gillnet index surveys (PO)

Katrina Audet[1], Arfa Khan[2], Margaret Docker[1]

[1]University of Manitoba, [2]Fisheries and Oceans Canada

Lake Winnipeg's fish stocks are annually monitored by the Province of Manitoba using gillnet index surveys, a traditional approach requiring fish to physically be caught for assessment. Environmental DNA (eDNA) is a non-invasive technique that requires water sampling within the fishes' ranges. Eight freshwater fishes in Lake Winnipeg were selected based on their: economic and recreational importance in Manitoba; value as a prey species; or elusiveness to being caught in gillnets. Walleye (*Sander vitreus*), sauger (*Sander*

canadensis), emerald shiner (*Notropis atherinoides*), spottail shiner (*Notropis hudsonius*), rainbow smelt (*Osmerus mordax*), yellow perch (*Perca flavescens*), trout-perch (*Percopsis omiscomaycus*), and lake cisco (*Coregonus artedi*) were assessed. Water samples were collected prior to gillnet placement to prevent introduction of any DNA that may be present from past sampling events, and filtration and processing of the samples were performed in a sterile manner to avoid contamination. Species-specific TaqMan probe-based quantitative polymerase chain reaction (qPCR) assays were designed to determine the presence or absence of these eight species across 41 sites in Lake Winnipeg, and this will be compared to the catch results from the annual gillnet index surveys at each sampling site. The results of this project can be used to analyse the biases in detection of certain species, estimate species relative abundance, and compare costs between eDNA and gillnetting techniques. Results from this research may help industries in recreational and commercial fisheries find a better monitoring system of fish stocks in Lake Winnipeg with fewer mortalities, thereby ensuring the conservation of important fish species.

"Gone Fishing": Using environmental DNA (eDNA) and citizen science to assess the presence of stocked fishes in FortWhyte Alive's lakes (PO)

Arfa Khan[1], Brooklynne Litke[2] , Claire Risbey[2], Katrina Audet[2], Morgan Anderson[2], Margaret Docker[2]

[1]Fisheries and Oceans Canada, [2]University of Manitoba

Environmental DNA (eDNA) sampling refers to a promising, non-invasive surveying technique that can detect a species in an environment through the presence of their shed DNA. The ease of use of eDNA sampling allows for its incorporation as a tool in citizen science projects, encouraging public participation in science while facilitating meaningful research that can inform fisheries and conservation management decisions. In collaboration with the International Institute for Sustainable Development-Experiment Lakes Area (IISD-ELA) and 10 high-school students, we used eDNA to assess fishes stocked in the lakes at FortWhyte Alive (Manitoba) as a part of the IISD-ELA eDNA pilot program: monitoring eDNA and learning ecology with youth (MeDLEY). Four of the five lakes have been stocked over the past 50 years with several species for catch-and-release fishing, including: northern pike (*Esox lucius*), walleye (*Sauger vitreus*), yellow perch (*Perca flavescens*), and black crappie (*Pomoxis nigromaculatus*). To detect the eDNA, we developed TaqMan probe-based quantitative polymerase chain reaction assays for black crappie and walleye that target the cytochrome oxidase subunit I (COI) gene and used existing COI assays for the remaining fishes. Using water samples collected from the five lakes and filtered by the students, and the above assays, we non-invasively assessed the presence of the stocked fishes in each lake. While gaining insight regarding fish stocking at FortWhyte Alive, this project also demonstrates an example of effective citizen science; encouraging youth participation and curiosity in science and the natural world, and providing future scientists with meaningful hands-on experiences.

"Where are they now?": Using environmental DNA (eDNA) to track freshwater fishes of conservation concern in Manitoba (PO)

Brooklynne Litke[1], Arfa Khan[2], Margaret Docker[1]

[1]University of Manitoba, [2]Fisheries and Oceans Canada

Freshwater ecosystems contain some of the most imperilled habitats on Earth. Assessing the distribution and abundance of the species in these ecosystems is an essential step in the effective monitoring of the well-being of their populations and the biodiversity within the ecosystems. However, some freshwater fishes are often overlooked during traditional surveys due to their elusiveness, size, rarity, and/or range. Over the past decade, environmental DNA (eDNA) surveying has emerged as a promising, non-invasive solution to this issue in aquatic ecosystems as it can detect the presence of fish without their physical capture. We developed five TaqMan probe-based quantitative polymerase chain reaction assays that target the cytochrome oxidase subunit I gene and can detect the DNA of six freshwater fishes of conservation concern: bigmouth buffalo (*Ictiobus cyprinellus*), chestnut lamprey (*Ichthyomyzon castaneus*), golden redhorse (*Moxostoma erythrurum*), hornyhead chub (*Nocomis biguttatus*), and paired species northern brook (*I. fossor*)/silver (*I. unicuspis*) lampreys; and one genus-specific assay that distinguishes *Ichthyomyzon* spp. lampreys from other genera of lampreys. Using water samples collected from over 55 sites across Manitoba, we are using these eDNA assays to help efficiently and cost-effectively map the distribution of these species in Manitoba and determine if the detectability of these fishes using eDNA is affected by body size, life-history traits, or habitat characteristics.

Large-scale validation of an environmental DNA monitoring protocol for larval invasive sea lamprey (*Petromyzon marinus*) (PO)

Cameron Brown[1], Robert Hanner[1], Margaret Docker[2]

[1]University of Guelph, [2]University of Manitoba

Environmental DNA (eDNA) is a species detection method that is a cost and labour-effective alternative to traditional capture-based sampling. Previous development of a quantitative polymerase chain reaction (qPCR) assay for sea lamprey (SL) has made eDNA monitoring possible, but this method had not yet been tested through large-scale field validation. Current control efforts and the support for continued monitoring provided an excellent opportunity to leverage pre-existing infrastructure to optimize and validate an eDNA monitoring protocol for larval SL across various riverine habitats and seasons. Working with the US Fish and Wildlife Service and Fisheries and Oceans Canada, we conducted paired eDNA and electrofishing (EF) surveys at three to five stations on 24 rivers in 2021 and 2022, where we expected larval SL to be absent or at low, medium, or high density. We sampled each station in the summer, fall and winter. We then analyzed the

samples using a qPCR assay with an internal positive control to test for inhibition. Our current results demonstrate the optimal parameters (filter type, sampling effort, etc.) for eDNA monitoring of SL. We also compared EF to eDNA monitoring, identified the detection probability of eDNA, and determined under which conditions eDNA is more sensitive to SL presence. Our standardized and rigorously tested eDNA protocol for SL monitoring will greatly assist current control efforts in reducing invasive SL's ecological and economic damage and assessing the effectiveness of lampricide treatments. Combined with an occupancy modelling framework, this dataset will allow novel insight into the interactions between the SL, eDNA, and the environment.

Zooplankton and associated microbiome response to simulated oil spill and remediation efforts (VPO)

Phillip Ankley[1], Yuwei Xie[1], Lauren Timlick[2], Madeline Stanley[3], Markus Brinkmann[1], Markus Hecker[1], John Giesy[1,4], Vince Palace[2]

[1]University of Saskatchewan, [2]International Institute for Sustainable Development-Experimental Lakes Area, [3]University of Manitoba, [4]Baylor University

Oil spills can disturb aquatic ecosystems, with best practices for restoration of impacted environments continually improving. Nutrient enrichment and floating wetlands can stimulate microbial degradation of petroleum constituents, assisting in the recovery of impacted aquatic ecosystems. Zooplankton provide essential functions to aquatic ecosystems and can serve as useful indicators of ecological health, while zooplankton-associated microbiome and their responses to oil remediation practices are largely unknown. In this study we applied DNA and RNA COI, and 16s rDNA metabarcoding to profile zooplankton and associated microbiome response to simulated oil spills, and select remediation practices. The objectives were to assess the response using diversity metrics and compare DNA and RNA metabarcoding to measure change in the respective communities to ecosystem perturbations. Model oil spills of conventional heavy crude were applied to isolated shorelines in a boreal lake, and following primary recovery efforts, two remediation practices were employed which included enhanced monitored natural recovery and engineered floating wetlands, with five sampling time points taken over the summer of 2021. The overall dominant zooplankton genera included Bosmina, while the dominant prokaryote family was Comamonadaceae. Differences in RNA and DNA alpha and beta diversity profiles existed between zooplankton and associated microbiomes, while RNA and DNA exhibited similar results for alpha diversity response to treatments for prokaryotes, but not for zooplankton. Engineered floating wetlands had the largest negative effect on alpha diversity for the zooplankton-associated microbiome. Future steps include comparison of zooplankton metabarcoding with morphological data, incorporation of environmental chemistry data, and a recovery time point.

Special Session: 30 Years of Environmental Effects Monitoring (EEM)

The history of the development of the Environmental Effects Monitoring (EEM) program (PL)

Kelly Munkittrick[1,2]

[1]*University of New Brunswick,* [2]*University of Calgary*

Environmental effects monitoring (EEM) started to develop in the late 1980s. There were a variety of stimuli, including a desire for more equity in monitoring requirements across the country. Most western Canadian mills developed post-1972 *Pulp and Paper Effluent Regulations* (PPER) and most had detailed monitoring programs. The 1992 revisions to the PPER were under development, and would stimulate older mills to implement secondary effluent treatment and a number of process changes to meet the more stringent requirements. Our research studies had shown that we could detect impacts on fish reproductive development and sexual maturity with mills that would comply with the new 1992 regulations, and at mills that did not bleach using chlorine. The EEM program evolved a number of times between 1989 and the 1992 inclusion in the regulations, and the objective focused to determine how often, when mills were in compliance with the 1992 regulations, did we still see changes of concern in fish, fish habitat, and fish use. There has been a lot of confusion, frustration, and resistance to EEM as an effluent discharge evaluation tool. This presentation will give an overview of the development, implementation, and success of the program for its designed intention.

Incorporating environmental effects monitoring in baseline development improves environmental impact assessments and post-development monitoring (PL)

Carolyn Brown[1,2], R Allen Curry[2], Gord Yamazaki[2], Kelly Munkittrick[2,3]

[1]*Wilfrid Laurier University,* [2]*University of New Brunswick,* [3]*University of Calgary*

Environmental impact assessments (EIA) have been criticized for decades for focusing on stressor-based approaches (chemical and physical aspects of habitat that may affect biota) and excluding or minimizing effects-based approaches (biological indicators). Although effects-based approaches are often required during monitoring, the failure to consider operational monitoring requirements during baseline assessment means that developments typically fail to utilize Before-After Control-Impact study designs. There is often confusion in the role monitoring plays in adaptive management – the EIA process needs to provide the information on which management decision will be made. But those

management decisions are more than just "should the development proceed?". They should be broadened to include post-development adjustments and verification as to whether EIA predictions were met, and whether there are unanticipated consequences of development. Environmental effects monitoring (EEM) has the potential to integrate and align site-specific EIA monitoring with watershed management by providing the baseline information required to develop monitoring and forecast triggers to drive decision-making during operational phases. An adaptive monitoring process based on monitoring and forecast triggers requires good baseline data and a link to modelling predictions about the potential impacts of development. Multiple years of baseline sampling has occurred to develop data prior to a potential EIA for the refurbishment of the Mactaquac Generating Station on the Saint John River, New Brunswick. This presentation will discuss how consideration of post-development monitoring early in the EIA process can help with the assessment and monitoring.

Environmental effects monitoring in Canada's offshore oil and gas sector: Observations on a science policy tension (VPL)

Ian Stewart[1]

[1]*University of King's College*

Environmental effects monitoring (EEM) connected to offshore oil and gas development has been in effect for over two decades in Canada's Atlantic waters, being integrated into Canada's impact assessment (IA) legal frameworks and regulatory oversight mechanisms. Methods for monitoring in particular the ecotoxicological effects of hydrocarbons and other potential pollutants related to routine operations have evolved, punctuated also by intense periods of research on accidental oil spills following disasters such as the Deepwater Horizon tragedy of 2010 in the Gulf of Mexico. This paper will briefly survey that history, and then describe a present-day science-policy tension. On the one hand, as a review of EEM reports and IA documents associated with offshore development projects makes clear, after so many years, EEM appears to be confident of itself, contributing to what appears to be a trend towards relaxing the IA regime for Canada's offshore. On the other hand, concerns about our abilities to monitor and thus understand in particular the toxicological effects of routine operations on the ecotoxicology of fish and fish habitat features centrally in the record of community concerns about offshore development, including Indigenous communities in Newfoundland, Labrador, and Nova Scotia. The concerns only increase with respect to effects of major spills due to accidental releases from wellheads or transport. This presentation concluded with some science-policy considerations regarding the continued role that Canada's ecotoxicology community can play to address this tension, and as Newfoundland and Labrador seek to expand their offshore hydrocarbon developments.

Environmental effects monitoring (EEM) in support of *Wastewater Effluents Systems Regulations* (WSER) (PL)

Gerald Tetreault[1], Mark Servos[2], Mark McMaster[1]

[1]Environment and Climate Change Canada, [2]University of Waterloo

In 2012, the Canadian federal government approved the *Wastewater Effluents Systems Regulations* (WSER) for wastewater systems that deposits effluent via its final discharge point into the Canadian receiving environment, under certain conditions. In order to evaluate the effectiveness of the regulations, our research team followed the environmental effects monitoring (EEM) guidelines, and additional endpoints, to evaluate the health of sentinel fish species collected in wastewater effluent receiving environments of facilities that did or did not currently meet the WSER criteria. Fish responses, in terms of energy storage (condition factor, liver size), energy utilization (gonadosomatic indices) and reproduction (*in vitro* sex steroid, intersex) were assessed at each site. Overall, fish collected downstream of effluent discharges did not demonstrate consistent differences in condition and liver somatic indices. The most consistent response observed in fish collected downstream of municipal wastewater effluent (MWWE) discharges which did not meet WSER criteria, was increased incidence of intersex in male fish in contrast to very low occurrences of this condition in upstream agricultural and urban reference sites. The increased intersex coincided with reductions in gonadosomatic indices and capacity to produce steroids, demonstrating the ability of MWWE to alter the reproduction of these fish. After facility upgrades, disruptions in key fish health response variables were no longer statistically different from the upstream reference sites. Results from these studies confirm the effectiveness of WSER and support the rational for remedial actions for to improve effluent quality at facilities yet to meet the WSER.

Environmental effects monitoring for the Premier Gold Project (PL)

Dave Huebert[1]

[1]Palmer

The Premier Gold Project is a recently reactivated and permitted mine located in northwestern British Columbia, primarily in the Cascade Creek watershed. During operations, the mine will be required to design and complete an Environmental Effects Monitoring (EEM) program for compliance with the requirements of the *Metal and Diamond Mining Effluent Regulations* (MDMER). Assessment of the benthic invertebrate community in the downstream receiving environment will be one of the required components of the EEM program. Environmental monitoring for both permitting and compliance requirements has been undertaken at the site and over the past decade has consistently included collection of surface water samples and benthic invertebrate community data at numerous sites in Cascade Creek and within the watershed. The benthic

invertebrate samples were collected using the Canadian Biomonitoring Network (CABIN) protocol and assessed against the 114 reference sites in Group 2 of the 2015 BC Central/North Coast Reference Model. The results indicate that the benthic invertebrate community was consistently within reference condition or mildly divergent for samples that were collected at both control and impact sites. The results also indicate that the benthic invertebrate community was consistently within reference condition for the endpoints: Taxon Richness, EPT Richness, Simpson's Evenness, and EPT Relative Abundance. Based on these results, the CABIN protocol will be used in the EEM program to assess the health of the benthic invertebrate community. The protocol provides a robust experimental design, unlike traditional Control-Impact designs, and provides simple data interpretation without the complications of more traditional statistical analyses.

30 years later: do we still need environmental effects monitoring (EEM)? (PL)

Kelly Munkittrick[1,2], Mark McMaster[3], Ashley Mahaffey[4]

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Environmental effects monitoring (EEM) is a form of adaptive monitoring that is more than 30 years old. It began as an industry-funded, cyclical monitoring program designed to evaluate the effectiveness of *Fisheries Act* regulations by determining how frequently impacts on fish, fish habitat, and fish use existed in receiving environments when industry was in compliance with the discharge limits. EEM is very successful in freshwater aquatic environments at accomplishing what it was designed to do. While EEM requirements have expanded to include some other industry sectors, it does not exist for all sectors, and there is still confusion, frustration, and disappointment with an EEM approach and challenges with its implementation. Our research has been focusing on how to align or interface various types of regional monitoring designs into an integrated regional framework. The framework demonstrates an integral role for cyclical, adaptive monitoring in driving decision-making about the focus, intensity, and effort of regular monitoring, and embraces a clear role that consistent data should play in improving regional modelling, management, and regulatory development. A regional integrated monitoring and management system can be driven by an EEM approach, but it would require improvements in alignment within and across regional stakeholders and increased clarity, transparency, and availability of monitoring data collected across a range of types of environmental monitoring programs. EEM can evolve to become a regional management tool with a minimum of changes to its design and philosophy.

Testing effluent samples from Canadian pulp and paper mills using the standard *Ceramium tenuicorne* ISO test method and Canadian add-on procedure (PO)

Sarah Costantini[1], Cintia Glasner Regis[1], Anna Sobaszek[1], Vanessa Chanes[1], Carla Gibbs[1], Lisa Taylor[1]

[1]*AquaTox Testing & Consulting Inc.*

As indicated in the *Pulp and Paper Effluent Regulations* under the *Fisheries Act*, sublethal toxicity testing for effluent discharged into a marine or estuarine environment must be conducted using the required test methods with marine algae species. Environment and Climate Change Canada (ECCC) is investigating the use of the red macroalgae, *Ceramium tenuicorne*, for sublethal testing following the ISO 10710:2010 method. To develop this method for the Canadian context, the Method Development and Applications Unit (MDAU) of ECCC has drafted an add-on procedure that provides supplementary methods and conditions specifically for Canadian laboratories. AquaTox Testing & Consulting Inc. (Puslinch, Ontario) has been mandated by ECCC to test the viability of this method and add-on procedure by using effluents voluntarily submitted by Canadian pulp and paper facilities. To best reflect the Canadian pulp and paper industry, effluent from a variety of process types will be tested, including chemical, recycling/paper making, and mechanical. Results of this testing will inform the modernization of the *Pulp and Paper Effluent Regulations*, with potentially including *C. tenuicorne* as a regulatory test species.

General Ecotoxicology: Soil, Sediment, Water, and Biota

A pan-Canadian assessment of chlorinated alkanes in whole body fish, and gull and starling eggs (PO)

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Chlorinated alkanes were defined as "toxic" under the *Canadian Environmental Protection Act* (CEPA) 1999, and their manufacture, use, sale, or importation are generally prohibited. Both fish and wildlife are monitored across Canada to determine if regulations are sufficient to reduce the risk of chemicals to the environment. We investigated the spatial and temporal distributions of alkanes in top predator fish, primarily whole-body lake trout (*Salvelinus namaycush*) and walleye (*Sander vitreus*), and in the eggs of European starlings (*Sturnus vulgaris*) and three congeneric gull species (*Larus* spp.) from nesting sites across Canada. Total short chain chlorinated alkanes (SCCAs) were measured in fish in 2013 from 12 sites in the Great Lakes and 9 sites from elsewhere across Canada. Short, medium, and long chain chlorinated alkanes (S/M/LCCAs) were analyzed in bird eggs collected across Canada in 2019, and from two gull and one starling colonies from 2009–2019. Concentrations of SCCAs in fish ranged from below the method detection limit (MDL) to 39.2 $ng \cdot g^{-1}$ (wet weight) and were highest in Georgian Bay and Niagara on the Lake, in Lake Ontario. Average SCCAs concentrations in fish at most sites were <MDL. MCCAs were detected in gulls, but SCCAs and LCCAs were mostly undetectable. Concentrations of CAs ranged from <MDL to 9.11 $ng \cdot g^{-1}$, and were found at only three gull colonies in the Great Lakes. Starling eggs had mostly <MDLs, but one colony in Nova Scotia had 670 $ng \cdot g^{-1}$ CAs. All concentrations of total SCCAs in fish were below Canadian Federal Environmental Quality Guidelines for mammalian wildlife diet and for fish tissues.

Firefighting water additives save forests from flames, but do they also pose a risk to aquatic biota? (PO)

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The use of firefighting water additives has become essential over the past decades in both wildland and municipal fires in order to maximize the extinguishing efficacy of water. Many past firefighting additives contained fluorinated constituents, however, due to their bioaccumulative potential and persistence in the environment they are no longer permitted in Canada. With greater concern of the environmental fate of firefighting water additives, new formulations have been developed that are meant to be "eco-friendly" alternatives for fire suppression. There is currently very little data on the toxicity of these new additives

towards aquatic biota. This study assessed the toxicity of nine different types of firefighting water additive on aquatic species. This included acute lethality testing of the aquatic species *Daphnia magna*, *Hyalella azteca*, and a 28-day reproductive test using *Tubifex tubifex*. This was followed by a deterministic risk assessment using a realistic application rate and four theoretical water bodies of differing depth. The *D. magna* portion of the study revealed potential hazards for all tested products in at least two exposure scenarios except for Eco-gel™ AB, FireIce®561, and TetraKO™ for which no hazards were found. All products tested presented a potential hazard to *Hyalella azteca* with exception of Eco-gel™ and FireIce®561. Finally, LC95A was the only product found to pose a potential hazard to *Tubifex* survival, while both LC95A and WD881C demonstrated a potential hazard to reproduction. The results of this study highlight the potential hazards that firefighting water additives could pose to aquatic organisms.

Using a novel photoelectrochemical oxygen demand (peCOD) analyzer to estimate concentrations of venlafaxine in freshwater (PO)

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Detection of contaminants in the environment is a primary concern in remediation and monitoring efforts. Photoelectrochemical oxygen demand (peCOD) is an analysis technique which may provide utility in detection of organic contaminants in aquatic systems. Venlafaxine is a prescribed antidepressant that is transported into the environment in wastewater. In an experiment to assess the fate and toxicity of venlafaxine in aquatic ecosystems, 10 mesocosms were deployed in Lake 239 at the IISD-Experimental Lakes Area (IISD-ELA). A regression design was employed with target concentrations ranging from 100 000 ng·L^{-1} to 4.18 ng·L^{-1} with three control mesocosms. A peCOD analyzer was paired with this experiment to assess its efficacy at determining accurate estimates of venlafaxine concentrations in the higher concentration treatments. Water for venlafaxine analysis was analyzed by ultra-high performance liquid chromatography mass spectrometry (UHPLC-MS), while subsamples were analyzed for peCOD. Sampling occurred 12, 24, 48, 96, and 168 hours post-treatment. There were correlations between peCOD analysis in the two highest treatments (100 000 ng·L^{-1}, 19 306 ng·L^{-1}) and the UHPLC-MS results. Considering the high background DOC in the sample (8.0 ± 1.0 mg·L^{-1}), these preliminary findings warrant further investigation into use of peCOD as a technique for assessing high concentrations of organic analytes in boreal water matrices. While the ability for qualitative determination of organic analytes through peCOD is still being investigated, quantitative determination of organics in aquatic samples appears to be possible in appropriate conditions.

Developing a populational normalization tool to better correlate SARS-CoV-2 concentrations in wastewater with clinical cases (PO)

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Wastewater-based epidemiology (WBE) has been used to estimate clinical cases of COVID-19 in various regions around the world with varying degrees of success, with unique challenges in smaller municipalities. This passive approach covers a large population with fewer costs than clinical diagnostic testing. The ability to accurately estimate clinical cases depends on the method used, notably the method of normalizing SARS-CoV-2 concentrations in wastewater. Five municipalities in Québec, Canada, with a population range of 2000 to 49 000, were sampled during the spring of 2021. Normalization markers used were the pepper mild mottle virus (PMMoV) and wastewater treatment plant (WWTP) flow rate. According to the data, the use of different normalization markers could lead to a more accurate model. Therefore, our objective is to quantify different markers that could better normalize SARS-CoV-2 concentrations and thus build a better model to estimate clinical cases in the population. Human mitochondrial DNA and crAssphage virus (dsDNA) will be quantified using real-time quantitative polymerase chain reaction (RT-qPCR) and 5-hydroxyindole-3-acetic acid (5-HIAA) and caffeine with gas chromatography-tandem mass spectrometry (GC-MS). Clinical cases will be correlated with SARS-CoV-2 concentrations normalized with each new marker. The main hypothesis indicates these four new markers will have an increased correlation coefficient when compared to the previously used markers for this region, which would lead to a more accurate estimate of clinical cases. This study will further help public health authorities in Québec with their management of the COVID-19 pandemic as well as any future pandemic.

Metformin and guanylurea reduce survival but have limited sublethal effects in larval zebrafish (PO)

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Metformin is the most common first-line oral therapeutic agent used in the treatment of type-2 diabetes, one of the most prevalent chronic diseases in North America. Metformin is partially bio-transformed by bacteria into guanylurea during wastewater treatment. Both metformin and guanylurea are released in wastewater effluent and are frequently detected in aquatic environments. Studies on the effect of metformin and guanylurea on early life

stage fish are limited. The aim of this study was to examine the potential impact of metformin and guanylurea on development (survival, hatching, abnormalities, and growth), cardiometabolic responses, and behaviour in larval zebrafish. Embryos were exposed to environmentally relevant (0.4, 4, 40 $\mu g \cdot L^{-1}$) and supra-environmental (400 and 4000 $\mu g \cdot L^{-1}$) concentrations from 3 hours post-fertilization to 5 days post-fertilization. Importantly, metformin and guanylurea exposure caused an increase in mortality at all concentrations tested, with guanylurea having the larger effect. Metformin increased abnormalities (spinal bends, yolk sac edema, and pericardial edema), and decreased certain morphometric parameters (head diameter, eye diameter, and snout-vent length) but only in the highest concentration tested. Metformin and guanylurea exposure did not alter hatchability, time to hatch, visual motor response, thigmotaxis, startle response, general swim metrics, heart rate, or oxygen consumption. The results suggest that these compounds impact survival and spinal abnormalities but sublethal effects are limited with exposure during early embryonic development prior to first feed.

Effects of treated wastewater on fathead minnows (*Pimephales promelas*) across a multi-basin wetland in southern Alberta (PO)

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Wetlands have been used for treating anthropogenic effluent for decades due to intense biogeochemical cycling in processing and removing nutrients. Frank Lake wetland, located in southern Alberta, is a multi-basin complex that has received treated municipal wastewater from the town of High River and treated agro-industrial wastewater from the Cargill beef processing facility, since late 1980s. Two ephemeral creeks also discharge water to Frank Lake during spring. Frank Lake discharges into the Little Bow River. Based on a previous study, 17.3 tones of phosphorus (P) and 244 tones of nitrogen (N) enter the wetland from effluent and the wetland can remove about 50% P and 95% N before water is discharged to Little Bow River, annually. However, the composition of anthropogenic organic contaminants in Frank Lake, and the toxicological potential of surface water, has not been characterized. The goal of our work is to expand understanding of the impacts of effluent wastewater on the health of Frank Lake using a toxicological approach. Monthly water samples of each effluent input (Cargill and High River), and the outflow of each basin are collected, and the organic fraction is isolated by use of solid phase extraction. The chemical composition of each extract is characterized by use of non-target high-resolution Orbitrap mass spectrometry. A fish embryotoxicity (FET) assay with fathead minnows (*Pimephales promelas*) is performed to determine toxicity of extracts. This study represents an important step towards characterizing health of the Frank Lake complex and its ability to remediate effluent associated toxicities.

Assessing alone and in-mixture effects of aquaculture formulations on non-target marine invertebrates to set environmental quality standards (PO)

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Marine finfish aquaculture sites use drugs and pesticides for management and maintaining high standards of fish health. Products may be administered with food or as a bath treatment. However, chemicals not absorbed or metabolised by the fish may impact other aquatic organisms and their habitats. Compounds may disperse in the water column, far from aquaculture sites, or accumulate in sediments, where their persistence vary from months to years. Pesticides and therapeutants that enter the marine environment interact with marine non-target species with unknown effects. This leads to concerns regarding the environmental effects of these compounds. The composition of the formulated product may enhance the physico-chemical properties of the active ingredient, therefore the data derived from the active ingredient alone may not be appropriate when predicting environmental fate and effects. Moreover, considering the widespread usage of chemicals in aquaculture along the Canadian coast, it is crucial to assess the impact of formulations alone and in mixtures. Synergistic, antagonistic, and additive effects may be revealed by assaying the combined presence of these stressors. To this end, the toxicological effects of active ingredient and formulation of the three pesticides SLICE® (active ingredient = Emamectin B1a), IVOMEC® (Ivermectin) and SALMOSAN® 50WP (Azamethiphos) and the antibiotic oxytetracycline were tested on the larval stage of the American lobster and the green-sea urchin. The results will provide information on hazards posed by environmental concentrations of these compounds and will serve as valuable input when establishing environmental quality standards or monitoring thresholds.

A critical review of toxicity data for species sensitivity distributions of chloride salts in freshwater (PO)

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The concentrations of salts in freshwater systems have been increasing globally, making the understanding of the ecological impacts of salinization a pressing concern. With numerous potential pathways for dissolved salts to enter surface waters (e.g., via road de-icing, wastewater effluents, agricultural practices, and resource extraction), there is a need for high quality toxicity data to inform risk assessors when making regulatory decisions for freshwater protection. Additionally, there are concerns about the quality and reporting of toxicity data for use in risk assessment (e.g., weak experimental design, improper controls) that can add significant uncertainty to decisions around risk and guideline derivation. To address these issues surrounding the reliability and ecological relevance of ecotoxicological

literature, we conducted a critical review of 335 articles, with the overall objective to assess the current state of toxicological laboratory data as it relates to chloride ions associated with freshwater salinization. To assess the quality of the data, studies were scored using a set of transparent and objective *a priori* metrics, with a rubric for strength of the study design, and a rubric for ecological relevance of the endpoints used to evaluate toxicity. Criteria for these rubrics were selected to address common issues found in the peer-reviewed literature regarding statistical power, ecological relevance, study reliability and repeatability, and the utility of the results. Based on these scores, data of the highest quality were used to create species sensitivity distributions to estimate water quality protection thresholds. Furthermore, the results obtained through this study will help to guide future studies by identifying specific species and exposure scenarios that might be lacking reliable and ecologically relevant data.

Omics Approaches: From Laboratory to Field Studies

Using EcoToxChips to characterize and compare the ecotoxicity of benzo[a]pyrene in amphibian, bird, and fish embryos (PL)

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This study aimed to apply a novel standardized reduced transcriptome assay (the EcoToxChip system, www.ecotoxchip.ca) using short-term embryonic exposures to compare molecular response patterns across model species representing three vertebrate taxa (amphibian, bird, fish). Animals were exposed to benzo[a]pyrene (BaP), a well-characterised polycyclic aromatic hydrocarbon (PAH) and indicator of industrial pollution. Embryos of *Xenopus laevis* (XL), fathead minnow (FHM), and Japanese quail (JQ) were exposed to graded concentrations of BaP for 4 to 9 days prior to independent feeding (XL/FHM) or pre-hatch (JQ), and sampled for assessment of gene expression using species-specific EcoToxChips. A subset of the animals was then grown out for several weeks to assess apical and histological outcomes to anchor molecular responses. Reduced transcriptome data was analyzed using EcoToxXplorer.ca, an intuitive online bioinformatics tool. EcoToxChip analysis revealed 22, 53, and 269 differentially expressed genes in XL, JQ, and FHM, respectively. Specific genes involved in xenobiotic metabolism (e.g., CYP1A, AhR, etc.) and molecular pathways associated with various forms of metabolism were commonly dysregulated across all taxa. In XL and FHM, a main apical response was seen in the liver with increased liver size and histological alterations (e.g., atypical hepatocyte nuclei). There were no apical effects observed in JQ; however, this could be attributed to differences in route and length of exposure (single egg injection vs semi-chronic waterborne exposure). In conclusion, this study suggests that reduced transcriptome profiling after short-term embryonic exposures can be used to identify later but specific biological outcomes across diverse vertebrate taxonomic groups.

Use of Japanese quail EcoToxChips to derive transcriptomic points of departure for chlorpyrifos in a multi-lab ring test (PL)

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Transcriptomics dose-response analysis (TDRA) is a promising new approach in the field of ecotoxicogenomics. Here, we perform TDRA using a targeted qPCR (quantitative

polymerase chain reaction) array (EcoToxChip) and evaluate the repeatability of the results in a ring test involving three independent laboratories. In each laboratory, solvent control (DMSO) and seven doses of the pesticide chlorpyrifos (0.04 – 40 $\mu g \cdot g^{-1}$ egg nominal) were injected into the air cell of fertilized Japanese quail (*Coturnix japonica*) eggs on embryonic day 0 (ED0). Liver was harvested on ED9 and hepatic gene expression was assessed using the 384-gene Japanese quail EcoToxChip (n=5 per dose). Standard operating procedures, reagents, chlorpyrifos stock solutions, and dose-response analysis using EcoToxXplorer (www.ecotoxxplorer) were common between the three laboratories. The strain of quail used, and the qPCR instrumentation differed. Preliminary results from two labs points to a large difference in the number of genes for which benchmark doses (BMDs) could be derived (39 vs 70). Despite this difference, transcriptomic points of departure for the first mode (tPODmode) were similar (20.3 $\mu g \cdot g^{-1}$ vs 16.8 $\mu g \cdot g^{-1}$) and corresponded to the tPODmode previously calculated from RNASequencing data (21.6 $\mu g \cdot g^{-1}$). All tPODs were below our previously published organismal LOAEL of 41.1 $\mu g \cdot g^{-1}$ for chlorpyrifos in Japanese quail. Overall, our results suggest that performing TDRA on the reduced gene set provided by EcoToxChips is a valid approach that can produce repeatable tPODs that are protective of adverse apical outcomes.

Transcriptomic response variability to environmental contaminants in four species of darter (Etheostomatinae) (PL)

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As humans continue to influence aquatic environments through the introduction of contaminants, it is crucial to understand how species and populations will respond. Waste water treatment plant effluent (WWTPE) is a commonly studied contaminant that has demonstrated deleterious effects on population dynamics and species richness. However, in some cases, individuals of an effected species persist in these contaminated environments. Little is known about how individuals within fish species vary according to tolerance of environmental stressors, and the underlying mechanism that drive this diverse inter- and intraspecific response is not fully realized. Darters (Etheostomatinae spp.) represent one of the most diverse clades of freshwater fish in North America, and have demonstrated a range of tolerances to environmental protuberance. This study will take advantage of this variation to investigate the effects of WWTPE on four darter species: fantail (*E. flabellare*), greenside (*E. blennioides*), johnny (*E. nigrum*), and rainbow darter (*E. caeruleum*). Darters were collected upstream and downstream from the Waterloo municipal wastewater treatment plant effluent discharge site on the Grand River (Ontario, Canada). The effects of effluent exposure on each population was assessed through RNA-Seq, *de novo* transcriptome assembly, and *in silico* differential expression analysis. Gene set enrichment and pathway analysis was then completed to help contextualize the functional impact of WWTPE exposure on each species. Overall, exploring this question may reveal

novel molecular or biochemical coping mechanisms, and could help us infer the success of the species in response to environment protuberance.

Effects of chiral venlafaxine on reactive oxygen enzymes of two sentinel fish species, the fathead minnow and rainbow darter (PL)

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A wide diversity of emerging contaminants of concern are released as complex mixtures into the Canadian environment through municipal wastewater effluent outfalls. Despite implementation of secondary treatment, many chemicals are poorly or only partially removed and are therefore released in relatively high concentrations where they can interact with aquatic ecosystems and potentially have adverse impacts on fisheries resources. Antidepressants, such as venlafaxine and its metabolites, are an example of a group of pharmaceuticals that may act together in complex mixtures to cause subtle but important effects on fish physiology and metabolism. Further, venlafaxine exists in the aquatic environment as a mixture of chiral forms (racemic) and differences in metabolism, fate, and potency of each form, including their metabolites, can lead to different action/potency in exposed organisms. This study evaluated the effects of an environmentally relevant concentration of venlafaxine on two sentinel species, fathead minnow (*Pimephales promelas*) and rainbow darter (*Etheostoma caeruleum*). Fish were chronically exposed for two weeks at 1 $\mu g \cdot L^{-1}$ of the various forms of venlafaxine; the R-enantiomer or S-enantiomer. Specifically, this study evaluated the effects of venlafaxine of the R and S forms impacting antioxidant enzyme activity in the liver and gill tissue of the two fish species. Overall, the findings indicated that R-enantiomer had a stronger influence reactive oxygen scavenging enzyme activity, although limited impact on transcriptional responses. This study takes a novel approach towards understanding the impacts of specific chiral forms of pharmaceutical contaminants and identifying which chiral forms should be of greater concern for further evaluation.

Plasma metabolome profiles in rainbow trout exposed to stormwater outfall and municipal wastewater effluent in the Bow River (PL)

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Contaminants in municipal wastewater effluent (MWWE) and urban surface runoff (stormwater) enter the Bow River as it flows through Calgary, Alberta. Our understanding of the effects of these complex contaminant mixtures on stress response, energy substrate

allocation, and metabolism remains limited. We investigated whether MWWE and stormwater exposure leads to metabolic disruption in juvenile rainbow trout (*Oncorhynchus mykiss*). We used stress response and active metabolic rate (AMR) as a readout of potential performance disruption due to the mixture exposures. Fish were caged in the Bow River at three sites, including upstream from Calgary (reference site), upstream from wastewater inputs, and downstream from a wastewater diffuser for 21 days. Following the exposure, fish from each site were subjected to an acute stress response *in situ*, while another subset of fish were transported to the laboratory for a swim performance test. Plasma was sampled from all these fish to characterize the metabolome profile using hydrophilic interaction liquid chromatography (HILIC). Component analysis indicated that the metabolite profiles in the stressed fish were significantly different compared to the unstressed fish at all sites. Metabolite profiles following MWWE exposure were significantly different in stressed and unstressed fish as well. The AMR of fish exposed to MWWE and stormwater was reduced compared to the reference site, while liver triglyceride and plasma glutamine concentrations in MWWE and/or stormwater exposed fish was higher. These results suggest that exposure to these contaminant mixtures leads to metabolic costs and compromised stress and/or swimming performance, leading to reduced fitness for fish.

What role do mitochondrial microRNAs play in response to wastewater effluent stressors in freshwater fish (*Etheostoma* spp.)? (PL)

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Freshwater fish are exposed to many anthropogenic stressors, and living downstream of a wastewater treatment plant (WWTP) has been shown to impact darter (*Etheostoma* spp.) metabolism in the Grand River, Waterloo, Ontario. The mechanisms driving this change in metabolism are not fully understood, so we hypothesized that microRNAs play a role in modulating that response. MicroRNA are small RNA molecules that post-transcriptionally regulate gene expression, and in mammalian cell culture, microRNA can be transported into the mitochondria where they are predicted to regulate expression of mitochondrial genes, namely electron transport chain protein subunits. Despite these findings in mammals, no studies have demonstrated the presence or predicted mechanisms of mitochondrial microRNAs *in vivo* or in fish. In this study, rainbow, fantail and johnny darters were collected upstream and downstream of the Waterloo WWTP, then liver mitochondrial fractions were isolated. Candidate microRNAs and their target mitochondrial mRNAs were quantified by qPCR (quantitative polymerase chain reaction), then cytochrome c oxidase (COX) activity and COX2 protein abundance were measured to predict the mechanism of mitochondrial microRNAs. Four microRNAs, miR-1, miR-20, miR-122, and let-7a, were identified in darter mitochondria for the first time, and each darter species had differing responses to effluent exposure. As hypothesized, miR-1 was

predicted to silence expression of its target mitochondrial mRNA, resulting in a decrease in COX activity in rainbow darters. Overall, this research helped describe the role that mitochondrial microRNAs may play in regulating mitochondrial gene expression in fish, and future work could define those specific regulatory mechanisms.

Assessing the toxicity of bisphenols in the freshwater snail *Planorbella pilsbryi*: Relating fitness components to metabolomic responses (PL)

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Bisphenol A (BPA) is a precursor to plastic polymers used in consumer products, including some food containers, food packaging, adhesives, and paper coatings. Increasing concerns about its endocrine activity and leaching from plastics have led to the ban of BPA in the production of baby bottles. However, BPA remains in use and alternative compounds with similar structures are being developed and/or used in consumer products, which could pose comparable health hazards. In the present study, we assessed the toxicity of BPA and alternative compounds BPF, BPS, and BPAF (bisphenols F, S, and AF) in three life stages of the freshwater snail *Planorbella pilsbryi*: 96-hour assays with adult snails, 7-day assays with juvenile snails, and 13-day assays with snail embryos. We also assessed the chronic toxicity of BPA and BPAF in a 28-day assay with adult snails, followed by a 21-day assessment of the F1 generation. The relative toxicity of BPA and its alternatives was BPAF > BPA > BPF > BPS. The juveniles from the F1 generation exposed to BPA were more sensitive than juveniles from the F0 generation, suggesting potential multigenerational effects. The recently completed testing will also determine whether BPAF induces multigenerational effects. The metabolomic response of snails was assessed by non-targeted nuclear magnetic resonance spectroscopy (96-hour assays) and mass spectrometry (28-day assay with BPA), to gain a better understanding of potential mechanisms of action, and to relate observed fitness components (e.g., altered growth and reproductive output, decreased embryo survival) to alterations in the metabolome.

Transcriptomic and genomic approaches to assess the potential for lampricide resistance to evolve in invasive sea lamprey (PL)

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Sea lamprey (*Petromyzon marinus*) is an invasive species in the Laurentian Great Lakes of North America that has caused severe ecological and economic damage. Lampricide

applications in rivers, specifically 3-trifluoromethyl-4-nitrophenol (TFM), have been a major sea lamprey control method in its invasive range. The main mechanism of toxicity of TFM in fishes is through an uncoupling of oxidative phosphorylation in the mitochondria. While TFM is relatively specific against sea lamprey, its toxicity can vary among fishes. However, the cellular mechanisms behind the differences in TFM sensitivity between species is poorly characterized. We conducted a laboratory experiment where we exposed sea lamprey and a tolerant species, bluegill (*Lepomis macrochirus*), to their species-specific TFM 24-hour LC_{10} concentration and then used RNA-Seq to compare transcriptomic responses with physiological markers of TFM exposure. We used the genes that were responsive to TFM to inform our assessment of genes that may be important for evolving resistance to lampricide exposure in wild sea lamprey. We then used whole-genome sequencing of wild-caught sea lamprey from their invasive and native ranges in North America to test for evidence of adaptation to a strictly freshwater life-history and the potential for evolving resistance to TFM exposure. Genomic scans of single nucleotide polymorphisms (SNPs) for loci involved in local adaptation revealed strong evidence for selection on genes associated with transcriptomic and physiological responses to lampricide exposure. This work also highlights the presence of putatively adaptive loci that could be targeted for genetic control in invasive sea lamprey.

Transcriptomes of free-ranging ringed seals differ by region and contaminant burden (PL)

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Ringed seals (*Pusa hispida*) are a circumpolar Arctic marine mammal species used for various national and international monitoring programs and are an important food source for Indigenous communities in the North. Much is known about spatiotemporal contaminant trends in ringed seals, but we have limited knowledge on the contaminant related health effects. In this study we explored the use of full genome transcriptomics as a tool in wildlife ecotoxicology research, focusing on disparate populations of ringed seals in the Canadian and European North with wide-ranging contaminant burdens. *De novo* transcriptome assembly followed by low count filtering produced a total of 32 503 unique gene transcripts for male ringed seals. Principal component analysis showed that the transcriptomes of samples (n=34) differed by geographical location, where the first component separated seals from Labrador (Canada) and those from Svalbard and the Baltic Sea (Europe), and the second component further separated seals from the Baltic Sea and Svalbard. Using multiple linear regression models on the full transcriptomes we found thousands of genes that differentially expressed according to geographical region (factor: Canada vs Europe) and contaminant levels (continuous variable). Ongoing work will characterize the functional profile of differentially expressed genes to understand the biological implications of contaminant exposure. This work will advance our understanding

of the complexities involved in transcriptomic ecotoxicology research in wildlife populations.

Using transcriptomics to examine beluga health across two populations (VPL)

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Using gene transcript profiles has become a powerful tool in detecting contaminant exposure and even toxicity in animals. There are many genes that have been isolated down to function and gene transcription, which is dependent on the contaminant the animal has been exposed to. These become biomarkers, which are measurable features used to study physiological processes as well as for diagnostics and health monitoring. Canada is home to eight beluga populations that range in status from not at risk to endangered. Several beluga populations remain central to the health and well-being of Inuit who continue to have traditional subsistence harvests of beluga whales. In efforts to address concerns on beluga health and to have the ability to detect early warning of stress, novel approaches to beluga health monitoring has included the use of transcriptomics. Here we worked closely with two Inuit communities, Tuktoyaktuk, Northwest Territories, and Arviat, Nunavut, to collect tissue samples for contaminant and transcriptomic measurements. The expression of 17 genes in skin samples were obtained from over two summers (i.e., 2020, 2021) for two beluga populations, the Eastern Beaufort Sea (EBS) and Western Hudson Bay (WHB). These included genes that are indicators of stress and inflammation as well as contaminant exposure. While current results need to be interpreted with caution due to small sample size for Arviat, an exploratory principal component analysis showed significant clustering along PC1 suggesting not only differences amongst populations but also interannual differences. Further analyses are currently underway to investigate interpopulation differences as well as population health.

Linking histological and morphological effects of ethinylestradiol with transcriptomic responses in early life stage Japanese quail (PO)

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The full utilization of ‘omics in toxicology necessitates linking molecular effects to higher-level outcomes. To promote the use of transcriptomics for avian early life stage toxicity tests, our study explores and links transcriptomic, histological, and gross morphological responses in embryonic Japanese quail exposed to ethinylestradiol (EE2). Exposures were conducted according to an avian egg injection protocol that we have proposed for standardization. EE2 was dissolved in dimethyl sulfoxide and injected into the

air cell of eggs prior to incubation at nominal concentrations of 0 and 3.33 $\mu g \cdot g^{-1}$ egg weight. At embryonic days 9 and 16, gross morphology of gonads was evaluated. Sequentially, livers and gonads were collected from five embryos per sex per treatment group for RNA-sequencing and/or histology. While no significant effects of EE2 exposure on growth morphometrics were observed, signs of feminization in male embryos after EE2 exposure were evident based on gross morphology and histology of gonads at both embryonic days. The gonadal asymmetry and cortex thickness from the ongoing histological analysis will inform the paired transcriptomics data. A principal component analysis on the transcriptomics data revealed a clear distinction not only between males and females, but also between control and treatment groups. This study informs the standardized early life stage test for birds and contributes to the development of screening methods for estrogenic chemicals using avian embryos and transcriptomic approaches.

Changes in gene expression in embryo-larval fathead minnows exposed to oil sands area wetland waters (VPO)

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Oil sands areas of northern Alberta contain bitumen deposits that can make up some river and wetland sediments. Groundwater flow and aerial deposition are also pathways for some bitumen-related contaminants to enter rivers and wetlands. Wetlands (sampled in 2019) were selected to be close to industrial development, inside the surface bitumen deposit area, or far from bitumen deposits and industry. Fathead minnows were exposed to 100 % wetland waters for 6 days from embryo to hatch. Gene expression in fathead minnow fry was examined to determine if patterns of toxicity differed among 10 oil sands wetland waters. The expression of 375 fathead minnow genes was measured using a targeted qPCR (quantitative polymerase chain reaction) array (Ecotoxchip). Changes in gene expression were tallied and linked to pathways affected by the exposures to wetland waters. Six of ten wetlands affected some genes (either increasing or decreasing them significantly over 2-fold compared to controls). Two of the most potent wetlands affected 8 to 11 genes, and these were the most potent wetland waters causing decreases in hatch success of the exposed fathead minnow embryos. Most affected genes were in the Aryl hydrocarbon receptor (AhR) pathway. Activation of the AHR pathway has been linked with larval mortality in fish. This pathway is affected by polycyclic aromatic compounds (PAHs) and alkylated PAHs in the wetlands, and possibly by naphthenic acids/other bitumen-derived organic compounds. Work will continue assessing mechanisms of action for the wetland waters in fish, in order to determine the contaminants that cause the majority of effects.

Comparing molecular and apical dose response in early life stage rainbow trout exposed to benzo[a]pyrene (PO)

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New approach methods (NAMs) are urgently needed to address the high cost, low throughput, and significant ethical concerns associated with the use of live animals in the current toxicity testing framework. A transcriptomic points-of-departure (tPOD) approach from short-term early life stage fish studies offers a promising approach to address such issues. However, developing NAMs requires careful calibration through multiple lines of evidence linking the transcriptomic data to apical outcomes. Thus, this study aimed to estimate and compare tPODs from short-term rainbow trout (RBT; *Oncorhynchus mykiss*) embryo assay to benchmark doses derived from apical outcomes in chronic exposures (aPOD), and to provide weight-of-evidence in the use of tPODs in estimating aPODs. Embryos of RBT were exposed for 4 to 28 days post-hatch (dph) to benzo[a]pyrene (BaP) at measured concentrations of 0.079, 0.35, 1.5, 7.4, and 28.6 $\mu g \cdot L^{-1}$ and solvent control of 0.01% DMSO. Benchmark dose analysis of toxicogenomic data (RNASeq) at 4 dph yielded tPODs of 0.02, 0.15, 1.8, and 0.07 $\mu g \cdot L^{-1}$ BaP based on statistical groupings. After 28 days, morphometric analysis showed significant growth inhibition at >7.4 $\mu g \cdot L^{-1}$ BaP, with notable decreasing trend in body weight. Molecular pathways, biochemical responses, histological alterations, and physiological responses supported the canonical BaP toxicity pathway model. This study showed that molecular perturbations at 4 dph lead to biological responses at more advanced life stages, providing a strong support for the derivation and use of tPODs to estimate benchmark doses that are associated with adverse outcomes.

Investigating the effects of per-and poly-fluoroalkyl substances on *Planorbella pilsbryi* in laboratory and *in situ* utilizing a multi-omic approach (PO)

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Per- and poly-fluoroalkyl substances (PFAS) are widely used in consumer and industrial applications, including non-stick cookware, personal care products, disposable food packaging, stain resistant coatings, and firefighting formulations. These compounds are notoriously environmentally persistent due to the chemical stability of their fluoro-carbon bonds. PFAS can enter waterways and threaten aquatic life by potentially leading to bioaccumulation and exerting a broad range of toxic effects that can impair growth, development, reproduction, mobility, and survival. Thus, PFAS has become a global health concern worldwide. The present study focuses on the effects of a chronic exposure to PFAS

on the survival, growth, and fertility of freshwater snails (*Planorbella pilsbryi*). Toxicity effects were assessed in a 28-day *in situ* exposure at two sites (reference vs contaminated) located in Hamilton, Ontario. In addition, a 28-day laboratory chronic exposure to perfluorooctane sulfonate (PFOS) was completed. For both studies, snail tissues were collected pending non-targeted proteomic and semi-targeted metabolomic analyses. These 'omic applications are used to study the effects of chemicals on organisms in order to better understand the biological and molecular pathways and how they may be changed. This study will build upon the current knowledge of PFAS and further explore which proteins and metabolites are affected by PFAS exposure.

Transcriptomic effects of sediments from flooded agricultural fields on early life stage yellow perch (*Perca flavescens*) (PO)

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Floodplains of fluvial lakes make attractive spawning habitat for many freshwater fishes. However, when flooding takes place over agricultural fields, water may become contaminated with pesticides and negatively impact the health of fish embryos and larvae. In the present study, we used a sediment contact assay to assess transcriptomic effects of embryonic exposure to pesticide-contaminated soils in yellow perch (*Perca flavescens*). Soils were collected from forested areas (forest treatment) or agricultural fields (soybean treatment) within the floodplain of Lake St. Pierre (Québec). We also assess three different UV conditions (control, UVA and UVB removed, UVB removed) for a total of six treatments with five replicates per treatment. Water and perch embryos used in the sediment contact assay were collected from sites expected to be relatively free of pesticide contamination. After a 13-day exposure, water and larvae were frozen and stored for chemical and transcriptomic analysis. Pesticides were detected at higher levels and more frequently in the water conditioned with soil from the soybean treatment than the forest treatment. Atrazine, metolachlor, imazethapyr, and clothianidin were consistently detected in all agricultural samples, sometimes at levels exceeding toxicity thresholds for the protection of aquatic life. Two pesticides, thiamethoxam and clothianidin, were also detected at levels exceeding toxicity thresholds in water samples conditioned with forest soil, but only in one of the five replicates. Results suggest pesticides can transfer from sediment to lake water and may become bioavailable to ELS yellow perch. Analysis of organismal and RNA sequencing data from larval perch is ongoing.

Studying the effects of Contrecoeur's contaminated sediments on planorbid snails using multi-omic analyses (PO)

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Contrecœur, Québec, is a small municipality located in the fluvial area of the St. Lawrence River, across an archipelago. In 1981, the federal government established Contrecoeur's islands as a National Wildlife Area. However, the city also has a history of strong industrial development. Previous environmental studies reported key areas where sediments contain high concentrations of butyltins and several heavy metals. This research aims to study the impact of the contaminated sediments on Planorbid snails' survival, reproductive output, and behaviours. It also aims to investigate the influence of water temperature and sediment disturbance in this context. In a two-week *in situ* exposure, we assessed the effect of the contaminated sediments on *Planorbella trivolvis*. We assessed the snails' survival, behaviour, and reproductive output throughout the study. We did not alter the water temperature or the sediment disturbance; we only recorded what naturally occurred. After the exposure, we cryo-preserved the snail tissue samples for proteomic and metabolomic analysis. In a laboratory exposure, we assessed the effect of the contaminated sediments on *Planorbella pilsbryi*. In addition to assessing the same variables as the *in situ* exposure, we considered the added effects of water temperature and sediment disturbance. Once completed, we preserved the snail tissue samples for proteomic and metabolomic analysis. The proteome and metabolome analyses will highlight any differences in molecular functions and biological processes between the control and the contaminant-exposed snails. Previous research studying similar contaminants showed that functions affected were locomotion and reproduction.

Linking impaired reproduction performance of adult fathead minnow with dysregulation of gene expression in fathead minnow larvae exposed to contaminated groundwater (VPO)

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Traditional risk assessments rely on resource intensive animal testing to determine the toxicity of contaminants. Mechanistic toxicology approaches are progressively seen as a promising way to address these limitations. However, the lack of clear and consistent linkages between effects at molecular and apical levels curtails adoption of such approaches. The objective of this study was to test the effects of complex mixtures of contaminants on fish reproductive performance and determine the correlation, if any, with

effects on the expression of relevant genes using a reduced transcriptome assay (EcoToxChips). To do this, two laboratory experiments were conducted exposing adult and early life stages (ELS) of fathead minnows (FHM; *Pimephales promelas*) for 21 and 7 days, respectively, to increasing concentrations of groundwater collected from a legacy contaminated site. In the first study, adult FHM showed a significant decrease in the cumulative number of eggs produced, a reduction in the number of nuptial tubercles of males, and suppression of ovary development in females. In the second study, the relative expression of genes of ELS FHM were assessed using EcoToxChips to determine the toxicity pathways of these mixtures via the differential expression of 376 genes involved in a variety of biological processes. Results obtained revealed that groundwater mixtures activated several adverse outcome pathways such as aromatase reduction or estrogen receptor antagonism pathways linked to reproductive dysfunction and impaired fertility of FHM. Therefore, the molecular outcomes were predictive of apical outcomes and this study could help to increase the confidence in the use of 'omics data for regulatory purposes.

1H-NMR-based metabolomics provides insight into effects of four BZT-UVs on livers of zebrafish (*Danio rerio*) (PO)

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Benzotriazole ultraviolet-stabilizers (BZT-UVs) are plastic additives used to prevent damage caused by UV-radiation. BZT-UVs have been detected in freshwater, sediment, and biota, but little is known about adverse effects of these emerging contaminants of concern. Here we used 1H-NMR-based metabolomics to investigate potential effects of four BZT-UVs: UV-P, UV-090, UV-9, and UV-234. Sexually mature zebrafish were exposed to each chemical, individually, via their diet for 10 days. Using univariate, multivariate, and machine learning analysis, changes in abundances of 59 metabolites were identified across all treatments. Of the 59 metabolites, 30 were common to all chemicals. Exposure to low, medium, and high UV-090 caused greater abundance of 5, 5, and 0 metabolites and lesser abundance of 30, 16, and 3 metabolites, respectively. Exposure to low, medium, and high UV-234, caused greater abundance of 6, 5 and 7, and lower abundance of 29, 2, and 43 metabolites, respectively. UV-9 exposure caused greater abundance of 7, 42, and 4 and lesser abundance of 3, 9, and 5 metabolites, respectively. UV-P exposure caused greater abundance of 5, 5, and 8 and lesser abundance of 31, 2, and 40 metabolites, respectively. There were differences among chemicals in perturbed pathways. Nicotinate and nicotinamide metabolism was perturbed only in fish exposed to UV-090. Metabolism of β-alanine and biosynthesis of pantothenate and CoA were different in fish exposed to UV-090, -234, and -P. Pyruvate metabolism and Glycolysis/Gluconeogenesis were different in fish exposed to UV-234, -9, and -P. Potential effects of alterations to the metabolome will be discussed.

Advances in Ecotoxicology Research on Aquatic Oil Spills

Stable and radio-carbon isotope analyses reveal minimal assimilation of petrogenic carbon into a freshwater food web after experimental oil spills (PL)

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Following an oil spill into water, some bacteria can biodegrade petroleum hydrocarbons, which could lead to petrogenic carbon assimilation by aquatic biota. Changes in the isotope ratios of stable ($\delta^{13}C$) and radio- ($\Delta^{14}C$) carbon can be used to measure petrogenic carbon assimilation as hydrocarbons from crude oil tend to be ^{13}C- and ^{14}C-depleted relative to most organic C sources to lakes. We examined the potential for assimilation of petrogenic carbon into a freshwater food web following experimental spills of diluted bitumen (dilbit) into a boreal lake in northwestern Ontario, Canada. Different volumes (1.5, 2.9, 5.5, 18, 42, 82, and 180 L) of Cold Lake Winter Blend dilbit were applied to seven 10-m diameter littoral limnocorrals (approximate volume of 100 m^3), with two additional limnocorrals serving as controls. Particulate organic matter and periphyton from oil-treated limnocorrals had lower $\delta^{13}C$ (up to 3.2‰) than the control at every sampling timepoint. Dissolved organic and inorganic carbon (DOC and DIC) had lower $\Delta^{14}C$ than the oil-treated limnocorrals relative to the control. Giant floater mussel (*Pyganodon grandis*) housed for 25 days in aquaria containing water from the limnocorrals did not show significant changes in $\delta^{13}C$ values of muscle tissue. Overall, the changes in $\delta^{13}C$ and $\Delta^{14}C$ observed indicated small amounts (up to 11% in DIC) of oil carbon incorporation into the food web. Researchers should be cautious when using isotope analysis to investigate assimilation of petrogenic carbon in freshwater biota because $\delta^{13}C$ of study organisms can be variable and similar to that of crude oil. $\Delta^{14}C$ is a more sensitive tracer of oil carbon, but other carbon sources can have similar $\Delta^{14}C$ values, complicating the interpretation of results.

Bioaccumulation and toxicokinetics of alkylated and non-alkylated polycyclic aromatic compounds and metals in giant floater mussels (*Pyganodon grandis*) exposed to a simulated diluted bitumen spill (PL)

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Canadian bitumen is mainly transported with a diluent via pipeline to reach global markets. This transportation comes with a risk of accidental releases of this diluted bitumen (dilbit) in the environment. In the summer of 2018, the Boreal Lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) project was conducted at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) to understand the fate, behaviour, and potential toxic effects of dilbit in a freshwater boreal lake. We conducted a series of controlled dilbit spills into seven 10-m diameter limnocorrals, and two additional limnocorrals not treated with dilbit were studied as controls. Here we present the bioaccumulation and toxicokinetic parameters of polycyclic aromatic compounds (PACs) and various metals in giant floater mussels (*Pyganodon grandis*) exposed *ex situ* for 25 days to water from the limnocorrals treated with dilbit. Significantly greater total PACs concentrations in mussels exposed to water contaminated with dilbit (24.09–36.36 $\mu g \cdot g^{-1}$ lipid) were detected compared to mussels from the control treatment (0.64 $\mu g \cdot g^{-1}$ lipid). Uptake (24.65–0.66 $L \cdot g^{-1}$ day-1 lipid) and depuration (0.012–0.37 day^{-1} lipid) kinetic rate constants, as well as bioconcentration factors (7.15–1309.69 $L \cdot g^{-1}$ lipid) were determined for the 29 PACs that accumulated in mussel tissue. This study responds to the need for improved toxicokinetic models for compounds derived naturally weathered dilbit and demonstrates the utility of mussels to monitor oil pollution in a freshwater ecosystem.

Characterizing the impacts of diluted bitumen spill remediation methods on boreal lake littoral freshwater benthic macroinvertebrates – Part 2: Changes in the benthic community using artificial substrates over a 3-month period (PL)

Danielle Desrochers[1], José Luis Rodríguez-Gil[1,2], Vince Palace[2], Mark Hanson[1], Tyler Black[1]

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There is uncertainty regarding the impacts of current and proposed oil remediation methods for freshwater ecosystems following spill events. To address some of these gaps, the Freshwater Oil Spill Remediation Study (FOReSt) was conducted in a boreal lake at the IISD-Experimental Lakes Area in summer 2019 (Ontario, Canada). The primary focus was two remediation approaches: enhanced monitored natural recovery (EMNR) and a shoreline washing agent (SWA; Corexit™ EC9580A). A total of 16 five by ten-metre

shoreline enclosures were installed into two substrate types: peat organic or rock cobble. There were eight enclosures per substrate with a triplicate of each treatment, in addition to two reference enclosures. All enclosures underwent a simulated oil spill using weathered diluted bitumen and an industry standard clean-up response scenario; then, treated enclosures underwent one of the two treatments. The benthic invertebrate community was monitored via colonization of modified Hester-Dendy samplers before and after treatment for 69 days. Declines were observed in communities treated with the SWA, particularly Chironomidae in the peat organic substrate, and Hyalellidae in the rock-cobble substrate. The rock-cobble substrate was more sensitive to remediation strategies than the peat organic substrate. Total benthic colonization over the 69 days was significantly different between treatments for the rock-cobble substrate (reference > EMNR > SWA) but confounding factors may have contributed to these changes, which will be discussed. The observed results will contribute to knowledge on spill response strategies and for selecting appropriate remediation methods for spills within the framework of a net environmental benefit analysis.

Diluted bitumen, crude oil and UV radiation cause lethal and sub-lethal effects to *Hyalella azteca* through photo-enhanced toxicity (PL)

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Canada is one of the top oil producers globally with approximately 10% of the world's oil reserves, crude oils and diluted bitumen (dilbit) being the major products. The rate of oil spills has declined over the past decade, however, many pipelines and proposed pipelines cross freshwater, and more knowledge is required about how oil behaves in these systems. Previous studies have evaluated the toxicity of crude oils and dilbit, but many overlook the photo-enhanced toxicity of oil constituents. Photo-enhanced toxicity is a synergistic interaction between the toxicity of a contaminant and UV radiation. The Freshwater Oil Spill Remediation Study (FOReSt) at the IISD-Experimental Lakes Area was designed to study the effects of oil spills in a shoreline environment and compare the efficiency of non-invasive methods to remediate freshwater shorelines after oil spills. This study was conducted with Cold Lake Blend dilbit in 2019 and conventional heavy crude oil in 2021. To examine the photo-enhanced toxicity of these oils, *Hyalella azteca* were exposed to water accommodated fractions (WAFs) of dilbit (2019) and crude oil (2021) from oil weathering experiments and shoreline enclosures. The test was duplicated to expose individuals to low (10%) and high (90%) UV exposures. Mortality was documented throughout a 5-day exposure, and photographs were taken at the end to measure growth and deformities to identify and categorize sub-lethal impacts of remediation measures and photo-enhanced toxicity. Overall, both dilbit and crude oil exhibited photo-enhanced

toxicity to *Hyalella azteca* as indicated by increased mortality and deformities, and decreased growth.

Assessing the effects of minimally invasive freshwater oil spill remediation techniques on multiple life stages of wild small bodied fish (PL)

Lauren Timlick[1], Lisa Peters[1], Sonya Michaleski[2], Juan Manuel Gutierrez-Villagomez[3], Valerie Langlois[3], José Luis Rodríguez-Gil[1,2], Madeline Stanley[1,2], Gregg Tomy[2], Vince Palace[1,2]

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IISD-Experimental Lakes Area (Ontario, Canada) has been host to studies examining the effects and efficacy of minimally invasive methods of oil spill remediation since 2018. The Freshwater Oil Remediation Study (FOReSt) and Floating Wetlands to Enhance Remediation (FLOWTER) project have assessed the impacts of both diluted bitumen and conventional heavy crude oil spills on boreal freshwater lakes and their biological communities by treating large (10 m x 5 m) shoreline enclosures with subsequent remediation methods. All enclosures underwent shoreline washing and sorbent collection followed by application of either engineered floating wetlands or nutrient enhanced monitored natural recovery. Wild collected fish eggs (*Pimephales promelas*) were exposed to enclosure water in a lab setting while small-bodied freshwater fish (*Phoxinus neogaeus*) were exposed within the enclosures. For the embryo-larval exposures, upregulation of cytochrome P450 was assessed, and common malformations associated with PAC exposure were scored. In both conventional heavy crude and dilbit exposures there were significantly more malformations present than reference enclosure regardless of the remediation method applied. Within the adult fish we will assess whether this effect is present in adult fish as well by assessing bioaccumulation of polycyclic aromatic compounds and histological analysis of the liver, gills, and thyroid to assess exposure and sublethal effects.

Engineered floating wetlands for the bioremediation of oil spills: Research at the IISD-Experimental Lakes Area, Canada (PL)

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As a top producer and exporter of crude oil, and host to 20% of the world's freshwater, Canada needs to be prepared with effective remediation strategies in the case of an accidental oil spill. Current conventional methods can be damaging to sensitive environments and do not fully recover spilled oil, leaving habitats altered and with chronic

contamination. Biologically-mediated methods may be an effective and less invasive alternative to mechanical or chemical remediation, using natural microbial processes to degrade oil compounds. The IISD-Experimental Lakes Area is exploring the use of engineered floating wetlands (EFWs) as a non-invasive remediation method for freshwater oil spills, as part of the FLOating Wetland Treatments to Enhance Remediation (FLOWTER) study. EFWs are platforms of vegetation that float on the water surface, with a vast suspended root network and associated microbial biofilm. Plants can stimulate microbial colonization and increase contaminant interaction in the water column, with potential to enhance biodegradation. Since 2019, researchers have been exploring changes to the root microbial community on EFWs exposed to model spills of diluted bitumen and conventional heavy crude oil using in-lake shoreline enclosures or mesocosms. Changes to microbial community structure and the effect of different plant and nutrient ratios on crude oil degradation have been examined. Future research will confirm whether plants enhance metabolism of phenanthrene, a three-ring polycyclic aromatic compound, in a small-scale microcosm experiment. Results will be synthesized to offer guidance on EFW design and use for spill remediation, with efforts to enhance ecosystem recovery.

Effects of elevated water temperature on the toxicity of diluted bitumen to embryonic coho salmon (*Oncorhynchus kisutch*) (PL)

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Diluted bitumen (dilbit), the major crude oil export product from Canada's oil sands, causes developmental and long-lasting effects in various species of fish, including Pacific salmon that are at risk of exposure from a pipeline spill. To accurately predict the toxicity of dilbit in a real-life spill situation, interactions with other environmental variables should be investigated. We examined whether a modest increase in water temperature altered the toxicity of dilbit to early life stage (ELS) coho salmon (*Oncorhynchus kisutch*) using a suite of molecular and whole-animal endpoints. Fish were reared in uncontaminated water (control) or in one of two environmentally-relevant concentrations of dilbit (~0.2 $\mu g \cdot L^{-1}$ and ~1 $\mu g \cdot L^{-1}$) from fertilization to swim-up at each of two water temperatures, ambient and ambient +3°C. At swim-up, a subset of fish from each treatment were reared under common conditions (uncontaminated, ambient water) for an additional 6 weeks. ELS coho salmon exposed to dilbit experienced higher mortality and reduced cardiorespiratory performance. These effects were exacerbated at the higher temperature, and persisted for at least 6 weeks post-exposure. A latent reduction in body mass and condition appeared following the 6-week grow-out in clean water. Combined, these results indicate that environmental conditions at the time of a spill, such as water temperature, can greatly influence the biological response of exposed fish in ways that could impact their populations. As the demand on Canada's crude oil grows, understanding the interactions

between dilbit toxicity and environment is critical for spill response, species impact, and risk assessments.

Examination of chemical and biological changes related to aged oil from historical shipwrecks (PL)

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Heavy marine oil was recently observed leaking from the wreck of the M/V Schiedyk that sank in 1968 near Bligh Island, British Columbia. The two fuel tanks contained Bunker "C" type fuel oil and marine diesel oil, both of which were mixed with seawater and weathered. Both types of oil were recovered from the wreck and used in a series of experiments measuring changes to the hydrocarbons and microbial dynamics at different temperatures, using water collected from Bedford Basin, Nova Scotia. Microcosms with seawater containing either Bunker C or diesel oil collected from the Schiedyk were set up between 6 and 18°C. The change in hydrocarbon composition was monitored through the use of GC-MS, fluorescence spectroscopy, and Microtox, while microbial dynamics were determined using 16S rRNA sequencing and flow cytometry. At a water temperature of 18°C, 16S rRNA sequencing results showed a Marivita-dominant community from day 7 that peaked at day 28 in Bunker C. For the diesel treatment, the microbial community switched from a Glaciecola-dominated community (days 4–7) to Pseudophaeobacter- and Altererythrobacter-dominant communities (days 14–21). Marivita genus became more dominant community in day 28 in the diesel treated samples. These microorganisms have been implicated in biodegradation in oil-contaminated marine environment. Further analyses will provide important insights on hydrocarbon compositional and microbial dynamic changes during the incubation period. Although the Schiedyk spill has been addressed, evidence-based advice can be generated from this study regarding future emergency spill response specifically targeting historical shipwrecks that may release aged oil in the future.

Still potentially toxic after nearly forty years: Polycyclic aromatic hydrocarbon residues within crude oil in Arctic beach sediments at the Baffin Island Oil Spill (BIOS) site (VPL)

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The Baffin Island Oil Spill (BIOS) project (Cape Hatt, Baffin Island, Canadian Arctic) hosted numerous experimental oil spill studies in the early 1980's to monitor the short and

long-term physical, biological, and chemical impacts of crude oil, when left subject to natural attenuation processes within an Arctic setting. In 2019, selected sites of the BIOS project were revisited to collect surface and subsurface beach sediments from the 1981 nearshore surface oil spill intertidal area (Bay 11), from 1980 supratidal oiled control plots (Crude Oil Point), and from 1982 supratidal oiled mixing plots (Bay 106). Samples were analyzed for polycyclic aromatic hydrocarbons (PAHs) and alkylated congeners via gas chromatography mass spectrometry (GC-MS). Pre-spill baseline studies indicated total mean PAH concentrations of low to sub $\mu g \cdot kg^{-1}$, whereas our results denote concentrations of 50 to 14 000 $\mu g \cdot kg^{-1}$ for total PAHs, and of 3 to 3000 $\mu g \cdot kg^{-1}$ for the 16 EPA (United States Environmental Protection Agency) priority PAHs. Concentrations presented in the Interim Marine Sediment Quality Guidelines (SQGs) were exceeded by various PAHs in samples from all three sites. Potentially toxic levels of PAHs were detected in all supratidal samples. Future monitoring of the BIOS sites is essential for determining a timeline for oil degradation and the decline in toxicity over time. Thank you to Patrick Lambert and Ben Fieldhouse of ECCC for the sample of Lagomedio crude oil. A very special thanks to those who assisted with sample collection and processing including Esteban Gongora Bernoske, Ianina Altshuler, Madison Ellis, Margaret Cramm, Fernanda de Matos Ferraz, Shiva Lashkari, Teresinha Wolfe, and Misuk Yun.

Application of machine learning algorithms to predict key water quality parameters in oil sands demonstration pit lake based on real time sensor data (PL)

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Wireless sensor networks (WSN) have been gaining popularity globally as a means to obtain continuous data and higher data frequency. This, combined with regression-based machine learning (ML) techniques, can provide a powerful approach to predicting key water quality parameters in real time. The aim of this study was to develop an approach to characterize the spatio-temporal changes in water chemistry and predict key water quality parameters in oil sands demonstration pit lake (DPL). To do this, a WSN system was used to monitor multiple physicochemical and limnological parameters in the oil sands DPL, Suncor's Lake Miwasin near Fort McMurray, Alberta. Sensor probes were deployed in Lake Miwasin at four different depths [shallow (~0.3 m), medium (~2.5 m), and deep (~3.5 and 4.0 m) depths] and hourly data were obtained for a period of 29 days and 119 days during summer/early fall 2020 and 2021, respectively. Simultaneously, manual water sampling and field measurements were carried out at all four depths to validate the sensor data. In this study, advanced bagging and boosting-based ML models were developed and compared with other conventional data-driven models. The performance of each model in both training and testing phases was assessed using statistical metrics. Modeling results

showed that advanced ML models outperformed the other models in predicting Chl-a (chlorophyll a) and NH_4^+ (ammonium) concentrations, but all models yielded similar performances for prediction of pH. Generally, the ensemble ML models successfully captured both the low and high levels of the water quality variables, particularly for NH_4^+ concentrations.

Biodegradation of polycyclic aromatic compounds by engineered floating wetlands: Optimizing plant species composition and nutrient additions for oil spill bioremediation (PO)

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Conventional oil spill cleanup methods can further damage affected shoreline and near-shore areas and leave residual oil in the system, creating a need for less-invasive alternatives. Research conducted at the IISD-Experimental Lakes Area in 2021 investigated the use of engineered floating wetlands (EFWs) for non-invasive remediation of freshwater oil spills. EFWs are vegetated floating platforms – conditions in the underwater root system promote microbial colonization and contaminant biodegradation. This study aimed to determine the capacity of EFWs to degrade oil-derived polycyclic aromatic compounds (PACs) and to identify how this process is affected by changes to the plant species composition used or the carbon to phosphorus ratio achieved by augmenting the system with fertilizer. Twenty-six 1600-L mesocosm tubs containing EFWs were spiked with 16 L of water accommodated fraction (WAF) produced by weathering conventional heavy crude oil. Concentrations of 44 PACs in the water column were measured over a 96-day exposure. Root samples were analyzed for microbial biofilm activity via adenosine triphosphate (ATP) assays and community diversity via 16S and 18S rRNA gene amplicon sequencing. The mean concentration of total PACs one day after WAF application was 176 ± 33 $ng \cdot L^{-1}$ which declined to baseline levels in all mesocosms by day 35. Preliminary ATP assay results indicate that biofilm activity was not affected by WAF addition. Ongoing analyses will identify the effects of EFW design on microbial diversity and PAC removal. Results of this research will be communicated to industry partners to support the use of EFWs in oil spill cleanup.

Characterizing the impacts of diluted bitumen spill remediation methods on boreal lake littoral freshwater benthic macroinvertebrates – Part 1: Emergence and post-study benthic community (PO)

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There is significant uncertainty around the impacts of oil spills and subsequent cleanup methods, specifically chemical and biological responses, in freshwater ecosystems. To address these gaps, the multi-year Freshwater Oil Remediation Study (FOReSt) was conducted at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) using littoral enclosures installed in a freshwater boreal lake on two different substrates (rock cobble and peat organic) in 2019. Specifically, we examined the behaviour, fate, and effects of weathered dilbit applied at 0.05 $L \cdot m^{-2}$ and subsequently cleaned using traditional response methods before the application of a secondary cleanup methods; either a shoreline washing agent (SWA) and enhanced monitored natural recovery (EMNR). Aquatic emergent insect communities were monitored weekly to biweekly, pre- and post-experimental spills using insect emergence traps. Benthic invertebrate communities were sampled by standard kick net sampling at the end of the exposure periods. We found that both SWA and EMNR showed no statistically significant effects on the total abundance or taxa richness for emergent insects and benthic invertebrates in both shoreline types relative to control enclosures. There was a statistically significant decrease in total diversity (Inverse Simpson) for the EMNR for emergent insects in the peat organic shoreline relative to control enclosures; however, the diversity endpoint was not statistically different for any other treatment-substrate combination relative to the control. Our results indicate minimal adverse impacts to the aquatic macroinvertebrate community with the addition of both cleanup treatments compared to a release of dilbit itself.

The impacts of hydraulic fracturing flowback and produced water on fathead minnow (*Pimephales promelas*) (PO)

Ebi Lari[1], Arian Farajizadeh[1], Greg Goss[1]

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Flowback and produced water (FPW) from hydraulic fracturing is a mixture of toxicants that can result in adverse effects on different fish species. The olfactory organ in fishes plays an important role in behaviours and activities in fishes such as foraging or aversive behaviours to negative cues. However, responses to FPW have not been evaluated. We showed that 24-hour exposure to (1%) FPW resulted in negative effects on the fathead

minnow olfactory system. We also demonstrated using a behavioral maze that fathead minnows exposed to low doses (1%) of FPW do not show avoidance to trichloroacetic acid (TCA), a substance normally shown to result in avoidance behavior. We used an electro-olfactogram (EOG) to test the neurons responsible for the avoidance response. Olfactory neurons could not detect the aversive clue, showing no specific EOG reaction to the TCA concentration. Filtration has been proposed as a means of treating FPW to reduce any potential effects and reduce the toxicity of FPW. Filtration of FPW through a 0.45 μm mesh has been shown in previous studies to reduce the adverse effects of FPW. Results show that the adverse effects on the olfactory system are abrogated by prior filtration. This result is supported by both behavioral maze assays whereby filtered FPW exposed fish had less prominent effects on the fish avoidance assay and the EOG system where filtering FPW resulted in the return of the neuronal response to TCA. In summary, filtering the particulate fraction out of FPW was associated with reduced adverse effects.

Investigation of the molecular mechanisms of detoxification in embryonic coho salmon exposed to dilbit in elevated water temperatures (PO)

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Although the association between the aryl hydrocarbon receptor (AhR) mediated detoxification pathways and diluted bitumen (dilbit) toxicity in teleost fish has been well documented, there are knowledge gaps regarding the influence of environmental conditions, composition of the crude oil mixture, and other physiological factors have on the mechanisms of toxicity. Using a suite of molecular and whole-animal endpoints, we investigated the effects of a modest increase in water temperature on the toxicity of dilbit to early life stage (ELS) coho salmon (*Oncorhynchus kisutch*). Fish were reared in uncontaminated water (control) or in one of two environmentally-relevant concentrations of dilbit (~0.2 μg·L^{-1} and ~1 μg·L^{-1}) from fertilization to swim-up at each of two water temperatures, ambient and ambient +3°C. The concentration-dependent increase in the expression of phase I biotransformation enzyme CYP1A was consistent with the previous studies linking dilbit toxicity to phase I of biotransformation. However, in contrast to the temperature effect we observed on whole-body responses, fish exposed to dilbit in ambient temperature had greater CYP1A expression than the heated group across all concentrations. Expressions of the phase II enzyme gst and the quantification of lipid peroxidation did not show any concentration or temperature dependence. Our results suggest that the whole-body toxicity of dilbit may not be directly linked to oxidative stress induced during the phase II of biotransformation pathways, and environmental factors such as water temperature can have varied effects on the physiological mechanisms of dilbit toxicity.

How to improve the reporting and communication of oil toxicity testing (PO)

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Results from oil toxicity studies performed with established testing protocols generate data that could be integrated into models and databases that support oil spill planning, response, and environmental assessments. To foster transparency, facilitate repeatability, and maximize use and impact, outcomes from toxicity tests published in the peer-review literature need to be clearly reported and communicated. Thus, the primary motivation of the current work is to provide guidance and encourage effective disclosures of key reporting elements from aquatic toxicity studies. Specific recommendations are provided regarding key reporting elements (i.e., experimental design, test substance and properties, test species and response endpoints, media preparation, exposure conditions, chemical characterization, reporting metric, data quality, standards and statistical methods, and raw data). An outcome of this work is a checklist that can be used to assess the completeness of reporting elements or to guide study design. Improving reporting, science communication, and access to critical information would enable users to assess the reliability and relevance of study outcomes and increased their incorporation into oil spill response tools. This effort represents one contribution of the Modernizing Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) Aquatic Toxicity Testing International Forum.

The post-spill impacts of hydraulic fracturing flowback and produced water on benthic *Lumbriculus variegatus* (PO)

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The toxicity of hydraulic fracturing flowback and produced water (FPW) has been studied and reported for various aquatic species to predict the potential impacts of an FPW spill on the receiving environment. However, the post-spill impacts on benthic species exposed to FPW-contaminated sediments are not well studied. In the present study, we investigated the post-spill effects of FPW in a lotic environment by exposing *Lumbriculus variegatus* to FPW-spiked sediment in a flow-through setup. Lethality was measured in a 7-day exposure to sediments spiked with a range of geometrically ascending concentrations of FPW. The sub-lethal effects were also measured in response to 5% FPW spiked sediment for 21 days. LC_{50} from sediment spiked with FPW was calculated as 24% FPW with a NOEC at 5% FPW-spiked sediment. Longer-term exposures to the 5% FPW-spiked sediment reduced both growth and metabolic rate in *L. variegatus*. However, no significant accumulation of FPW metal components, activation of oxidative stress responses, or EROD activity in the organisms was detected. The present study results

indicate that *L. variegatus* could efficiently withstand the accumulation and harmful impacts of an FPW spill except at very high concentrations. However, the detoxification of FPW components created an energy deficit in *L. variegatus*.

Fate of Macro- and Microplastics in the Environment: Sources, Sinks, and Biological Implications

International interlaboratory intercalibration study for microplastics measurement in environmental media (PL)

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Microplastics are ubiquitous emerging environmental contaminants. Their presence has prompted recent legislation aimed at assessing and mitigating risks of microplastics to humans and aquatic ecosystems. These require the State of California to develop standardized methodologies to characterize exposure to microplastics in order to compare among measurements, a foundational component needed for assessing exposure and therefore risks, that has long eluded the scientific community. Accordingly, forty laboratories in six counties participated in an intercalibration exercise to address these needs, by analyzing blind samples with particles of varying polymer types, sizes (1–1000 μm), colors, and morphologies. Extraction and analysis were performed on surrogates for drinking water, ambient water, tissue, and porous media, using visual microscopy, Fourier transform infrared spectroscopy, and Raman spectroscopy. Inter- and intra-laboratory performance and operator experience were assessed using draft standard operating procedures (SOPs) followed by labs. Processing and measurement method performance was optimized through permutations in SOPs for a subset of labs. Costs associated with training, time, equipment, and consumables were analyzed. Particle recovery was 92 ± 12% (s) in clean water, and decreased dramatically only for the smallest particles (1–20 μm). Performance was reduced for more complicated matrices that had to be removed, but not to the extent observed for 1–20 μm particles. Both forms of spectroscopy were accurate and precise, with no significant differences between novice and experienced labs. Training improved precision of measurements considerably. Results from this work has helped to develop the first regulatory monitoring program for microplastics in the world, and informed methods and QA/QC for microplastics work worldwide.

Optimization of the isolation and quantification method for the analysis of microplastics in wastewater treatment plants (VPL)

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The accumulation of microplastics (MPs) in environmental systems have attracted increased attention in recent years for their adverse impacts on ecosystem and human health. The effluent discharge and sludge disposal from wastewater treatment plants (WWTPs) have been identified as one of the main sources of MPs to surface water and agriculture land. However, due to the chemical complexity and organic richness of wastewater and sludge samples, there is still a lack of standard procedures for the isolation and characterization of MPs. Since the current quantification of MPs commonly rely on visual observation under microscope, the efficiency of isolation, which determines how visible the MPs are, will impact the accuracy of quantification. Moreover, the further characterization of MPs using instruments such as FTIR (Fourier transform infrared) and Raman spectrometry also requires the MP particles to be isolated sufficiently to diminish interference. As a result, a well-established and standardized isolation method is vital for the quantification of MPs, as well as their identification and characterization. In this study, an isolation method combining pre-treatment, WPO and EDTA treatment, was developed and optimized for the removal of organic mater and iron particles in wastewater and biosolid samples, contributing to the investigations on the fate, exposure, and environmental risk of MPs. Furthermore, the maximum probability number (MPN) method was introduced for the quantification of MPs. Compared to the direct visual counting method, the MPN method accounts for random variation of sampling and avoids direct number counting; thus, it is more reliable, simpler, and less laborious.

Three-dimensional whole mount imaging of microplastics using lightsheet microscopy (PL)

Jun-Ray Macairan[1], Brian Nguyen[1], Nathalie Tufenkji[1]

[1] *McGill University*

With ever-growing concerns over plastic pollution, there is a need to understand the impacts of plastics in the environment. Studying plastic pollution requires an effective methodology to detect micrometer sized polymer particles in environmental samples and organisms. To date, detecting and quantifying microplastics in a biological system generally required liquefying biomass via digestion. While digestion can effectively recover microplastic, it results in the loss of spatial information on the distribution of microplastic within an organism. Fluorescence microscopy allows visualization of microplastic uptake but requires fluorescently labeled microplastic. Unfortunately, these labeling strategies are non-specific and label biomass, which prevents effective visualization of the embedded

microplastic. In addition, studies using pre-labelled microplastics (prior to exposure studies) can affect the viability of the organism and could potentially impact the overall uptake mechanism of the plastics. To address these shortcomings, we developed a method that utilizes non-specific labeling in conjunction with a tissue clearing technique. In brief, tissue-bound fluorescent dye is removed, while the structurally intact organism is rendered transparent, and the fluorescent dye remains bound to plastic. This process results in a sample with fluorescently labeled plastic that can be rapidly imaged in three dimensions with light sheet microscopy. We demonstrate this technique with model aquatic organisms including amphioxi (lancelets) and *D*. *magna*.

Microplastics loadings in the St. Lawrence River (Québec) (VPL)

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Microplastics (MPs) are synthetic or semisynthetic polymers ubiquitously found in freshwater ecosystems and deleterious to living organisms. We quantified and characterized the floating MP fraction at 11 sites through the St. Lawrence River (SLR). The SLR forms the primary drainage outflow of the Great Lakes Basin to the North Atlantic Ocean and crosses the province of Québec. Sampling occurred from May to July 2021 using two sampling nets with different sizes, structural construction, and mesh size: Manta (40 x 20-cm rectangle metal frame with a 300-µm mesh net) and Poly-Mer (33 x 15-cm diameter rectangle wooden frame with a 100-µm mesh net). The nets were towed in parallel from a boat for 20 minutes in the top 50 cm from the surface at each of the sampling sites (n=2–4). Samples were prepared and analyzed for fibres, fragments, and spheres using an optical microscope. Each particle was annotated using Fourier transform infrared spectroscopy (FTIR) and library standards. Data confirmed the presence of MPs at each of the sampling sites along the SLR with MP concentrations ranging from 125 to 861 particles of plastics per million litres (PPML) according to the studied site. The most abundant categories of MPs found were the fibres, followed by the fragments, and lastly, the spheres. The five main material found were polyester, polyethylene, polypropylene, nylon, and polystyrene for both nets. This work provides valuable information about the distribution of MPs in aquatic ecosystem in North America for water resource protection and management.

Estimated discharge of microplastics via stormwater runoff (PL)

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In urban environments, impervious surfaces, such as rooftops and roadways, prevent precipitation from soaking into the ground; rather, the runoff flows into storm sewers and is eventually discharged into surface waters, often without treatment. Urban stormwater runoff is a source of pollutants and sediments to surface waters and is thought to be an important vector for the introduction of microplastics into the aquatic environment. However, there is a paucity of data available on the prevalence of microplastics in urban runoff, limiting assessment of its role in transporting microplastics and preventing any form of risk assessment or mitigation strategies to be implemented. This study investigates the concentration and characteristics of microplastics in urban stormwater runoff from the city of Calgary, Canada. Samples (n=75) were collected under both baseflow and rain event conditions from 15 sampling locations. Microplastics were found in all samples at concentrations ranging from 0.7 to 200.4 pieces·L^{-1} (mean=31.9 pieces·L^{-1}), with microplastics <125 µm being the dominant size class. Consistent with studies in other freshwater environments, fibers were the predominant microplastic morphology identified, followed by fragments. Total concentrations, dominant morphologies, and particle sizes differed between rain events, baseflow conditions, and morphology, and size and color distribution differed amongst land use types. We estimate that individual outfalls discharged between 1.9 million and 9.6 billion microplastics to receiving waters per rain event. This study adds to the growing knowledge that urban stormwater runoff is a significant pathway by which microplastic particles and anthropogenic fibers enter aquatic environments and will provide a baseline for future monitoring and mitigation studies.

Assessing wastewater treatment plants as sources of microplastics using freshwater bivalves in the Grand River, southern Ontario (PL)

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Microplastics monitoring in aquatic organisms is critical to understand exposures and potential risks of these emerging contaminants in freshwater ecosystems. The objective of this study was to assess microplastic accumulation in bivalves near wastewater treatment plants (WWTPs), a known point source of microplastics to the environment. Sampling was conducted upstream and downstream of three WWTPs and at two reference sites in the Grand River watershed. Flutedshell mussels (*Lasmigona costata*; n=80), fingernail clams (Sphaeriidae; n=400), and sediment were collected in August 2021. Whole clams and

mussel hemolymph were digested in KOH for 14 days, and sediments were dried and underwent density separation in $CaCl_2$ to isolate microparticles. Particles were counted and characterized by colour and morphology. A subset of particles will be sent for chemical confirmation by FTIR (Fourier transform infrared) spectroscopy to identify which are plastic. Microparticles in all bivalve samples were dominated by clear fibers. Fingernail clams had a mean of 1.4 ± 0.8 and a maximum of seven microparticles per clam (blank corrected). These results and preliminary data for mussels show significant variation of microparticle concentration across sites. Microparticles were elevated in clams downstream of one WWTP and in mussel hemolymph downstream of another WWTP when compared to the upstream sites. The potential for freshwater bivalves to act as bioindicators will be evaluated by comparing MPs collected from tissues to sediment samples collected at the same sites. This project will increase our understanding of the sources and environmental fate of microplastics, and whether bivalves can be effective biomonitors for MPs.

The importance of water and diet as sources of microplastics to American bullfrog (*Rana catesbeiana*) tadpoles (VPL)

Samantha Gene[1], Natalie Czajka[1], Abigail Mathews[1], Hareena Sidhu[1], Barbara Katzenback[2], Jennifer Provencher[3], Chelsea Rochman[4], Diane Orihel[1]

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Microplastics are a pollutant of concern, prevalent in the habitats of many amphibians. Our study compared the relative importance of two exposure routes in a 12-mesocosm experiment. We compared how the presence and absence of microplastics in water and diet impacted microplastic concentration in bullfrog (*Rana catesbeiana*) tadpoles using a 2×2 factorial design (control, contaminated water, contaminated food, and both contaminated water and food). To assess water exposure, white polyethylene fragments were added to the mesocosms' water (containing 90 L of lake water) at a concentration of 100 particles·L^{-1}. To assess food exposure, green polyethylene fragments were added to a mixture of agar, spinach, and commercial tadpole food at a concentration of 3.4 μg·g^{-1}. Tadpoles (Gosner stage 25; n=60) were captured from a natural lake and exposed to treatments for 10 days. Tadpoles were collected and dissected every 48 hours for microplastic quantification. Microplastics were isolated using a 20% potassium hydroxide solution, followed by a 10% hydrogen peroxide solution for gastrointestinal tract (GI) samples. Waterborne and diet-borne microplastics were detected after 48 hours of exposure. Waterborne exposure affected microplastic concentrations in the GI and remaining tissues ($p<0.05$). Diet-borne exposure affected microplastic concentration in the GI ($p<0.05$) but not significantly in the remaining tissues ($p>0.05$). There is evidence to suggest that multiple exposure routes can influence microplastic concentrations in tadpoles. Our study provides insights into where – and how quickly - tadpoles acquire

microplastics in their environment, which is important for modelling the risk of microplastic pollution to amphibian populations.

Exposure to microplastics alters gene regulation in developing North American wood frogs (*Rana sylvatica*) (PL)

J. Drew Thompson[1], Samantha Gene[2], Diane Orihel[2], Jennifer Provencher[3], Chelsea Rochman[4], Barbara Katzenback[1]

[1]University of Waterloo, [2]Queen's University, [3]Environment and Climate Change Canada, [4]University of Toronto

Limited information exists about whether exposure to microplastics affects amphibian immune function. To address this research gap, North American wood frog (*Rana sylvatica*) embryos were exposed throughout development to each of 0, 50 000, or 500 000 particles·L^{-1} of a microplastics mixture consisting of equal parts polypropylene, polystyrene, and polyethylene terephthalate microplastics (40–1400 µm shortest dimension) in quadruplicate 300-L aerated outdoor mesocosms containing 200 L of filtered lake water at Queen's University Biological Station. RNA was isolated from dorsal skin tissues collected from one individual from each mesocosm after six (Gosner stage (GS) 31–35) and eight (GS 40–45) weeks of exposure and as individuals reached the end of metamorphosis (GS 45). NEBNext Ultra II Directional polyA mRNA library preparation and 150 base paired-end sequencing on a NovaSeq™ 6000 were performed by The Centre for Applied Genomics (Toronto, Ontario), yielding 3 048 043 472 read pairs. Using the Seq2Fun and edgeR tools, we found a total of 894 genes were differentially expressed across all timepoints and treatments, with the most seen after six weeks of exposure. Gene set enrichment analysis reveals alterations in KEGG pathways related to glycine, serine and threonine metabolism, biosynthesis of secondary metabolites, neuroactive ligand-receptor interaction, and carbon metabolism. Immune genes involved in cell communication (interleukins), antiviral signaling (TRIM13), Toll-like receptor signaling (MYD88), and inflammasomes (NOD1) were also differentially regulated between control and treatment groups as well as between treatments. These data suggest exposure to microplastics affects metabolic activity and immune regulation in amphibian skin. [Funded by ECCC-IKPP and NSERC DG grants]

Life in plastic, is it fantastic? Accumulation and toxicity of environmentally relevant microplastics exposures in freshwater macroinvertebrates (PL)

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Microplastics (MPs) (<5mm) pollution has quickly become one of the most pressing environmental issues to date. However, freshwater macroinvertebrates are vastly

underrepresented within the current MPs literature, despite their extensive use in aquatic biomonitoring. Therefore, this study aimed to address this gap through ecologically realistic exposures using freshwater macroinvertebrates. For the experiments, three macroinvertebrate species, *Tubifex tubifex*, *Planorbella pilsbryi*, and larval *Hexagenia* spp., and two MPs types, polystyrene microbeads (6µm) and polyester microfibers (100µm), were used. For each species, four tests were conducted: pristine microbead and microfiber exposures, and "aged" microbead and microfiber exposures, across a range of concentrations. MPs "aging" was incorporated into this experiment to replicate the microbial attachment and biofilm formation on MPs within the environment. To assess toxicity, reproduction and survival were measured across all tests following MPs exposure. To date, no significant effects to reproduction or mortality have been observed for any of the three species, or for either type of MPs, aged or pristine. Accumulation and retention of MPs within the organisms is being assessed to predict the potential for MPs bioaccumulation. Additionally, impacts of MPs on the gut microbiome, used in assessing overall organism health, will be determined. This study will provide new insights regarding the potential impacts of MPs to freshwater macroinvertebrates to determine the risks of implications of MPs contamination in freshwater ecosystems.

Toxicity of microplastics and released chemicals from single-use surgical face masks to the model aquatic organism, *Daphnia magna* (VPL)

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[1]*McGill University*

More than 1.5 trillion single-use face masks are used annually to curb the spread of the COVID-19 pandemic. The indiscriminate disposal of these masks has become an important source of plastic pollution in the environment and several studies have shown that these masks can release millions of microplastics and other harmful chemicals when exposed to environmental conditions. However, there is still limited knowledge of the impacts of the released microplastics and chemicals to aquatic organisms. In this study, we carried out acute and chronic toxicity tests on *Daphnia magna*, using chemicals and microplastics released from single-use face masks during a simulated weathering experiment. The acute toxicity tests were conducted on three different life stages of *Daphnia magna* (neonates, juveniles, and adults). The toxicity of the microplastics alone, chemicals alone, and mixture of microplastics and chemicals was compared. The results reveal that the chemicals alone and mixture of chemicals and microplastics reduced the survival rate of neonates and juveniles but did not impact the survival of adults. Microplastics alone had no effects on the survival of the neonates but significantly affected the survival of juveniles and adults. Also, microplastics alone did not impact survival during chronic exposure but caused significant impacts on growth and reproduction. In contrast, chemicals alone and mixture of chemicals and microplastics significantly decreased survival during 21-day chronic exposure. This

study shows that the released microplastics and chemicals may have different impacts on Daphnia, and the observed impacts may depend on the life stage of the exposed organism.

Effects of microplastics on freshwater zooplankton communities in a large scale mesocosm study (PL)

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Microplastics (plastic particles <5 mm) are ubiquitous contaminants in aquatic ecosystems, but the ecological implications for freshwater environments are largely unknown. Laboratory studies have shown that microplastics have the potential to negatively impact zooplankton at the individual and population level but the effects for natural zooplankton communities have not yet been studied. To better understand the potential effects of microplastics on freshwater ecosystems we conducted a large-scale in-lake mesocosm study at the International Institute for Sustainable Development-Experiment Lakes Area (IISD-ELA) in northwestern Ontario in 2021. We added a mixture of three types of common microplastics (polyethylene, polystyrene, and polyethylene terephthalate) to 10-m diameter by 2-m deep mesocosms in an experimental lake. The concentrations of microplastics were added in an environmentally relevant gradient using a regression design (0, 6, 24, 100, 414, 1710, 7071, 29 240 particles·L^{-1}). The zooplankton community was monitored for changes in abundance, biomass, and species composition for 10 weeks. Preliminary data indicate that microplastics did not have a negative impact on the zooplankton community, but after five weeks of microplastic exposure, zooplankton abundance and biomass were positively correlated with increasing microplastic concentrations.

Impact of UV stabilizers on gene expression and development in Japanese quail embryos (PL)

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UV stabilizers are ubiquitous in the environment during manufacture, waste disposal, and leaching from microplastics. UV stabilizers have been detected in bird eggs, but their impact on avian development has yet to be studied. Here, we assess the effects of four UV stabilizers in Japanese quail (*Coturnix japonica*) embryos. Eggs were injected with solvent control or five doses (10, 1, 0.1, 0.01, and 0.001 μg·g^{-1}) of UV-9, UV-P, UV-328, and UV-329 dissolved in DMSO. The initial sample size was 10 eggs per dose per chemical. On

embryonic day 9, liver was sampled to analyze the hepatic expression of genes relevant to quail toxicity pathways using EcoToxChips (custom 384-well qPCR arrays). Heart tissue was collected for DNA sex determination. We also noted changes in weight and color of the liver and heart, the size and weight of the embryo, and some deformities. A pilot study suggested that there was no dose-dependent increase in mortality or physical deformities at concentrations of the UV stabilizers up to 100 times greater than those previously detected in the eggs of wild birds ranging between 1 and 50 $ng \cdot g^{-1}$. This work will provide information on the toxicity and potential mechanism(s) of action of four UV stabilizers in avian embryos and potentially permit the ranking of their potency.

The pELAstic Project: Mesocosm- and ecosystem-scale experiments on the fate and effects of microplastics in freshwater ecosystems (PO)

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Despite rapid advances in elucidating the implications of microplastic exposure on aquatic organisms, the fate and effects of these contaminants in freshwaters at the community- and ecosystem-scale remain poorly understood. As such, our team has embarked on an ambitious, decade-long study across multiple ecological scales to determine: (i) the physical, chemical, and biological fate of microplastics in lakes and their watersheds; (ii) how microplastics impact aquatic ecosystems across all levels of biological organization; (iii) how ecosystem processes and functions, such as nutrient cycling and photosynthesis, are affected by MPs; and (iv) the recovery of an ecosystem exposed to microplastics, including how they break-down and/or transform over time. Our study, named the pELAstic Project, involves mesocosm, limnocorral, and whole-lake experiments situated at the IISD-Experimental Lakes Area (ELA), Queen's University Biological Station, and the Rochester Institute of Technology. Since our study began in 2019, we completed two mesocosm studies and four limnocorral studies using color-coded microplastics, and conducted baseline monitoring in two study lakes in preparation for a whole-lake manipulation. In this presentation, we provide an overview of the objectives, study design, and anticipated results of each experiment. The pELAstic Project will ultimately advance

our scientific understanding of microplastics in freshwater ecosystems, develop new tools and protocols for characterizing these contaminants, and inform risk assessments and policies in Canada and beyond. Funded by NSERC, Fisheries & Oceans Canada, Environment and Climate Change Canada, Loblaws and the Ocean Conservancy.

Low-cost offline pyrolysis GC-MS for the analysis of microplastics in environmental samples: a proof of concept (PO)

Matthew Ross[1], Miya Matz[1], Ryan Duchnowski[1], Roland Lee[1]

[1]*MacEwan University*

Chemical characterization of microplastics extracted from environmental samples typically utilize either spectroscopic (e.g., FTIR (Fourier transform infrared) or Raman) or thermoanalytical (e.g., pyrolysis GC-MS) techniques. Thermoanalytical techniques enable identification and quantification of microplastics, but necessitate instrumentation not widely available in laboratories. Our goal was to develop a low-cost, off-line pyrolysis GC-MS method capable of identifying and quantifying microplastics in environmental samples. As a proof-of-concept, the method was developed using virgin polyethylene, polystyrene, and polypropylene. Plastic particles were pyrolyzed in an inert environment within a sealed tube using a sand bath. The generated pyrolysis products were extracted with dichloromethane, concentrated, and analyzed by GC-MS. The expected characteristic pyrolysis products for each polymer was identified by mass spectrometry, enabling identification of individual polymer types within mixed samples. Calibration curves for all polymers were linear (R^2>0.99) over 2–3 orders of magnitude and the limits of detection were polymer dependent, ranging from 0.05 to 0.4 mg. These preliminary results suggest that this methodology provides comparable results to online pyrolysis GC-MS techniques and may provide a low-cost entry point for microplastics analysis. Future work will be aimed at expanding the range of polymer types investigated and the application of the method to quantify and identify microplastics in stormwater runoff.

Development and application of a device for one-pot digestion and extraction of microplastics (PO)

Matthew Ross[1], Tianna Groeneveld[1], Zach Vydra[1]

[1]*MacEwan University*

Typical workflows for the isolation of microplastics from environmental matrices utilize a combination of density flotation to separate microplastics from more dense matrix material, and chemical or enzymatic digestion to remove organic material. This multi-step workflow necessitates transferring samples between multiple containers, increasing particle loss and risk of contamination. To overcome these issues, we developed a device that enables both digestion and density flotation steps to take place within the same

apparatus. The device is constructed of inexpensive and widely available stainless steel components. Recoveries were assessed in sand by spiking samples with fluorescent microbeads and consumer plastic fragments which ranged in size from 63 µm to 1 mm and in density from 1 to 1.4 $g \cdot cm^{-3}$. Extractions of microbeads from sand produced acceptable recoveries (>75%) for beads >250 µm in diameter. Compared to typical methodologies, our novel device produced significantly better recoveries for small microplastics (<75 µm) and a reduction in contamination. The modular design also provides flexibility and adaptability to a range of sample matrices, and the technique was further applied to quantify microplastics in biosolids, river water, and sediment.

Impacts of polyethylene microplastics on the growth, behaviour, and cognition of juvenile convict cichlids (PO)

Miguel Eduardo Felismino[1], Grant Brown[1]

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Microplastics (MPs), particles <5 mm in size, are ubiquitous in the environment and lead to a variety of adverse effects when consumed by aquatic organisms. However, despite the wealth of studies addressing the effects of MPs on aquatic organisms, the impact of the consumption of MPs on fish growth, behaviour, and cognition are still poorly understood. Here, we tested the impacts of the consumption of ecologically relevant concentrations of MPs on the foraging competition, growth, and cognition of juvenile convict cichlids (*Archocentrus nigrofasciatus*). Under laboratory conditions, we manipulated the levels of MP exposure in shoals of juvenile cichlids by feeding them shrimp (*Artemia* spp.) exposed to 0, 10 or 100 $MPs \cdot mL^{-1}$ of polyethylene microspheres. Cichlids were fed shrimp exposed to one of three levels of MPs for 10 days, during which we analyzed their foraging and aggressive behaviour. Cichlids were measured pre- and post-exposure to assess growth and body allometry. We also tested the impacts of MPs on the cichlids' exploratory behaviour and cognitive ability by observing their performance through a simple two-day maze trial. Our research explores novel perspectives (exploration, boldness, learning) on the effects of MPs on aquatic organisms and combines them with previously used metrics (growth, morphology, foraging). This work shows that the consumption of virgin polyethylene MPs affects the cichlids' cognition and exploratory behaviour but not their growth or foraging rates. By determining how MPs affects this model system, we can improve our understanding of the threat that plastic pollution poses to freshwater communities worldwide.

Factors influencing the variation of microplastic uptake in demersal fishes from the upper Thames River, Ontario (PO)

Colleen Wardlaw[1], Patricia Corcoran[1], Bryan Neff[1]

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Microplastics (plastic particles <5 mm) are a widespread form of pollution that is abundant in aquatic ecosystems. Microplastics have previously been reported in a variety of aquatic biota, however few studies examine the variation of microplastic uptake by freshwater fishes, especially through field studies on demersal fishes that are at risk to ingest microplastics retained in benthic sediments. Microplastics were examined in demersal fishes—white sucker (*Catostomus commersonii*) and common carp (*Cyprinus carpio*)—across 11 sites in the Thames River, Ontario, Canada. Microplastics were found in 44% of white sucker, ranging from 0 to 14 particles per fish, and 31% of common carp, ranging from 0 to 128 particles per fish with body mass positively related to number of microplastics ingested by the fish. For both species, the number of microplastics was higher in urban sites than rural sites, and there was a positive relationship between the number of microplastics in the fish and the abundance of microplastics in the sediment. Microplastics analyzed by Fourier transform infrared spectroscopy revealed that the types of microplastics found in fishes were among the most commonly produced plastics, and that most fibres were natural in composition. Additionally, a large proportion of particles found in fishes were suspected tire wear particles, which are known to be abundant in the environment but rarely reported in fishes. Together these results provide insight into environmental and biological factors that may influence the susceptibility of demersal fishes to ingest microplastics.

Microplastic ingestion in ringed seals (*Pusa hispida*) from the Canadian Arctic (PO)

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Plastic pollution in the form of microplastics is present across the world's oceans, including the Arctic. Ringed seals (*Pusa hispida*) play an essential role in Arctic marine food webs as predators for fish and invertebrates and as prey for polar bears and humans. Their position in the food web may lead to the ingestion and accumulation of microplastics within their gastrointestinal tract, which poses risks to their overall health. However, limited data exists on the accumulation of microplastic within seal species from the Canadian Arctic. To better understand the extent of microplastic accumulation in ringed seals, 35 seal stomachs were collected in collaboration with Inuit hunters in 2021 from Arviat and Resolute, Nunavut, Canada. Stomachs were dissected and contents were passed through a series of sieves to fractionate contents based on size (53–1000 μm). The material

in each sieve was digested for 5 days using potassium hydroxide and microplastics were isolated using vacuum filtration. Visual microscopy and Raman microspectrophotometry will be used to count and categorize plastics on size, color, morphology, and chemical composition. This data will provide baseline information for future plastic pollution monitoring programs in the circumpolar Arctic. Acknowledgments: We'd like to thank the Resolute Bay Hunters and Trappers Association and Arviat Hunters and Trappers Organization for help with sample collection, and NSERC and the Undergraduate Student Research Award for funding.

Assessing the biological risk of exposure to aged microplastics in *Mytilus galloprovincialis*: Preliminary results using a multibiomarker approach (PO)

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Microplastics (MPs) are increasingly present in aquatic environments worldwide, reaching coastal areas and oceans as the ultimate sink. Therefore, marine organisms, and in particular filter-feeders such as bivalves, are continuously exposed to MPs, which can then enter the food web. Aged MPs could be more harmful than pristine ones due to the microorganisms growing on them and the pollutants that can be adsorbed on their surface. In this project, polyethylene (PET) MPs were obtained by grinding water bottles and sifting them to get the desired size range (40–250 μm). PET-MPs were aged for 8 months under natural environmental conditions in a flow-through controlled setup. Mussels (*Mytilus galloprovincialis*) were placed in the tanks at the concentration of 10 000 particles·L^{-1}. The exposure lasted 7 days, after which MP accumulation was assessed. In addition, immune parameters (total haemocyte count, haemocyte diameter and volume, lysozyme, acid/alkaline phosphatase activities) were assessed in haemolymph. Biomarkers of antioxidant defence, detoxification and neurotoxicity (superoxide dismutase, catalase, glutathione reductase, glutathione-S-transferase, acetylcholinesterase, butyrylcholinesterase), and oxidative damage (protein carbonyl content, lipid peroxidation) were assessed in gills and digestive gland. Haemocyte characteristics and immune enzyme activities did not change following MP exposure. Induction of antioxidant enzymes and increase of oxidative damage were observed in the digestive gland. In gills, only inhibition of the superoxide dismutase activity was detected. These results were not expected as gills are the first barrier to the presence of MPs. However, aged MPs could have brought into the mussels, microbes and pollutants that easily reached the digestive gland.

A microplastic feast: Measuring ingestion in freshwater mussel *Lampsilis siliquoidea* (PO)

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Plastic debris polluting our waterways has been a concern for decades. Recently increased attention has been placed on microplastics (MPs) in aquatic ecosystems. These small plastic particles (<5 mm) have been observed in marine and freshwater ecosystems globally. To date, freshwater studies have focused on the presence and/or concentration of MPs in surface waters. To assess their risk, there is a need to compare environmental concentrations of MPs to concentrations that cause adverse effects. Freshwater mussels are a group of filter-feeding organisms that have experienced a decline due to habitat destruction and poor water quality, and they are under-represented in MPs research. In this study, four 28-day exposure tests followed by a 7-day depuration period were conducted with ~2 cm juvenile *Lampsilis siliquoidea* (fatmucket mussel). Tests were performed with MP spheres and fibers of varying polymer type and size. Algal food stocks were spiked with polystyrene (6, 90 µm) spheres, polyethylene (28 µm) spheres, or polyethylene fibers (60 µm), with treatment concentrations ranging from 100 to 150 000 000 MP·L^{-1}. Ingestion of each type of MP was determined by whole tissue digestion of individual mussels. Tissues from the depuration stage will be compared to non-depurated mussels to assess whether juveniles have the potential to eliminate MPs within a 7-day period. In each MP exposure, there was <10% mortality observed, and burial ability was not affected as the concentration of MPs increased within treatments. Such findings will inform the risk assessment of MPs to freshwater biota, specifically mollusks which can be sensitive to contaminants.

A source of plastics to terrestrial ecosystems: Characterizing the microplastic content of biosolids and biosolid-amended fields in southern Ontario (PO)

Nicholas Letwin[1], Gladys Stephenson[1], Adam Gillespie[1], Ryan Prosser[1]

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Microplastics (<5 mm diameter) are an emerging concern to the health of various ecosystems. To date, the overwhelming majority of microplastic research has focused on their impacts on aquatic ecosystems, leaving a knowledge gap surrounding their effects on terrestrial ecosystems. One source that could potentially contribute a significant amount of microplastic contamination to soil ecosystems are biosolids. Biosolids are nutrient-rich field amendments that are derived from wastewater treatment processing. Crude biosolid samples were collected from eight wastewater treatment plants in hopes of characterizing the magnitude and type of microplastic within biosolids that are used for land application. Additionally, in Spring 2022, soil samples were taken from thirteen agricultural fields that

had been amended with biosolids within the last four years. To ensure proper random sampling, a statistical model that incorporated the slope, slope length and steepness factor (LS-factor), and topographical wetness index of each field was used to generate 20 sampling points per field. Microplastic quantification was performed via density separation with olive oil, followed by vacuum filtration. The resulting filters were then analyzed under a microscope to count the number of microplastics per sample. This data will be used to determine a quantitative assessment of the exposure levels of microplastics in biosolid-amended fields. These results will then be coupled with data from microplastic toxicity tests with *Eisenia andrei* and *Eisenia fetida* to perform an environmental risk assessment. Toxicity tests will be performed with varying types (polystyrene, polyester, polypropylene) and sizes of polymer, ranging from 6 to 1000 μm.

Fate of microfibres from single-use face masks: Release to the environment and removal during wastewater treatment (VPO)

Oluwadamilola Pikuda[1], Mathieu Lapointe[1], Olubukola Alimi[1], Dimitrios Berk[1], Nathalie Tufenkji[1]

[1]*McGill University*

Single-use face masks are used globally to mitigate the spread of the coronavirus. However, the indiscriminate disposal of billions of masks has resulted in widespread environmental contamination. Various studies have shown that these face masks can release microfibres when exposed to certain environmental conditions. Still, the extent of microfibre release from masks in urban settings is not known. Moreover, the fate of the released microfibres during wastewater treatment is not well understood. In this study, we exposed new and used single-use face masks to UV irradiation and mechanical friction and quantified the release of microfibres during the exposure. Furthermore, we assessed the removal efficiency of the released microfibres using a simulated coagulation/flocculation wastewater treatment process. We find that UV irradiation and mechanical friction significantly increase the number of microfibres released from both new and used masks. Also, new masks release significantly higher number of microfibres compared to used masks, in the presence of UV irradiation and mechanical friction. Microfibres released from the used masks show higher removal efficiency in simulated coagulation/flocculation process, compared to microfibres released from new masks. FTIR (Fourier transform infrared) and XPS (X-ray photoelectron) analysis shows significantly higher carbonyl indices and silica percentage, respectively, in the microfibres from the UV-exposed used masks. Combining the results of this study with available data, it is conservatively estimated that 4 to 47 million microfibres released from single-use face masks may end up in natural waters per day in an urban environment with a population density of 4300 persons·km^{-2}.

Non-lethal/Non-invasive/Non-animal Methods in Ecotoxicology

Characterizing the lethal and sublethal effects of environmentally relevant pesticides using the rainbow trout gill (RTgill-W1) cell line (PL)

Sophie Emberley-Korkmaz[1], Niladri Basu[1]

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Ethical concerns and increasing demands for chemical testing has called for the need to explore new approach methods (NAMs) to reduce animal testing and address resource limitation issues. The rainbow trout gill cell line (RTgill-W1) was recently standardized by the Organization for Economic Co-operation and Development (OECD) to assess the cytotoxicity of chemicals. However, little is known of how results from this *in vitro* test compare with the whole organism and the test's focus on cytotoxicity does not provide much information on a chemical's mechanism of action. The first objective of this study was to use OECD Test No. 249 to characterize the cytotoxicity of 19 pesticides considered important to Canadian ecosystems, and to compare these *in vitro* data with lethal concentration 50 (LC_{50}) results from whole animal studies. The second objective was to derive transcriptomic points of departure (tPODs) values for these same pesticides to gain a deeper understanding of the impacts these pesticides have on biological mechanisms. Cytotoxicity results from fluorescent dyes assessing cell viability found chlorothalonil, carbaryl, and diquat as the most toxic with LC_{50}s of 21.2, 97.0, and 100.4 µM, respectively. Of the 19 chemicals tested, 10 of them obtained LC_{50}s below 500 µM. There was concordance between these *in vitro* values obtained and *in vivo* values from 96-hour rainbow trout fish experiments. Studies are underway to derive tPOD values. This research demonstrates the potential for alternatives to animal testing strategies that are faster, more cost-effective, and yield more information.

Using the RTgill-W1 cell line to investigate cytotoxic and molecular effects of sediment extracts from the Agbogbloshie electronic-waste site (PL)

Krittika Mittal[1], Jingyun Zheng[1], Stephane Bayen[1], Julius Fobil[2], Niladri Basu[1]

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Electronic-waste (E-waste) sites are notoriously contaminated with complex mixtures thus challenging environmental monitoring and management activities. Additionally, there is increasing awareness that traditional whole-animal-based toxicity tests are resource-intensive, expensive, and unethical. Given that E-waste sites are typically situated in poorly resourced countries, there is a need to develop more efficient techniques for application in

such settings. The objectives here were to A) perform screening of plastic related contaminants in extracts prepared from 35 soil samples collected at the Agbogbloshie E-waste site (Accra, Ghana; classified as upstream:6, downstream:2, community:3, trade-site:8, dump-site:13, and burn-site:3) through liquid chromatography coupled to hybrid quadrupole time of flight mass spectrometry, and B) characterize cytotoxic and molecular effects of these extracts on the rainbow trout gill cell line (Rtgill-W1), following OECD Test No. 249. Gill cells were exposed to concentrations equivalent to 9.38, 4.69, 2.34, 1.17, 0.59, and 0.29 mg dry weight of extract (eQsed)·mL^{-1}. Many samples from various site types caused a decrease in cell viability ranging from 2.2 to 77.4%. Trade-site samples were among the most cytotoxic of which trade-site #8 was the most cytotoxic (cell viability: 74.2% to 2.2% at 0.29 to 9.38 eQsed·mL^{-1}). Plastic-related contaminants such as Bisphenol A and dibutyl phthalate were measured at high concentrations of up to 255.62 and 384.83 ng·g^{-1} dry weight, respectively. Further chemical analysis, and exposures to examine molecular effects of the sediment extracts on the gill cells are underway. This work is expected to support ongoing efforts in establishing the use of efficient alternative testing strategies in ecotoxicology.

The use of fish scale hormone concentrations as a non-lethal biomonitoring tool in teleost fishes (PL)

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Investigation of the use of fish scales as a medium for non-lethal biomonitoring has recently commenced. Fish scales have been shown to incorporate cortisol over long periods of time and thus provide a promising means of assessing long-term stress in many species of teleost fish. However, while cortisol is a major mediator of the stress response in fishes, the quantification of additional hormones could allow for the expansion of this area of research. In addition to cortisol we quantified dehydroepiandrosterone (DHEA) and cortisone, which were elevated alongside cortisol in scales after two weeks of physiological stress challenges in rainbow trout and goldfish. In another study we successfully quantified gonadal steroids (progesterone, 17- α-hydroxyprogesterone, testosterone, 11-ketotestosterone and estradiol) to allow for the assessment of the effects of elevated stress on reproduction. With information on multiple scale hormones these methods also allow for calculation of hormone ratios which can be considered more robust markers of altered physiology than single hormone concentrations. For example, the cortisol:DHEA ratio, which is used in mammals to diagnose chronic stress, was significantly different in scales from stressed rainbow trout and goldfish when compared to controls. Additional ratios between cortisol and gonadal steroids which are currently used to assess the impacts of long-term stress on reproduction can now also be quantified using scales. Thus, while these methods still require further validation, fish scale hormone concentrations

appear to provide a novel and informative tool in the assessment of long-term stress and resulting effects on reproduction in teleost fishes.

Tissue microchemistry using LA-ICP-MS: an accurate, precise, faster, and greener (no harsh chemicals) alternative to conventional bulk analysis (PL)

Jennie Christensen[1], Geriene LaBine[1]

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Certain challenges and inefficiencies are apparent in environmental monitoring programs for industry. Specifically, in many cases lethal sampling (e.g., fish tissue) is necessary to obtain enough sample volume for bulk tissue metal analysis. Additionally, collecting enough smaller organisms such as periphyton or invertebrates is challenging, and the smaller volumes typically result in substantially poorer analytical detection limits. Sediment contamination is also a significant confounding factor when collecting periphyton and benthic invertebrates, and can create inaccurate estimates of actual metal accumulation in the food web. Here, we highlight our novel method using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to quantify metals in these biological matrices, and demonstrate the method's accuracy, precision, and comparability to conventional bulk analysis using acid-digestion ICP-MS.

Transcriptomic monitoring of *Onchorhynchus kisutch* smolt exposure to and recovery from low sulphur marine diesel seawater accommodated fractions (VPL)

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Oil spills pose considerable risks to social, ecological, and commercial interests in marine environments. Sensitive and rapid biomonitoring tools are critical for ascertaining contamination and remediation effects on species of interest and local habitats. This is important as water-soluble components of oils, such as polycyclic aromatic hydrocarbons (PAHs), can persist post-remediation. We previously demonstrated that 3-methylcholanthrene responsive cytochrome P450-1a (CYP1A) transcript abundance in the liver and caudal fin of coho salmon smolts (*Onchorhynchus kisutch*) was sensitive to low sulphur marine diesel (LSMD) seawater accommodated fraction (seaWAF) exposures. We expanded upon this paradigm to assess tracking LSMD seaWAF exposures and subsequent recovery (depuration) in clean seawater by measuring CYP1A abundance in coho smolts using quantitative polymerase chain reaction (qPCR). Livers and caudal fins were dissected from 40 smolts per group after 4 days of 100 $mg \cdot L^{-1}$ LSMD seaWAF exposure and at 1-, 2-,

4-, and 8-days post-depuration from seaWAF-exposed and control seawater conditions (ntotal=400 smolts). PAH-induced CYP1A transcript levels decreased one day after depuration in the liver and caudal fin of sex-genotyped females and males. CYP1A abundance in the female caudal fin recovered by depuration day 1, while the male caudal fin and female and male liver tissues recovered after 2 days. These results support CYP1A measurements as a biomonitoring tool for contamination and remediation. Additionally, sex-dependent differences in recovery should be comprehensively explored on a transcriptome-wide basis. The present study demonstrates the value of using the caudal fin as a reliable, sensitive, and non-lethal sampling and monitoring tool.

Non-lethal mucus and caudal fin sampling of fathead minnow to assess oil exposure (VPL)

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The objective of this work is to investigate if mucus and caudal fin punches can be used to detect exposure to oil. Male adult *Pimephales promelas* were exposed for 4 days to water accommodated fractions (WAF) of Cold Lake Blend dilbit (6.25, 12.5, 25 and 50% v/v), to WAF + Corexit™ EC9580A (SHORE-WAF; 0.25, 0.5, 1, and 2% v/v) and water only control. Corexit™ EC9580A is a surface washing agent recommended by the USEPA as a shoreline cleaner after an oil spill. Polycyclic aromatic hydrocarbon (PAH) concentrations in the WAF treatments ranged from 6 to 104 $\mu g \cdot L^{-1}$ PAHs, while the SHORE-WAF ranged from 3 to 84 $\mu g \cdot L^{-1}$ PAHs. At exposure completion, no mortalities were observed and liver, mucus, and caudal fin were sampled for RNA-seq analyses. Results showed that WAF exposure significantly enriched up to 1510 GO terms in the liver of fish exposed to WAF treatments compared to the control. Similarly, WAF affected the enrichment of up to 1589 GO terms in the mucus and up to 1790 in the caudal fin compared to the control. The SHORE-WAF exposure also significantly altered GO terms, but to a lesser extent in the liver (up to 814), mucus (up to 813), and caudal fin (up to 1342) compared to the control. Ontology analyses showed that xenobiotic response, respiratory chain, and ribosomal function pathways were significantly enriched among the different tissues. These results suggest that fish mucus and caudal fin punches are robust non-lethal tissues to monitor oil exposure and ecosystem recovery.

Mercury chemoscape in the Gulf of St. Lawrence: Using northern gannets as a biological sampling platform (PL)

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Contaminants are ubiquitous in marine environments, but they are not evenly distributed. To better understand how an animal's use of its environment may determine its contaminant load, it is important to know where 'hotspots' of contaminants are located. The Gulf of St. Lawrence, Québec, Canada, is an economically and ecologically important ecosystem which hosts a high biodiversity of species, but it can be difficult to measure its level of contamination. To map the chemical landscape, or "chemoscape", of the Gulf of St. Lawrence, we collected fish regurgitations (n=54) from northern gannets (*Morus bassanus*) tracked using GPS devices, and analyzed total mercury and compound-specific stable nitrogen isotopes in amino acids (Aas) in the fish muscle. Using the d15N signatures in AA, we standardized fish mercury concentrations. Concentrations were assigned to the most recent gannet dive location and we mapped the chemoscape of mercury for the Gulf of St. Lawrence. Contrasting mercury profiles were found in the different regions due to differences in food web structure and trophic magnification. Demonstrating where contaminants accumulate more efficiently in the Gulf of St. Lawrence is crucial to understanding what risks wildlife are exposed to based on their habitat and feeding ecology.

New lines of inquiry: Fish epidermal mucus for non-invasive environmental effects monitoring (PL)

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Fish epidermal mucus is a viscous and dynamic biofluid that acts as a protective barrier to protect fish from injury and pathogens. Mucus contains many biological molecules and agents that could be informative about fish health and physiology. Our goals were to (1) develop a method to explore the fish epidermal mucus proteome and metabolome, and then (2) characterize the functions and pathways that are represented within the mucus proteome and metabolome. We tested different types of mucus sampling and collection methods and found that absorbing mucus onto a wipe and then using an ultrafiltration device allowed us to purify and concentrate mucus proteins. The filtrate was used for metabolomics. All samples were then analyzed using liquid-chromatography tandem high-resolution full scan mass spectrometry (Agilent 1260 Infinity LC with 6545 QTOF), and data were processed using Agilent Spectrum Mill (Version B.04.01.141), Skyline 20.2

(MacCoss Lab Software), a PCDL database (PCDL Manager B08.00), Agilent Profinder 10.0 software, and Metaboanlayst 5.0 software. We found that mucus contains thousands of proteins, including cytokines and signaling molecules, as well as a surprising number of neurological and sensory pathway proteins. We think these proteins may be coming from the lateral line, which is a new and exciting "line of inquiry" to explore for future behavioral toxicological studies. We also identified amino acids and will be expanding our metabolomic libraries to include hormones like cortisol and GABA for future studies. The potential of fish epidermal mucus will be discussed with linkages to how this could improve environmental effects monitoring and benefit society more generally.

Non-lethal analysis of F2-isoprostanes in the epidermal mucus freshwater fish species as an indicator of oxidative stress (PL)

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F2-isoprostanes (F2-IsoPs) are prostaglandin-like compounds found in all tissues and biofluids, and are referred to as the "gold-standard" for *in vivo* measurements of lipid peroxidation, a hallmark of oxidative stress. Despite their reliability, they are infrequently studied in fish, and never in epidermal mucus. This presentation describes the development of an extraction protocol and HPLC-MS/MS method for measuring F2-IsoPs collected non-lethally from the epidermal mucus of fish. We first confirmed the presence of F2-IsoPs in fish mucus in northern pike (*Esox lucius*) and lake trout (*Salvelinus namaycush*) using high-resolution HPLC-MS. In a second study, rainbow trout (*Onchorhynchus mykiss*) exposed to acute physical stress exhibited signs of oxidative stress in gill and liver: F2-IsoPs and glutathione were significantly lower in gills up to 48-hours post-stress, and F2-IsoPs elevated in the liver after 4 hours. However, the mucus extraction method proved inadequate to detect F2-IsoPs in these fish. In a third study, the method was modified and mucus F2-IsoPs extracted using solid phase extraction (SPE) were successfully quantified in mucus samples (n=64, massavg=44 ± 20 mg) of juvenile fathead minnows exposed to 0 to 30% preparations of diluted bitumen water accommodated fraction (dilbit WAF). Mucus concentrations of individual F2-IsoPs isomers ranged from 10 to 450 $pg \cdot g^{-1}$ protein. Liver F2-IsoPs ranged from 0.1 to 17 $ng \cdot g^{-1}$ tissue. Neither matrix responded to increasing dilbit WAF concentration, but polycyclic aromatic compounds (PAC) bile metabolites analysis indicated this may be due to low PAC uptake. Together, these studies suggest mucus F2-IsoPs have potential as non-invasive biomarkers of oxidative stress in fish.

Incorporating the 3Rs into the development of a standardized test method using a native amphibian species (PL)

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While there is growing demand for standardized tests using native amphibian species to inform risk assessment, tests with a mortality endpoint using vertebrates raise ethical concerns. Environment and Climate Change Canada (ECCC) has incorporated replacement, reduction, and refinement (3Rs) strategies during the development of its standardized test method for assessing contaminants with a native amphibian species (*Lithobates pipiens*, northern leopard frog). Two test options initiated with tadpoles are available depending on research objectives: a 14-day test for assessing changes in growth, and a 42-day test for assessing changes in development. Data from the literature and a regional ECCC laboratory support the use of rainbow trout acute toxicity data as a replacement for acute tests using larval *L. pipiens*, therefore only chronic definitive toxicity test procedures will be published for *L. pipiens*. Reducing the number of organisms used per experiment was accomplished by maximizing the amount of information (i.e., endpoints) obtained from each test, and optimizing the test design using power analysis. As well, ECCC is collaborating with the EcoToxChip project to determine the relationship between apical endpoints from chronic *L. pipiens* exposures and gene expression endpoints from acute early life stage *L. pipiens* exposures, and assess the use of EcoToxChip as a screening tool or alternative endpoint to support the ECCC test method. Lastly, the ECCC test method will refer to local animal care committees and Canadian Council on Animal Care publications for guidance on refinement practices specific to amphibians, and will include guidance on preventing and treating disease in *Lithobates pipiens*.

Increased temperature during rearing leads to long-term behavioural impacts in yellow perch larvae (PL)

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Water temperatures are expected to rise in the Great Lakes region from 1.7 to 3°C over the next two decades due to industrial practices and climate change. Increases in water temperatures have been linked to perturbations in neurodevelopment, which may manifest as behavioural disturbances in fish. This may be a concern for the yellow perch (*Perca flavescens*), a cool-water fish of importance to this region, however, no study has investigated the effects of early-rearing temperature on behavioural responses of this species. Here we tested the hypothesis that deviations from the optimal rearing temperature would impart disturbances on the behavioural profiles of yellow perch larvae.

We reared perch at 12°C, 15°C (optimal), and 18°C during embryogenesis, with hatchlings raised in common garden conditions (18°C). We additionally treated embryos to an ambient seasonal exposure, raising the temperature from 12 to 18°C (+1°C every 3 days), similarly to natural environments. Behavioural models were created for perch to assess movement (1, 5, 10, 20, and 30 days post-hatch [dph]), anxiety (1, 3, 10, 20, and 30 dph) in response to changing light level, and feeding responses (15 and 30 dph). At 1 dph, yellow perch reared at 12°C exhibit reduced movements relative to the other groups. By 5 dph, 12°C reared larvae match their 15°C conspecifics, with 18°C fish beginning to exhibit diminished movements. After 20 dph, the 18°C fish exhibit reduced movements relative to other groups, remaining diminished relative to 15°C perch at 30 dph. Using ethanol, we confirmed that the perch can exhibit anxiety-like responses at larval stages, but anxiety appears to not be influenced by rearing temperature. Importantly, our feeding trial demonstrates that 18°C fish consume less than half of the value of their conspecifics. Taken together, these results suggest that increasing temperature is of great consequence to the survival of the yellow perch.

Non-destructive biomarkers to assess pesticide exposure on bats (PL)

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Bat populations are dwindling worldwide due to anthropogenic activities like agriculture; however, the role that pesticide exposure plays on these declines is unclear. To address these research gaps, we first need to develop reliable methods to detect and monitor exposure to environmental pollutants and their effects. The use of biomarkers is a sensitive and informative tool to study sublethal effects in wildlife; however, it requires laboratory validation and integrative approaches to be applicable to free-living species. Here, we propose a set of non-destructive biomarkers to evaluate pesticide exposure on bats and validated their suitability with dose-exposure experiments in captivity. We selected three biomarkers widely used in vertebrate ecotoxicology which combined represent sensitive, specific, and ecologically relevant responses to pollutants: DNA damage, cholinesterase (ChE) activity, and leukocyte profiles. Micronuclei frequency (genotoxicity) and ChE activity (exposure and neurotoxicity) were robust indicators of toxicant exposure. The validity of these endpoints was supported by their consistent performance in laboratory and field experiments as well as by the significant correlation among them. Leukocyte profile (systemic stress) results were not consistent between laboratory and field studies, suggesting further evaluation of its suitability is needed. Integrative approaches, like the one we used, maximize the insights about toxicant effects by combining the information of single biomarkers into more meaningful inferences, which can be applied to environmental risk assessments. Furthermore, the use of non-destructive,

cost-effective biomarkers is imperative when assessing toxicant exposure and effects on vulnerable wildlife and it should be a priority in the field of wildlife toxicology.

Investigating toxicity of low concentrations of nickel in rainbow trout by examining proteomic profiles of skin mucus and blood plasma. (PO)

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Nickel production has been projected to increase since it is an integral component of batteries for electric vehicles. The Ontario Ring of Fire located in the James Bay Lowlands is a nickel rich ore with ongoing mining proposals. This raises the possibility of nickel contamination in one of Ontario's most precious freshwater regions and peatlands. However, impacts of nickel exposure on freshwater fish species remain elusive. Moreover, most studies use higher doses of nickel to study adverse impacts. To study the impacts in fish when they are exposed to low doses of nickel (1–46 $\mu g \cdot L^{-1}$), we examined temporal and concentration-dependent responses in protein profiles of skin mucus using untargeted proteomics. On the last day of the of the 30-day exposure, we sampled blood plasma in conjunction to mucus to compare significantly differentially abundant proteins between them. We found ~1800 and ~1200 proteins that were differentially abundant in mucus and plasma respectively. Findings suggest that the proteome of rainbow trout is sensitive to low doses of nickel. Particularly, proteins involved in neurological development, and kidney and immune function were impacted in plasma and mucus proteins. We also find an increase in nickel burden in blood plasma of trout at these concentrations. Additionally, the use of non-lethal blood sampling and non-invasive mucus sampling is becoming increasingly important as these methods do not require fish sacrifice to study toxicological effects. Future studies should examine impacts at low levels of nickel in longer-term exposures using non-lethal and non-invasive techniques.

Developing an *in vitro* avian red blood cell assay to assess toxicity of polycyclic aromatic compounds using a multi-omics approach (VPO)

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Polycyclic aromatic compounds (PACs) cause various health effects in exposed wildlife and there is a need to develop a sensitive toxicity assay where non-lethal sampling techniques can be used. Nucleated red blood cells (RBCs) of fish are known to be responsive to contaminant exposure, including the induction of DNA damage and specific protein changes with PAC exposure. However, it is unknown if transcripts, proteins, or

metabolites of avian RBCs may be indicative of PAC exposure. Therefore, the objectives of this study are 1) to assess the applicability of using avian RBCs as an *in vitro* bioassay for PAC exposure and effects and 2) to determine at what PAC concentration and exposure duration avian RBCs are sensitive to PAC exposure. RBCs, isolated from adult chickens (*Gallus gallus domesticus*), were exposed *in vitro* to relevant low (10 ppb) or high (10 000 ppb) concentrations of benzo[k]fluoranthene, a potent PAC in birds, compared to the vehicle control treatment. RBCs were sampled at 0.5, 4, and 8 hours and stored for non-targeted analyses to assess changes at multiple biological scales: transcriptomics (RNASeq), proteomics (LC-QTOF), and metabolomics (LC-MS QTOF). In a targeted pilot test, both time and treatment significantly contributed to the variation in cytochrome P450 1a4 transcript levels, a biomarker of PAC exposure. Ongoing 'omics analyses will be compared and combined to get a cohesive understanding of PAC toxicity in avian RBCs.

Mechanistic Ecotoxicology: Integrating Biochemical, Physiological, and Ecological Responses

Acute and chronic toxicity of new generation organophosphate flame retardants to the water flea (*Daphnia magna*) and rainbow trout (*Oncorhynchus mykiss*) (PL)

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Diphenyl phosphate (DPP) and triphenyl phosphate (TPP) are organophosphate flame retardants, and their use as a replacement of conventional flame retardants has increased significantly in the last decade. Recently, these compounds have also been identified in samples from flowback and produced water (FPW). FPW is a large-scale complex by product of hydraulic fracturing (HF), a drilling technique used to extract oil and gas from the underlying shale formation. Hydraulic fracturing has quickly become the dominant hydrocarbon extraction technique in North America with large volumes of contaminated wastewater being continuously generated. The appearance of these new generation organophosphate flame retardants in FPW samples from multiple Albertan wells coupled with the fact that to date, there is little information on the toxicity of these novel compounds is of potential concern. Our goal was to investigate the acute and chronic toxicity of both DPP and TPP to two model species: the water flea (*Daphnia magna*) and the rainbow trout (*Oncorhynchus mykiss*). Acute toxicity of TPP to *D. magna* was moderate (48-hour LC_{50}=1.96 mg·L^{-1}) while in chronic (21 days) experiments, effects in growth and reproductive output were seen in concentrations as low as 0.2 mg·L^{-1} TPP, indicating a greater potential for chronic toxicity. The 48-hour LC_{50} of DPP was much higher than the one found for TPP (>40 mg·L^{-1}). Data from DPP and TPP acute and chronic toxicity to rainbow trout are currently being collected and species sensitivity to these novel contaminants will be compared.

The brominated flame retardant, 1,2,5,6-tetrabromocyclooctane (TBCO), causes multigenerational effects on reproductive capacity of Japanese medaka (*Oryzias latipes*) (PL)

Chloe Devoy[1], Yamin Raza[1], Paul Jones[2], Jon Doering[1,3], Steve Wiseman[1,4]

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Exposure of fishes to endocrine disrupting chemicals (EDC) during early development can induce multigenerational effects on reproduction. The brominated flame retardant,

1,2,5,6-tetrabromocyclooctane (TBCO), is an EDC. The goal of this study was to determine whether TBCO has mutigenerational and transgenerational effects on the reproductive performance of Japanese medaka (*Oryzias latipes*). Sexually mature fish (F0 generation) were fed either a control diet or a low (40.6 $\mu g \cdot g^{-1}$) or high (1034.4 $\mu g \cdot g^{-1}$) diet containing TBCO and three generations of embryos were reared to determine reproductive performance. Concentrations of TBCO in eggs (F1 generation) from fish given the low and high diets were 711.3 and 2535.5 $ng \cdot g^{-1}$ wet weight, respectively. Cumulative fecundity of the F1 generation in the low and high treatment were reduced by 33.9% and 33.3%, respectively, compared to the control. In the F2 generation, cumulative fecundity of the low treatment returned to the level of the controls, but the high treatment was decreased by 29.8%. There was no decrease in cumulative fecundity in the F3 generation compared to controls. Mechanistically, cholesterol side chain cleavage enzyme (cyp11a), aromatase (cyp19a), and luteinizing hormone receptor (LHr) were differentially expressed in gonads from F1 females. However, concentrations of E2 in plasma and mRNA abundance of vitellogenin in liver were not significantly different compared to controls suggesting a mechanism other than disruption of steroidogenesis. Mechanistically, no effects were observed in the F2 or F3 generation. Overall, results suggest that TBCO has multigenerational effects on the reproductive performance of Japanese medaka.

1,2,5,6-tetrabromocyclooctane (TBCO) impairs oocyte maturation and reproductive performance of Japanese medaka (*Oryzias latipes*) (PL)

Yamin Raza[1], Chloe Devoy[1], Paul Jones[2], Jon. Doering[1,3], Steve Wiseman[1]

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The novel brominated flame retardant, 1,2,5,6-tetrabromocyclooctane (TBCO), is an endocrine disrupting chemical that decreases fecundity of Japanese medaka (*Oryzias latipes*), but the mechanism is not known. Using an *ex vivo* assay of maturation inducing hormone (MIH)-stimulated oocyte maturation with zebrafish (*Danio rerio*), a prior study demonstrated that TBCO might decrease fecundity by inhibition of oocyte maturation. This assay has not been developed for Japanese medaka. The objective of this study was to develop an *ex vivo* assay of MIH-stimulated oocyte maturation with Japanese medaka to investigate whether inhibition of oocyte maturation is a mechanism by which TBCO decreases fecundity. Ten stages of oocyte development were identified, and a time-course study demonstrated that 24 hours of MIH-stimulation was optimal for inducing maturation of stage IX oocytes. Results of *in vitro* exposure of stage IX oocytes to 0, 2, 20, and 200 $\mu g \cdot L^{-1}$ of TBCO demonstrated a significant decrease in MIH-stimulated maturation of oocytes. To assess whether disruption of MIH-stimulated oocyte maturation causes impaired fecundity, sexually mature fish were fed either a control diet, or a diet containing 100 $\mu g \cdot g^{-1}$ or 1000 $\mu g \cdot g^{-1}$ TBCO for 21 days, and fecundity and *ex vivo* oocyte maturation were assessed. Fecundity was significantly decreased by 50 and 55% in the low and high treatments. MIH-stimulated maturation of oocytes was not decreased in fish given the low diet but was

decreased by 44% in fish fed the 1000 $\mu g \cdot g^{-1}$ diet. Results suggest that inhibition of MIH-stimulated oocyte maturation contributes to decreased fecundity of Japanese medaka exposed to TBCO.

Can short-term data accurately represent long-term environmental exposures? Investigating the potential multigenerational adaptation of *Daphnia magna* to organic sunscreen ultraviolet filters (PL)

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Organic ultraviolet filters (UVFs) such as avobenzone, octocrylene, and oxybenzone are widely used in common sunscreens and other personal care products to protect against harmful ultraviolet radiation. Their use in sunscreens leads to widespread environmental contamination due to the leaching of these chemicals during recreational activities, posing a threat to many aquatic systems. Current research indicates that aquatic invertebrates are particularly sensitive to UVF contamination; however, data regarding the long-term outcomes of UVF toxicity at environmental concentrations is lacking. This study sought to model the long-term effects of chronic UVF exposure to the freshwater invertebrate, *Daphnia magna* over five generations of continuous exposure. Initial generations of Daphnia showed physiological impairment through both a 30% decreased reproductive output and a 10-fold greater proportion of non-viable offspring. In addition, 50% mortality was observed in the first two generations of oxybenzone exposures; however surviving daphnids proved capable of acclimating to long-term UVF exposure, ultimately returning to the control physiological state by the F4 generation. These physiological outcomes are further supplemented with non-target proteomic analysis to better characterize the changes that occur in these Daphnia populations across generations, shedding light on the possible mechanisms driving the observed long-term acclimation to the continued presence of UVF contamination. This data suggests that Daphnia are capable of overcoming sublethal concentrations of UVFs, indicating that perhaps current estimates of toxicity through short-term exposures are an overestimation of long-term toxicity in an environmental setting.

Effects of erythromycin on juvenile rainbow trout microbiome and fitness (VPL)

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Antibiotics are continuously discharged via wastewater effluent in urban environments including erythromycin, a commonly used broad-spectrum macrolide antibiotic in human medicine. Discharged antibiotics could potentially result in negative effects on ecosystem health, including the condition and health of exposed fishes. Gut microbiomes of fish serve central roles in the overall condition of hosts, and gut microbiome dysbiosis could have important implications for health of host fishes. A 7-day dietary exposure of erythromycin followed by a 7-day depuration period was conducted to understand the acute effects of erythromycin on juvenile rainbow trout gut microbiome and host health. Three levels of exposure (0.1, 10, and 1000 $\mu g \cdot g^{-1}$) and four collection time points (4-, 7-, 11-, and 14-day) were utilized for the study to investigate responses of juvenile rainbow trout using 16S rDNA amplicon sequencing of gut microbiome and mRNA-seq of host gut tissue. Bile samples were analyzed for changes in chemical concentrations to understand uptake and depuration of the antibiotic, with highest treatment having detectable levels of parent compound with levels dropping quickly at 11 days. Erythromycin was observed to decrease evenness of microbes during treatment followed by recovery at 11 days. Gut microbiome community composition of exposed fish also shifted in response to erythromycin treatment. mRNA-seq was anticipated to provide changes in gene expression leading to altered pathways using gene enrichment analyses. Results of this study provide insights into potential effects of changes in microbiome communities and impacts on the intestinal tissue due to antibiotic exposure, leading to altered fitness.

Effects of winter cold on acute copper bioaccumulation and toxicity in brook char (*Salvelinus fontinalis*) (PL)

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Disturbance of sodium (Na^+) regulation has been reported in freshwater fish acutely exposed to copper (Cu) concentrations above natural background levels. In addition, long-term acclimation of freshwater fish to near-freezing temperatures encountered during the winter has been shown to result in a decrease of plasma Na^+ and chloride (Cl^-) levels, suggestiing impaired ionic regulation. In addition, fish during winter month may have depleted energy reserves, making them more sensitive to contaminants. Yet, little is known regarding metal toxicity in winter acclimated fish. Thus, our research investigates cold temperature effects on acute Cu bioaccumulation and toxicity to brook char (*Salvelinus fontinalis*). Following long-term acclimation to two temperatures (3 and 14°C), we

determined gill-Cu bioaccumulation, net fluxes of Cl- and ammonia (NH_3), and net and unidirectional fluxes of Na^+ using a radioisotope (^{22}Na). Preliminary results show that Cu causes an inhibition of NH_3 and a net loss of Na^+ and Cl in brook char. Temperature affected baseline Na^+ and NH_3 regulation, as well as Cu toxicity in the fish, with warm acclimated fish being generally more affected by Cu than the cold-acclimated fish. This project will contribute to our understanding of how cold temperatures may exacerbate the effects of acute Cu toxicity and the potential risks for freshwater fish living in temperate waters.

Rearing temperature imparts cardiometabolic disturbances in embryonic yellow perch (PL)

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Rising temperatures in the Great Lakes region has led to concerted efforts to elucidate potential impacts to aquatic life. The yellow perch (*Perca flavenscens*) is of great cultural, economic, and ecological importance to the Great Lakes. Yellow perch breed a large ribbon of eggs once per year following ice break up. The temperature experienced by fish during embryogenesis is a critical factor controlling the development of the cardiac system and metabolism, and the yellow perch may be susceptible to increases in temperature imposed by industrial practices and climate change. With this backdrop, we tested the hypothesis that changes in temperature would lead to cardiometabolic changes in embryonic yellow perch. Yellow perch were reared at 12, 15 (optimal), and 18°C during embryogenesis, and with an ambient seasonal exposure (12 to 18°C; +1°C per 3 days) to mimic natural environmental incubation temperatures. Measures of heart rate and respiration were taken at three developmental stages (onset of heart rate, eye pigmentation, and hatch). Higher temperatures appear to lead to a stronger rhythm following the onset of heart rate; 15°C and 18°C groups had significantly increased heart rates compared to 12°C and seasonal groups. At eye pigmentation and hatch stages, fish reared at 18°C have the fastest heart rate, followed by seasonal and 15°C reared fish, and 12°C fish exhibiting the slowest heart rates. Respiration was recorded in individual larva and in ribbon, with the results suggesting alterations driven by rearing temperature. A Seahorse Bioanalyzer was used to discern possible pathways driving changes in metabolism. Rearing temperature possibly influences ATP production and the proton leak in the yellow perch. This study demonstrates that the cardiac performance and metabolism in embryonic yellow perch are altered by rearing temperature. Future work will attempt to specifically identify the mechanisms governing these changes.

Interspecies variation in sensitivity to activation of the aryl hydrocarbon receptor by polycyclic aromatic hydrocarbons (PL)

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Polycyclic aromatic hydrocarbons (PAHs) are naturally occurring chemicals that are ubiquitous in the environment, but alkyl PAHs are more abundant in certain environmental matrices. Some PAHs can exert toxicity to fishes by activation of the aryl hydrocarbon receptor (AhR). Although there is evidence that alkylation could increase potency of PAHs, these effects are not well characterized. Additionally, most research has focused on a small number of species, which likely does not capture interspecies variation in sensitivity. However, toxicity testing across numerous species is not practical due to limitations in time, cost, and challenges associated with certain species. As a step towards characterizing how alkylation affects potency of PAHs, zebrafish (*Danio rerio*) embryos were exposed to benz[a]anthracene (BAA) and three alkyl homologues via microinjection to assess lethality. Alkylation had position-dependent effects on potency. The most potent alkyl PAH, 8-methylbenz[a]anthracene, was 5.6-fold more potent than BAA, 7,12-dimethylbenz[a]anthracene was 1.4-fold more potent, and 4-methylbenz[a]anthracene was equally potent. Next, the potency for activation of the zebrafish AhR2 was quantified using a standardized *in vitro* AhR transactivation assay. These assays yielded relative potencies similar to those observed *in vivo*. Assay results from four other species showed the EC_{50} range exceeded 300-fold across all tested species-chemical combinations, with brook trout (*Salvelinus fontinalis*) being the most sensitive, and northern pike (*Esox lucius*) being the least sensitive. Due to the abundance of PAHs in the environment and the number of species to study, there is an urgent need to develop predictive tools to assess these toxicities more objectively.

Inflammatory response of gills following diurnal temperature fluctuations and antidepressant exposure in zebrafish (PL)

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Aquatic environments are continuously subject to anthropogenic stressors ultimately impacting aquatic wildlife. In particular, surrounding water temperatures are an indicator of ectothermic body temperature, and fish living within aquatic systems are in constant contact with environmentally relevant stressors. The impacts of contaminant exposure have become a particular area of interest in addressing how waterborne contaminants impact fish physiology. This study aims to address how contaminants from wastewater treatment plant (WWTP) effluent coupled with diurnal temperature cycling impact

laboratory zebrafish gill immune responses. Mixed-sex zebrafish were exposed to daily cycling temperature fluctuations from 27 to 37°C and an environmentally relevant concentration of the commonly prescribed antidepressant venlafaxine (VFX) (1 $\mu g \cdot L^{-1}$) for 96 hours. At 96 hours the fish were euthanized, and gill samples were collected. Key innate immune system and inflammatory transcript expression were assessed, and we determined that in the presence of VFX some immune and metabolic transcripts were differentially regulated. Presently, this study demonstrates that the combined effects of VFX and diurnal temperature fluctuations may modulate key immune and metabolic parameters within the gills of zebrafish. Understanding how diurnal temperatures impact fish will be indicative of how fish respond to daily thermal cycles in the presence of multiple stressors. Moreover, the investigation of the dual impacts of contaminant exposure and diurnal temperature changes represents a new direction in understanding fish

Soil ecotoxicology studies in support of site-specific soil remediation guidelines for hydrocarbon-impacted boreal soils (PL)

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A series of soil toxicity tests was conducted in support of a detailed risk assessment (DRA) in northeastern British Columbia. The toxicity testing investigated the light extractable petroleum hydrocarbon (LEPH) and volatile petroleum hydrocarbon (VPH) concentration effects on plants and invertebrates, using Environment Canada's Test Method for Terrestrial Organisms. Test organisms included seven plant species (*Populus tremuloides*, *Calamagrostis canadensis*, *Solidago canadensis*, *Betula papyrifera*, *Pinus banksiana*, *Picea glauca*, and *Picea mariana*), and two soil invertebrate species (*Eisenia andrei* and *Proisotoma minuta*). Plants and invertebrates were tested in natural background and impacted site soils, in artificial soils, and in natural background soils spiked by boric acid solution. Although clayish soil texture did not affect the springtail reproduction, it posed challenges for earthworm reproduction. To avoid losing the moisture within plant tests, and mimic natural soil horizons, a thin garden soil layer was added to each plant vessel. Substantial plant growth was observed in the hydrocarbon-impacted soils. Heterotrophic plate counts in the end of the test showed the highest bacterial number among test vessels with the greatest LEPH and VPH concentrations. Four impacted soils and one natural background soil were placed in the test containers with and without *P. tremuloides* seeds. Substantial decrease in LEPH and HEPH concentrations was observed in majority of sub-samples from seeded and non-seeded vessels. To investigate the potential combined effect of organic carbon, bacteria, and plants on the soil toxicity for biota, the soil microcosm testing (soil, plants, and springtails) was suggested.

Role of exposure and toxicity modifying factors in water quality benchmark derivations: A case study of nitrite toxicity (PL)

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Nitrite belongs to a class of compounds in which aquatic toxicity is affected by one or more exposure and toxicity modifying factors (ETMFs). The toxicity-modifying effect of chloride on nitrite toxicity to fish is well-studied. It is also likely that pH and fish size are important ETMFs for nitrite. Current protocols for the derivation of water quality guidelines by the Canadian Council of Ministers of the Environment (CCME) and United States Environmental Protection Agency (US EPA) support incorporation of ETMFs in guideline derivations. In this evaluation we used nitrite toxicity to aquatic organisms as a case study to demonstrate ETMF-driven benchmark derivation. The first part of the evaluation assessed the relationship between toxicity and ETMFs among species, approaches for incorporating ETMFs into species sensitivity distributions (SSDs), and how SSDs change with ETMF influence. The second part of the evaluation assessed the applicability of acute toxicity driven derivations (i.e., US EPA protocols) versus chronic toxicity driven derivations (i.e., CCME protocols). The acute toxicity of nitrite in fish is well studied, but relatively few acute toxicity data exist for other taxa, and very few chronic toxicity data exist for any species. Given the nature of the data for nitrite, the US EPA protocols are complementary to Canadian procedures used to derive benchmarks. Acute-to-chronic ratios and other extrapolation methods can be used to estimate SSDs from limited data and characterize the effect of ETMFs on those SSDs. This approach may be applicable to other compounds with ETMFs and data constraints similar to nitrite.

Understanding among-lake variability in fish mercury levels in lakes in the Dehcho region, Northwest Territories (VPL)

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In 2012, after 'A Return to Country Food' workshop was held in the Dehcho region of the Northwest Territories, communities approached researchers with several questions regarding fish mercury levels. People wanted to know why fish mercury levels were high enough in some lakes to warrant site-specific consumption advisories, whereas in nearby lakes the fish mercury levels were quite low. Since 2013, we have worked with Dehcho Guardians to sample 11 lakes for fish, water, sediment, benthic invertebrates, and zooplankton during annual on-the-land camps. Water and sediment chemistry have been evaluated, catchments have been characterized, food web structure has been assessed, and

biota have been analyzed for mercury and covariates of mercury. Piecewise structural equation modeling results reveal complex biogeochemical and ecological interactions – ranging in scale from individual organisms to whole ecosystems – that explain >80% of among-lake variability in fish mercury levels. The best predictors of fish mercury levels are fish growth rate and mercury levels at the base of the food chain, which are in turn driven by concentrations of dissolved organic carbon concentrations in water and mercury levels in sediment; and ultimately by catchment characteristics, such as type of forest cover and catchment slope. Our findings reveal which types of lakes and catchments may be most susceptible to stressor-induced changes in fish mercury levels, and the safest, healthiest sources of subsistence fish for local First Nation communities. Fish mercury data have also been used, through collaborations with public health scientists, to inform probabilistic risk models for human health.

Different mechanisms of chlorine toxicity and recovery in rainbow trout exposed to either hypochlorous acid or monochloramine (PO)

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Total residual chlorine (TRC) in the form of either added hypochlorous acid (bleach) or the longer-lasting chloramine has been used as a disinfecting agent in drinking water for decades. TRC is considered a special toxicant of concern whereby even acute, 15-minute exposures can cause death to fish. The determination of the underlying mechanisms involved in toxicity and the potential for recovery from either chlorine or chloramine toxicity at sub-acute doses is severely limited. We investigated the rate of methemoglobin MetHb formation and the rate of recovery effect from exposure to either hypochlorous acid or chloramine exposures (0.5 $mg{\cdot}L^{-1}$ and 1.0 $mg{\cdot}L^{-1}$). There was a significant difference in the mechanism of toxicity between hypochlorous acid and chloramine with MetHb formation being significantly faster and much greater in chloramine exposed fish when compared to hypochlorous acid exposed fish, while hypochlorous acid exposed fish had higher rates of lipid peroxidation as measured by TBARS formation, suggesting that gill damage from oxidative stress is the primary mechanism of toxicity. Swim performance was also measured at 3 hours post-exposure in trout exposed to 30 minutes of either of 0.5 or 1.0 $mg{\cdot}L^{-1}$ chloramine. These trout had a >50% reduction in both oxygen consumption and aerobic scope and a reduced swim performance despite a significant 60% recovery in blood MetHb by this time. These results suggest different mechanisms of action that could impact recovery from either hypochlorous acid or chloramine exposures.

Zebrafish models as an integrated approach to testing and assessment (IATA) while reducing animal use in chemical risk assessment (CRA) (VPO)

Lee Ellis[1], John Achenbach[1], Michael Morash[1], Cindy Woodland[2], Roberta Moore[2], Justin Lo[2]

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Zebrafish (ZF) embryo and larval models are being developed to evaluate for endocrine disruption and as an alternative to the 28-day rodent assay in chemical risk assessment. These models have potential application in both human health and environmental risk assessment and thus, supports initiatives to reduce animal use in toxicity testing. The incorporation of behavioral and transcriptome changes as toxicity biomarkers, along with absorption and metabolism assessments, have been key to this research. These ZF models were designed by the NRC Canada, and are based on the OECD fish embryo toxicity assay (TG 236), and include the NRC general and behavioural larval toxicity assay. Through a tiered integrated approach to testing and assessment (IATA), data generated in the first tier, e.g., gene expression, can be used to select the most informative line of follow-up testing with cell-based assays in higher tiers. In order to further develop zebrafish testing for the purpose of environmental assessment, testing must be conducted beyond the larval stages. The model is currently being expanded as a full life cycle model by including juvenile and adult stages such that evaluation of chronic population-level effects to environmentally relevant chemical concentrations can be evaluated. Additional relevant endpoints for environmental effects include behaviour along with changes in fecundity and fertility. The ZF model has proven to be a valuable platform for toxicity testing and is promising to be a fundamental part of IATAs for both environmental and human health risk assessment.

Mining and the Environment

Integrating health and well being into western risk assessment at mining properties (PL)

Harriet Phillips[1], Stacey Fernandes[1], Ken Froese[1], Ame-Lia Tamburrini[2]

[1]Canada North Environmental Services, [2]Hum Consulting

Mining activities have restricted the ability for Indigenous communities to participate in ceremony, to continue the traditional practices of learning about the land and caring for it, and this has affected physical, mental, spiritual, and emotional wellbeing. The reclamation of mine properties presents an opportunity to heal the land and in doing so help to restore health and wellness of the individuals, families, and communities. Western risk assessments are used for operations and reclamation at mining properties in order to fulfil regulatory requirements. However, in trying to address the concerns of Indigenous communities, western risk assessments need to broaden from the narrow approach to health to be more comprehensive and not just shoe-horn aspects of Traditional Knowledge into the regulatory risk assessment framework. Indigenous communities need to be engaged in the evaluation of impacts from mining projects so that they understand the issues and any remediation undertaken should occur in a manner that restores relationships with the land through culturally-based approaches. This presentation will discuss the limitations of the western science approach and provides some suggestions for the consideration of health in the risk assessment framework in a more holistic manner.

Eastern Athabasca Regional Monitoring Program 10-year summary (PL)

Mitchell Thorarinson[1], Kelly Wells[1], Cassandra Rees[1]

[1] Canada North Environmental Services

The Athabasca region of northern Saskatchewan is home to several Aboriginal communities and uranium mining and milling operations. During the past 25 years, there has been extensive community involvement in environmental sampling programs in the region. A key aspect of any successful community monitoring program is that sampling locations and media are selected based on their importance to the communities and the sampling is completed by, or with, local residents. The Eastern Athabasca Regional Monitoring Program (EARMP) is a collaborative industry-government-community partnership, long-term environmental monitoring program established in 2011 under the province of Saskatchewan's Boreal Watershed Initiative. The community portion of the program tests the safety of traditionally harvested foods by collecting and chemically testing water, fish, berry, avian, and mammal chemistry from seven communities located in the Athabasca region. It relies heavily on the participation of community members for

determining sampling locations and the collection of samples. The study design for the EARMP community program remains relatively consistent over time to collect a long-term dataset; however, the program is also adaptive and is refined in response to new information or changes associated with development in the region, community concerns, or community results. A large component of the EARMP is communication with the communities and in 2022 a plain language report is being prepared that will summarize the first 10 years of the project. This presentation will discuss the EARMP and summarize results from the past 10 years of the program.

Net ecosystem production of designed and opportunistic wetlands in reclaimed oil sands post-mining landscapes (PL)

Jan Ciborowski[1], Courtney Smith[1], Michael Wendlandt[1], Ashlee Mombourquette[1], Elizabeth Gillis[1]

[1]*University of Calgary*

Northeastern Alberta is dominated by a mosaic of wetlands and peatlands, reflecting hydrology, topography and successional processes operating since the last glaciation. Mining companies have created reclaimed watersheds harbouring seemingly productive and biodiverse wetlands. However, methods of assessing the effectiveness or 'functionality' of wetland reclamation are lacking especially during early succession. A candidate measure is net ecosystem production – the difference between gross primary production (GPP) and net ecosystem respiration (NER), some of which represents net carbon accrual. We placed data loggers in 40 wetlands to monitor temperature, photoperiod, dissolved oxygen (DO) concentration, specific conductance, and water depth in late summer 2021 to estimate underwater net ecosystem production (NEP) relative to wetland age, conductivity, and volume (before vs after a major mid-record recharge event). Loggers placed 15–30 m above the substrate in 40–75 cm of water recorded information at 30-minute intervals. Daily net primary production and respiration were inferred from day-night variation in DO. The first half of the recording period - the driest observed in the previous 20 years was followed by heavy rains that largely replenished wetlands. Young opportunistic wetlands appearing on reclaimed landscapes were significantly more biologically active than equivalent reference wetland counterparts, showing much higher GPP and NER than wetlands forming on less perturbed landscapes. NEP, GPP and respiration all declined in magnitude with increasing water salinity in reclaimed landscapes.

Characterizing hydrological and landscape features sustaining opportunistic boreal wetlands across a chronosequence using isotopologues of water and radon (PL)

Michael Wendlandt[1], Jean Birks[1], Nika Tovchyhrechko[1], Ashlee Mombourquette[1], Brenten Vercruysse[1], Elizabeth Gillis[1], Courtney Smith[1], Megan Mercia[1], Liam Mebesius[1], Ian Perry[1], Jan Ciborowski[1]

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Resource extraction and development in the Athabasca oil sands region (AOSR) has transformed the landscape and its local hydrology. Opportunistic wetlands (OW) form within newly created or altered landscapes where hydrological sources have been disrupted by natural events or local disturbance. Low relief landscapes found within the AOSR make it challenging to use traditional hydrological water budget methods. Alternatively, stable isotopologues of water and the presence of radon can be used to indirectly infer the water budget and its sources. In 2021, 40 stratified-randomly selected OW on reclaimed landscapes at Syncrude's Mildred Lake lease, and from selected reference OW in natural areas were visited; collecting surface and groundwater, soil properties, vegetation, bathymetry and local topography. The wetlands comprised a chronosequence (time since disturbance/formation) and gradient of salinity among opportunistic and constructed wetlands. A $\delta D/\delta^{18}O$ biplot of OW surface water samples revealed a wide range of water balances – spanning a continuum from wetlands with constant resident sources of water (permanent hydroperiod), and those that are heavily evaporative-enriched (ephemeral). Young wetlands seemed to dominate the extremes, with those on reclaimed landscapes exhibiting more ephemeral characteristics, consistent with predictions. The data from this study will aid in identifying landscape features that promote OWs with sufficient hydrological sources to maintain more permanent OW, compared to those that result in water balances parameters likely to transition to upland features. This information is needed to identify reclamation landscape practices that will create more hydrologically stable OW capable of continuous carbon sequestration after mine closure.

Effects of landscape age and salinity on plant community composition and productivity in ppportunistic and constructed wetlands in the Athabasca oil sands region, Alberta (PL)

Ashlee Mombourquette[1], Elizabeth Gillis[1], Michael Wendlandt[1], Jan Ciborowski[1]

[1] *University of Calgary*

Wetlands comprise up to 60% of the Athabasca oil sands region (AOSR) surface mineable area and thus support diverse flora (approximately 400 species). Increased anthropogenic land disturbance activities such as bitumen extraction will be accompanied

by postmining reclamation. Areas reclaimed using sodic overburden tend to be sodium-enriched compared to pre-disturbance landscapes. Up to 20% of reclaimed areas are becoming sites of opportunistic wetland (OW) formation. Thus, understanding how plant community composition and productivity in OW will change with age and salinity will be critical to predicting landscape reclamation success. In 2021, we sampled vegetation, soil and water properties, and hydrology at 40 stratified-randomly selected OW on postmining reclaimed landscapes and from selected reference OW in natural areas in the AOSR. The wetlands comprised a chronosequence (time since disturbance/ formation) and gradient of salinity among opportunistic and constructed wetlands. Preliminary surveys identified community composition and the six species most prevalent in wet meadow and emergent vegetation zones. We then contrasted variation in above ground biomass of these species in opportunistic and constructed wetlands of equivalent age and salinity. Biomass of dominant species appear to increase with age, whereas community composition over time shows no clear pattern of change. Soil salinity had no effect on biomass as expected because plants grow within their zone of tolerance. Preliminary results suggest that species present and their growth rates (productivity) are not influenced by the salinity of soils in the landscape.

Development of field ready assay for detection of low concentrations of toxic poly-acrylamide polymers in settling ponds (PL)

Greg Goss[1], Edyta Jasinska[1,2], Jesse Meints[2], Jerry Hannah[2]

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Coagulation–flocculation processes are among the most widely employed techniques to remove fine particulate matter from turbid waters. Adding positively charged polyelectrolyte polymers facilitates the settling and removal of negatively charged suspended particles (e.g., clays). The difficulty in measuring polymer concentrations in the field commonly results in dosing inaccuracy and the potential for excessive release of unbound toxic polymer. Cationic polyelectrolytes, at concentrations of >200 $\mu g \cdot L^{-1}$, pose a threat to fish in receiving waters because they bind to gills, hindering efficient oxygen transfer. The Clearflow Group has developed a proprietary reagent (CN369) that, when mixed in appropriate ratios with cationic polymer treated water, neutralizes the cationic polymer toxicity. A key issue is refining dosing protocols of the CN369 reagent and measurement of low concentrations of cationic polyelectrolytes. We have developed a straightforward, cost-effective colorimetric method for detection of cationic polyelectrolytes in real time at the field site. The polymer interacts with the test kit reagent and induces a distinctive colour change that can be quantified, thereby allowing for proper application of CN369. The colour change occurs at a low concentration of cationic polymer, as low as 100 and 150 $\mu g \cdot L^{-1}$ depending other water quality parameters. Therefore, cationic polymers can be detected in waters at the field site using simple visual inspection of this colour change. Important advances include the development of procedures to mitigate the

impact of dissolved organic matter and competing metal cations (e.g., Ca^{2+} and Mg^{2+}) that normally complicate cationic polymer detection.

Benthic invertebrate monitoring at the Con Mine legacy site: Seven phases of environmental effects monitoring (EEM) through changing climate conditions (PL)

Elaine Irving[1], Bryce Pippy[1], Hilary Machtans[1], Dwight Grabke[2]

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The Con Mine, which operated from 1938 to 2004, is now in post-closure, and WSP Golder works with Miramar Northern Mining Limited in a consulting capacity to support legacy operations, closure activities, and to meet compliance and regulatory monitoring requirements. The mine-site is located within the city limits of Yellowknife, Northwest Territories close to Great Slave Lake. To date, seven environmental effects monitoring (EEM) cycles were completed between 2003 and 2021, most recently with an Investigation of Cause study (2018), supplementary monitoring in 2019, and integration with the Aquatic Effects Monitoring Program in 2021. Monitoring of fish and invertebrates has focused on Jackfish Bay, which receives seasonal treated water from the mine-site, subject to water management and closure activities. The long-term benthic dataset includes community data collected from study areas within Jackfish Bay and a reference bay. Supporting sediment quality/toxicity, water quality, and habitat data were collected during the benthic surveys. Monitoring occurred through both extreme drought and wet conditions, with 2021 recorded as a record high water level year. Collectively, the multi-cycle dataset and supporting and regional data provided an opportunity to evaluate not just the potential influence of the mine effluent on benthic communities, but also local habitat conditions and regional climate factors, including pre-drought, drought, and post-drought conditions. We conclude that the pattern of invertebrate response has most likely been influenced by both effluent-related contaminants and climate-related factors. Northern regions are increasingly subject to an unpredictable climate which must be considered in addition to mine-related effects.

The relationship between cellular protein content and selenium accumulation in freshwater micro-algae (PL)

Courtney Bogstie[1], Melanie Gallant[2], Christopher Kennedy[1], James Elphick[2]

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In algal cells, an apparent threshold ceiling for selenium incorporation into amino acids suggests that protein content may determine the amount of selenium taken up or accumulated in these organisms, at the base of the food web. This directly impacts

bioaccumulation and subsequent toxicity observed in higher trophic-level organisms. In this research, the protein content of four different Chlorophyta species (*Parachlorella kessleri*, *Chlorella vulgaris*, *Raphidocelis subcapitata*, and *Tetradesmus obliquus*) was determined during the different phases of algal growth (lag, exponential, and stationary) and for *R. subcapitata* grown under different lighting intensities. Results from tissue analysis for selenium and protein following exposure showed no correlation between protein and selenium accumulation in the exponential phase, but a strong correlation in the stationary phase for *P. kessleri*, *C. vulgaris*, and *R. subcapitata*, as well as under different lighting intensities for *R. subcapitata*. These results suggest that considering protein content in site-specific primary producers is important in predicting ecological risk to higher trophic levels.

Using the periphyton-macroinvertebrate food chain to predict selenium body-burdens in freshwater fish: Do sampling methods matter? (PL)

Maira Peixoto Mendes[1], Beatriz Cupe-Flores[1], Karsten Liber[1]

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Selenium (Se) is an essential micronutrient known to bioaccumulate in aquatic food webs. Despite extensive research, Se bioaccumulation modelling still poses a challenge to Se risk assessment. This study aimed to predict Se body burdens in four fish species (northern pike, white sucker, nine-spine stickleback, and lake whitefish) inhabiting boreal lakes (Vulture Lake and McClean Lake) downstream from a Saskatchewan uranium mill. We investigated the effects of dietary sampling methods (artificial substrates vs grab samples) and seasons (summer vs winter) on the predicted Se concentrations in fish tissue (muscle and ovaries). In summer 2019, periphyton and benthic macroinvertebrates (BMI) were sampled using artificial substrates (n=4) and sediment grab samples (n=3) at two sampling stations in Vulture Lake, seven sampling stations in McClean Lake east basin and one reference site on the west basin. In winter 2021, the samples were collected through ice holes (n=3) using grab samples (n=4) at McClean Lake only. Invertebrates were sorted into the lowest taxonomic level, and trophic transfer factors (TTF) were calculated for each taxon based on dietary Se concentrations. At high effluent exposure sites (Vulture Lake), Se body burdens calculated using grab samples data, but not artificial substrates were comparable to measured Se concentrations in northern pike. At McClean Lake, predicted Se concentrations in northern pike, lake whitefish, and white sucker were overall similar to measured Se concentrations in these species. The lack of available information for stickleback (TTF and CFs) and associated measured Se concentrations, increased estimation uncertainty for this species.

Understanding the new *Metal and Diamond Mining Effluent Regulations* (MDMER) *Acartia tonsa* test method (PL)

Lisa Rozon-Ramilo[1], Colleen Prather[1], Jamie Quesnel[1], Thomas Genty[1]

[1]*Agnico Eagle Mines Limited*

An amendment to the *Metal and Diamond Mining Effluent Regulations* (MDMER) in June, 2021 resulted in the addition of a new invertebrate acute-lethality test method for effluent discharged to marine receiving environments. The *Acartia tonsa* test method was published in June, 2019 and came into force under the MDMER on December 1, 2021. Mine owners and operators in Canada discharging to the marine environment are required to comply with this new test method for effluents with salinities greater than 4 parts per thousand (ppt). The lack of baseline *A. tonsa* toxicity data available in the primary literature on real mine effluents; limited laboratory capacity to develop, acclimate, and manage multiple salinity cultures; limited number of laboratory staff trained in handling the species and performing the test; limited testing dates available to handle both regulatory testing and research, and limited commercial laboratories to run the test (due to lack of economic incentive), has made working with this new test species extremely challenging. Since 2020, Agnico Eagle Mines Limited has been working with several laboratories and consultants to gain a better understanding of this new test species. The present study demonstrates the lack of understanding of how *A. tonsa* reacts in the absence of effluent when basic things like pH, oxygen, temperature, and salinity are altered. The study team sees this as a key priority and first step to being able to effectively manage and treat the mine effluent while protecting the marine environment.

Using otolith and fin ray microchemistry to delineate temporal and spatial metal exposure (PL)

Jennie Christensen[1], Geriene LaBine[1]

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Otoliths and fin rays are calcified tissues found in fish that can grow throughout a fish's life span. Through micro-chemistry analysis, typically through laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), these tissues can provide a plethora of information about that fish. While otoliths are collected lethally from fish, fin rays can provide an effective non-lethal alternative for obtaining the same information. Here we show the different types of information you can collect from a fish otolith or fin ray by using different elements as indicators. For example, zinc can be used to determine the age of a fish. Barium and strontium can be used to ascertain the migratory history of a fish, even in a completely freshwater system. Metals, such as selenium, lead, or manganese can be used to determine the changes in exposure, and thus risk, over time. Pulling all the information together, through a variety of elements, a whole story can appear that reveals

not only the temporal, but also spatial, aspects to metal exposure in a fish. This can become a valuable too for identification, mitigation, and management of metals in an aquatic environment, and with the use of fin rays can work with a non-lethal advantage over otoliths.

Analysis of selenium in fish tissue: An interlaboratory study on weight constraints (PL)

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Fish tissue monitoring for selenium has unique sampling and analytical challenges that differ from monitoring for other constituents. Specifically, when compared to mercury sampling programs, selenium monitoring programs differ in the type of tissue analyzed (including egg/ovary), relevant concentration units (dry weight for selenium), and the types of fish species targeted (resident fish sensitive to selenium with limited home ranges). Similar to mercury monitoring programs, there is a growing emphasis on non-lethal sampling. These characteristics of selenium monitoring programs result in analytical challenges such as accurately quantifying selenium concentrations in tissues with varying lipid content (whole body, dorsal muscle, egg-ovary), meeting target detection limits, determining accurate dry weight concentrations, and analyzing small samples from small-bodied fish and non-lethal sampling. The objective of this study was to stress-test various conventional analytical techniques used by commercial laboratories in terms of their ability to maintain data quality objectives for selenium analysis in the face of sample weight constraints for ovary and dorsal muscle tissue. Four laboratories analyzed blind a suite of identical samples, and data were compared against *a priori* data quality objectives for accuracy, precision, and sensitivity. Data quality tended to decrease with decreasing sample weight, particularly when samples were less than the minimum weights requested by the participating laboratories; however, effects of sample weight on data quality were not consistent between laboratories or tissue types. This study has implications for fish tissue sampling programs that sample selenium, analyze egg/ovaries, and generate low-weight tissue samples.

Mining Association of Canada guidance on conducting studies of selenium in fish tissue under the *Metal and Diamond Mining Effluent Regulations* (PO)

Charles Dumaresq[1], Alexandra Duguay[2], Allison Merla[3], Charlene Burnett-Seidel[4], Helga Sonnenberg[5], Kelly Wells[6], Marko Adzic[7], Mary Murdoch[8], Rainie Sharpe[9], Sean Miller[10]

[1] Mining Association of Canada, [2]Rio Tinto, [3]Vale, [4]Cameco, [5]EcoReg Solutions, [6]Canada North Environmental Services, [7]Teck Resources, [8]Stantec Consulting Ltd, [9]Golder Associates Ltd., a member of WSP, [10]Bureau Veritas

The 2018 *Metal and Diamond Mining Effluents Regulations* (MDMER) under the *Fisheries Act* introduced requirements to conduct studies of selenium in fish tissue (muscle or whole body and ovaries or eggs, if practicable) if selenium concentrations in effluent exceed thresholds defined in the MDMER. The Mining Association of Canada has developed a guidance document to support mining companies conducting such studies. This guidance is intended to inform the collection of high-quality data in a consistent manner and is to be used in conjunction with Environment Canada's 2012 Metal Mining Technical Guidance for Environmental Effects Monitoring. It takes into account MDMER requirements for environmental effects monitoring (EEM) and best practices for monitoring selenium in fish tissue. This guidance addresses: MDMER-specific considerations, including integration of studies of selenium in fish tissue with other EEM requirements, particularly fish population studies; and technical considerations such as selection of species and tissues for analysis, collection of tissue samples, sample preparation and chemical analysis, and data analysis and reporting. The guidance advocates that, to the extent practicable, sampling of fish tissue for selenium analysis should be aligned with the conduct of EEM fish population studies, including the timing of sample collection and the species/individual fish collected. This approach will avoid further mortality to fish populations.

The comparative sensitivity of the acute *Americamysis bahia* and *Acartia tonsa* test methods to nickel and copper in seawater (PO)

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With the recent December 2021 amendments to the *Metal and Diamond Mining Effluent Regulations* (MDMER), there has been an increased interest in the newly developed acute lethality test method using the marine invertebrate *Acartia tonsa* (STB 1/RM/60). This method has been brought into force as a mandatory test for saline effluents released into marine environments, which are subject to scrutiny under the MDMER, Previously, investigators assessing acute toxicity of marine effluents to their receiving waters may have used *Americamysis bahia* as their model marine invertebrate, employing test methods described in the US EPA's "Methods for Measuring The Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (EPA-821-R-02-012). This has prompted a need to better understand the comparative sensitivities of the two test

methods. Over the course of 4 months, a series of side-side *A. bahia* and *A. tonsa* exposures were initiated under standard conditions using serially diluted natural seawater spiked with reagent grade nickel chloride and copper sulfate. LC_{50} data was derived for each test based on measured values of dissolved Cu^{2+} and Ni^{2+} in each test concentration.

Effect of technology critical elements Lanthanum (La), Neodymium (Nd), Praseodymium (Pr) and Yttrium (Y), individually and in mixtures to *Daphnia magna* (PO)

Celine Do[1], Scott Smith[1], James McGeer[1]

[1]*Wilfrid Laurier University*

Lanthanum (La), Neodymium (Nd), Praseodymium (Pr), and Yttrium (Y) are rare earth elements (REEs) that are increasingly being used in modern technologies and as such, there is increasing concern for potential environmental impacts associated with anthropogenic contamination. Canada's first REE mine began operation in 2021. There is very little data available for individual REEs, a lack of water quality guidelines, and also a lack of knowledge concerning mixtures. The latter is particularly relevant given REEs generally occur as mixtures. The objective of my study was to investigate the acute effects of La, Nd, Pr, and Y as single metals and as mixtures. Standard 48-hour acute tests with single metals, following Environment and Climate Change Canada methods were conducted with *Daphnia magna* neonates in an artificial soft water medium with a hardness of 50 mg $CaCO_3 \cdot L^{-1}$ and a pH of 6.8 with no added bicarbonate to determine the EC_{50} values. For example, the 48-hour EC_{50} for La was 0.52mg$\cdot L^{-1}$ (95% CI 0.41–0.65 mg$\cdot L^{-1}$), for Y was 0.54 (0.48–0.62) mg$\cdot L^{-1}$, and for Nd was 0.32 (0.03–0.56) mg$\cdot L^{-1}$. Mixture exposures were designed using a toxic unit (TU) approach, based on converting the EC_{50} concentrations to toxic units and applying a matrix isobologram approach. For example, TU combinations of 0.25, 0.5, and 0.75 were tested and hypothesized to follow an additive response. This research is supported via a NSERC Strategic Grant with funding from Environment and Climate Change Canada.

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

Sunny Choi[1], Chad Cuss[1,2], Chris Glover[3,4], Greg Goss[4], William Shotyk[1]

[1]*University of Alberta,* [2]*Memorial University,* [3]*Athabasca University,* [4]*University of Alberta*

Lake Miwasin is a constructed pilot-scale 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 one and two following lake construction, and to analyze trace metal body burdens for trace elements that may contribute to the observed effects. Acute 48-hour toxicity tests were conducted with mortality as the endpoint, and chronic 21-day toxicity tests were performed using reproduction and growth as endpoints. No mortality was observed over 48 hours; 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., Molybdenum, Nickel, Aluminum, Cobalt) burdens in daphnids. To mimic the effects of riparian runoff into the lake that will occur as this artificial 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.

Fate, Transport, and Monitoring of Agricultural Contaminants in the Environment

A celebration of the 15-year career of Dr. Claudia Sheedy at the AAFC Lethbridge Research and Development Centre (PL)

Annemieke Farenhorst[1]

[1]*University of Manitoba*

During her 15-year career as a research scientist at the AAFC Lethbridge Research and Development Centre, Dr. Claudia Sheedy made remarkable contributions to the environmental monitoring and modeling of pesticide active ingredients. Her sudden passing in July 2020 at 45 years of age, brought shock and sadness to many in our scientific community. This presentation is to celebrate the accomplishments of Dr. Sheedy by highlighting some of her key research findings. Dr. Sheedy played a major role in the development and refinement of the Indicator of Risk of Water Contamination by Pesticides (IROWC-Pest) under the National Agri-environmental Health Analysis and Reporting Program (NAHARP), eventually leading the initiative from 2010-2015. The IROWC-Pest helped governments to support the sustainable development of Canada's agri-food industry. Dr. Sheedy showed that pesticide mixtures were frequently detected in the water-column and bottom sediments of prairie surface waters. In contrast, there were only rare occurrence of pesticide mixtures in groundwater samples taken in prairie dryland and irrigated agricultural regions. Interestingly, occurrences of mixtures in ground water showed detections of 2,4-D in the fall versus detections of MCPA in the summer, despite these molecules only differing at position two of the benzene ring. The detected pesticide concentrations in these studies were generally well below applicable Canadian Water Quality Guidelines, but there were many pesticides detected that do not have these guidelines established. In addition, Dr. Sheedy led the use of biobeds in Canada to remove pesticide residues from rinsate affiliated with spraying equipment.

Towards the development of a National Water Monitoring Program for Pesticides (PL)

Cassie McLean[1], Janice Villeneuve[1], Alice Dove[1], Myriame LaFrance[1], David Blais[1], Peter Bradley[1], Jenna Davies[1], Denis Parent[2], Christine Garron[2], Mark Sumarah[3], Justin Renaud[3], Lyne Sabourin[3]

[1]*Health Canada,* [2]*Environment and Climate Change Canada,* [3]*Agriculture and Agri-Food Canada*

Health Canada's Pest Management Regulatory Agency (PMRA) considers comprehensive water monitoring data to inform its regulatory decisions on the potential

risk of pesticides to human health and the aquatic environment, when available. Abundant high quality monitoring data is required to estimate exposure concentrations of pesticides in aquatic habitats and drinking water; however, the quantity, variability, and uncertainty associated with existing monitoring data make it challenging to use these data to inform pesticide risk assessments. In August 2021, the Government of Canada committed to ensuring that PMRA makes timely, science-based decisions to support the safe and sustainable use of effective pesticide products in Canada. Under this commitment, multi-year funds were provided to enhance the availability of real-world data on pesticides including water monitoring. The PMRA is working closely with Agriculture and Agri-Food Canada (AAFC), Environment and Climate Change Canada (ECCC), provinces, academics, and other stakeholders to develop a framework that will provide consistent guidance for water monitoring programs across Canada and inform the implementation of a National Water Monitoring Program for Pesticides (NWMPP). The goal of this framework is to increase the availability of high-quality pesticide water monitoring data across Canada to better inform pesticide regulatory decision-making; increase public understanding, awareness and accessibility of information through the Government of Canada's Open Data Portal; and to expand partnerships and improve collaboration between regions to strengthen water monitoring programs across the country. An overview of the technical workshops, initial results, and lessons learned from Year 1 of the pilot program will be discussed.

Not-so-simple patterns of neonicotinoids and diamides in prairie streams (VPL)

Caitlin Watt[1], John-Mark Davies[2], Claudia Sheedy[1], Jonathan Challis[1]

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An increasing body of work shows the prevalence of neonicotinoids in streams and rivers year-round. There are few studies that have examined Canadian prairie streams, where neonicotinoids are commonly used and where there is a unique hydrology of depressions and wetland complexes that store or release water and contaminants. In 16 lower-order streams across south-central Saskatchewan, we monitored the concentrations of seven neonicotinoids between 2017 and 2019, with three additional neonicotinoids and two diamides added in 2018 and 2019. Approximately 45% of all samples (176 of 392) had at least one insecticide detected, with thiamethoxam detected most frequently. Most detection mixtures contained at least one of thiamethoxam, clothianidin, or imidacloprid (98% of detects). About 16% of samples between 2018 and 2019 had diamide detections reflecting their increasing usage in Canada. While thiamethoxam and clothianidin concentrations were similar between rain events and snowmelt, their average daily loads were greatest during snowmelt ($p<0.05$); suggesting overwintering and spring freshet as a significant source of neonicotinoids. Generally, sub-watersheds dominated by canola and cereals and agriculturally intensive sites had higher neonicotinoid concentrations, yet crop cover and sites only explained a small proportion of the variance. Neither site, crop, flow, or

year considerably accounted for the large variation in detections, suggesting many factors are responsible for insecticide detections in streams. The variation of these insecticides in streams reflects crop practices, precipitation, prairie hydrology, agricultural practices, and environmental conditions, and highlights the need for improved monitoring across Canada to better understand processes affecting their distribution and overall risk.

Risk assessment of pesticide mixtures within the southern Lake Winnipeg basin (PL)

Alistair Brown[1], Marufa Fatema[1], Claudia Sheedy[*], Annemieke Farenhorst[1]

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Estimating risk to non-target organisms within a complex fluvial system comprised of a diverse range of agriculture and urban activity is becoming more critical in protecting ecosystems. This study quantified 34 of 172 screened pesticides classified as either historical, non-registered, or registered within the southern Lake Winnipeg basin in Manitoba, Canada. Contributions from the Red River, Assiniboine River, La Salle River, and Seine River were assessed. Pesticides throughout the Canadian prairie growing season (May to August 2017) were quantified using both GC-MS and LC-MS/MS and external calibration curves. Mass loadings of the pesticides with the greatest mean concentrations were highlighted for specific sampling dates, and pesticide inputs and removals were calculated from the US border to discharge at Lake Winnipeg. The maximum measured concentrations were used in conjunction with ECOSAR v2.0 toxicity values to generate Tier 0 hazard quotients, hazard indices, and maximum cumulative ratios for each sampling point in time and space in accordance with Health Canada guidelines. The results indicate that regardless of sampling timepoint and location, cumulative elevated risk of pesticide mixtures to aquatic biota remained throughout the growing season. When risk was lesser, thiamethoxam was the primary driver of risk, and when risk was greater metolachlor was the most common driver. The results also highlight the significant residential and commercial pesticide usage in the urban environment. These contributions should not be overlooked during future risk assessments.

Mitigating point source pesticide contamination risk using biobeds in Manitoba. (PL)

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Biobeds are a Swedish invention intended to reduce point source pesticide contamination risk by treating rinsate produced at pesticide handling areas. Biobeds work by promoting the degradation, mineralization, and sorption of pesticides within a biomixture layer of the biobed, and are typically single-celled or dual-celled. This research

highlights a newly installed dual-celled biobed in Carman, Manitoba during the first two years of operation of the system in 2020 and 2021. This biobed research site is comprised of two biobed containers that operate in series and contain a biomixture comprised of straw, peat, and soil at a 2:1:1 ratio. Rinsate produced on the research farm from the routine rinsing of spraying equipment is collected and then treated by the biobed. Prior to the biobed installation, this rinsate would have been directly deposited onto the ground surface. The rinsate processed by the biobed contained 14 pesticide active ingredients that were detected above the limit of quantification (LOQ) with dicamba being the highest detected. Pesticide detections above the LOQ ranged from 2 to 1500 $\mu g \cdot L^{-1}$ in the stored influent, from 2 to 500 $\mu g \cdot L^{-1}$ after the first biobed, and from 2 to 200 $\mu g \cdot L^{-1}$ after the second biobed cell with some active ingredients not being detected above the LOQ in the effluent samples despite being detected in the influent samples.

Scrutinizing surficial sediment along a 600-km-long urban coastal zone: Occurrence and risk assessment of fipronil and its three degradates (PL)

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The coastal aquatic environment is the terminal sink for many anthropogenic chemicals; however, little is known about pesticide occurrence, variation, and integrative toxicity in urban coastline sediments. As part of the most recent five-year regional monitoring cycle of the Southern California Bight (SCB), we report results of a random stratified survey for fipronil and its three major degradates (-desulfinyl, -sulfide, and -sulfone; fiproles hereafter) in surficial sediments in five embayment habitats (strata) along the SCB coastline. Fiproles were present in a small areal extent (6.8%) of the SCB embayment, and detected in 14 of 174 stations with a total sum concentration ranging from 0.50 to 17.5 $\mu g \cdot kg^{-1}$ dry weight. Area-weighted mean concentrations were 3.16 ± 3.37, 0.584 ± 0.558, 0.071 ± 0.103, and 0.005 ± 0.009 $\mu g \cdot kg^{-1}$ in brackish estuaries, estuaries, bays, and marinas, respectively, and below detection limits in ports. Fipronil sulfone had the greatest detection frequency (8.05%) and highest mean concentration (3.24 ± 3.36 $\mu g \cdot kg^{-1}$) among the four compounds. A screening-level deterministic risk assessment for invertebrates found that, region-wide, fiproles generally posed an insignificant to low acute risk to *Eohaustorius estuarius* in 7.36% of the SCB embayment area. In addition, high risk to *Chironomus dilutes* was found in 77.5% of the fiproles-detectable area in the Los Angeles River's brackish estuary stratum. Fipronil sulfone was identified as the major contributor of these effects. These results establish a baseline of occurrence and toxicity potential for fiproles in coastal sediments of southern California, and highlight the utility of regional monitoring for contaminant occurrence and effects assessment.

Quantifying the transport potential of estrogens in spring-thaw snowmelt runoff from manure-amended cropland (PL)

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Livestock manure contains natural and administered synthetic steroidal hormones. Runoff from manured croplands can be a source of steroidal hormones in surface waters. Exposure to low concentrations of steroidal hormones can impact ecosystem function and human health. Given the abundance of liquid swine manure in Manitoba, it is widely used as a nutrient source, which poses the potential risk of soil and freshwater contamination. In Manitoba, approximately 75% of the annual water runoff occurs during the brief snowmelt period, when soils are frozen. Controls on the transport of steroids during this important hydrological period are not well understood but are critical for understanding the fate of these contaminants. Our study aims to quantify the dissolved 17-β estradiol (E2) in spring-thaw snowmelt runoff, in a lab simulation and field study, comparing two different manure application methods: surface and sub-surface. We collected snowmelt runoff in an agricultural field, pre-concentrated the snowmelt through solid-phase extraction, then quantified the concentrations and loads of E2 using ultra-high performance liquid chromatography-tandem mass spectrometry. The lab simulation assessed the E2 in snowmelt floodwater and soil porewater under controlled conditions using intact soil columns collected from manure-amended fields. We found no significant difference between manure application methods on the introduction of E2 into the environment through snowmelt. However, we did see a significant difference in the E2 concentration found in porewater (mean 1.60 ± 1.2 $\mu g \cdot L^{-1}$) and floodwater (mean 0.484 ± 0.57 $\mu g \cdot L^{-1}$). The E2 concentration increased in porewater over time but did not significantly change in floodwater.

Undetected remobilization of phosphorus in lotic systems and the myth of assimilation (PL)

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Phosphorus, in the environmental dosing that occurs in most of North America, is a persistent and accumulating environmental contaminant responsible for ecotoxicological and human health risks including those associated with cyanotoxins in surface waters including drinking water sources. Often, regulations and licensing policies overlook its persistence and accumulative properties, as well as the fact that “assimilated” phosphorus remains active in the receiving environment after release, with environmental impacts. This presentation draws on 25 years of experience on Canadian prairie rivers, including

empirical studies and modeling of water and sediments, to illuminate phosphorus fate and transport pathways that receive little attention in routine water quality monitoring programs or in local licensing policies. Pathways discussed include transport via large-particulate vectors upon seasonal detachment of vascular vegetation and macroalgae, as well as, and often coupled with, remobilization and scour associated with seasonal reservoir operations. Results of a recent modeling study, building on past work on a moderately-impacted river, show how the recirculative activation of historically-assimilated phosphorus represents an environmentally-active, impactful, and undetected effective dosing or loading similar in magnitude to the total annual loading detected in nutrient monitoring programs. Takeaways from the presentation speak to the "myth" of phosphorus assimilation and suggest that regulations and licensing should not focus merely on immediate impacts but on whether regulated releases might cause or contribute to an overall net positive or negative flux in the receiving waters, with an eye to historical baseline conditions and future effects.

Characterization of dissolved organic fraction from swine wastewater to identify their effect on soil sorption of sulfamethoxazole (PO)

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Dissolved organic (DO) fraction (<0.45 μm) in swine wastewater can play a pivotal role in veterinary antimicrobial soil sorption mechanisms. The objective was to characterize the components in the DO fraction to identify the effect on soil sorption of sulfamethoxazole. Thus, the DO fraction was examined using (i) fluorescence excitation-emission spectroscopy, (ii) the molecular weight distribution by size exclusion chromatography using relative absorbances at UV 254 nm, (iii) solid state ^{13}C-NMR, (iv) Fourier transform infrared spectroscopy, and (v) total dissolved organic carbon content. Fluorescence data revealed DO fraction having major peaks at (Ex 200–250 nm)/(Em 280–380 nm) (aromatic protein-like), (Ex 200–250 nm)/(Em 380–600 nm) (fulvic acid-like) and (Ex 250–500 nm)/(Em 380–500 nm) (humic acid-like). A wide range of molecular weights (from 0.0044 to 10.419 kDa) exhibited peaks for low molecular weight components (<0.9 kDa), building blocks for refractory organic matter (0.9–1.8 kDa), high molecular weight fulvic acid (1.8–4.0 kDa) and humic acid (>4.0 kDa). The organic carbon content is 1944.75 mg·L^{-1}. The $SUVA_{254}$ value of 11.45 L·mg^{-1}m^{-1} indicates the hydrophobic nature of the DO fraction. Isolation of low molecular weight hydrophobics, humic, and fulvic acids from the DO fraction will be performed by DAX-8 resin extraction method, and their structural physico-chemical characteristics will be obtained using solid state ^{13}C-NMR and infrared spectroscopy and will be presented. The next step of this research is to elucidate the effect of each DO component on the sulfamethoxazole sorption mechanisms onto 2:1 type smectite clays.

Can biofilms be used as an alternative pesticide monitoring tool in aquatic environments? (PO)

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Monitoring the presence and concentrations of pesticides in Ontario's aquatic systems is necessary to understand the impacts on the aquatic environment. Ontario's streams are susceptible to pesticide pollutants which are transported outside of the intended area of application from surrounding agricultural fields. Biofilms are a collective of microorganisms that grow on hard surfaces in aquatic ecosystems and have been shown to bioconcentrate pesticides in water. Biofilms are highly responsive to chemical and biological changes in the environment, and therefore have the potential to act as a cost-effective, integrated sampling tool to monitor pesticide exposures in aquatic ecosystems. The objective of this study is to determine whether biofilms can be used to provide an accurate representation of pesticide exposure in Ontario's aquatic systems. Ten sites across southern Ontario were sampled between May-September 2021. At each site, water, sediment and biofilm, colonizing both artificial and natural substrate, were collected and analyzed for the presence of ~500 pesticides. This data will be used to determine the distribution of pesticides in the three matrices (water, sediment, and biofilm) and will provide an assessment of how well each matrix characterizes the streams' exposure to pesticides. It is hypothesized that the partitioning of pesticides within water, sediment, and biofilm will be related to the physicochemical properties of the detected pesticide. The development of a novel and cost-effective sampling tool would benefit Ontario's current pesticide monitoring program and may greatly improve the reporting on pesticide pollutants and water quality in southern Ontario's aquatic environments.

Behavioural Responses to Chemical Pollution

Towards standardized behavioural endpoints for ecotoxicology studies: Antipredator chemical communication in wood frog tadpoles is influenced by naphthenic acid fraction compounds (PL)

Chris Elvidge[1], Chloe Robinson[1], Diane Orihel[1]

[1]*Queen's University*

Behavioural studies can provide desirable non-invasive assessments of sub-lethal ecotoxicological effects but the diversity of available behavioural endpoints limits generalizations across contexts, species, and life-history stages. We argue that antipredator behaviours and specifically responses to damage-released chemical alarm cues in the presence of environmental pollutants are ideal endpoints as they are based on ecologically relevant cues critical to individual survival and therefore largely dissociated from artefacts imposed by behavioural assays and experimental apparatus. We exposed wild adult wood frogs (*Rana sylvatica*) to either clean lake water (control) or naphthenic acid fraction compounds (NAFCs) from oil sands process affected water (OSPW) from an active tailings pond at nominal 5 $mg \cdot L^{-1}$ concentrations during captive mating events. Egg clutches were incubated and tadpoles maintained in their respective water types for ~40 days post-hatch and tadpoles (Gosner stage 25–31) were then individually exposed to one of six alarm cue (AC) stimuli solutions in clean water. Antipredator responses differed in graded fashion with ACs from control tadpoles eliciting the greatest response, water the least, and NAFC-tadpole ACs intermediate. Further, ACs delivered in NAFC water elicited greater latencies to resume behaviours in NAFC-exposed tadpoles but not in control tadpoles. These equivocal results in both AC production and response are consistent with our findings of no survival differences at this level of NAFC exposure and greater growth rates in the NAFC group compared to the controls. Critical antipredator behaviours appear to be reliable and accurate indicators of sub-lethal ecotoxicological effects across life history stages.

Incorporating social complexity in aquatic ecotoxicology (VPL)

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Behaviour is a valuable endpoint in ecotoxicology, as it provides a link between mechanistic, proximate processes and ultimate, ecosystem or evolutionary outcomes. Most organisms interact socially with conspecifics at some point over their lives, but this fundamental aspect of animal biology is often overlooked in ecotoxicology. I will present recent work by myself and my research group that both outlines conceptual approaches to incorporating social complexity in ecotoxicology, and results from recent experiments

using more complex social environments following pollutant exposure. Specially, I will highlight findings from our experiments assessing how the pharmaceutical pollutant oxazepam affects fish social behaviours. Using both brown trout and African cichlid fish as model systems, I will show how exposure changed dominant-subordinate behavioural relationships, making subordinate fish more competitively successful. I will further discuss what consequences these exposures had on fish fitness.

Effects of imidacloprid exposure on learned recognition of predatory stimuli by larval *Lestes* sp. (Odonata: Zygoptera) (VPL)

Prashani Arachchilage[1], Christy Morrissey[1], Adam Crane[1], Maud Ferrari[1], Douglas Chivers[1]

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Many aquatic organisms are highly responsive to slight changes in the physicochemical nature of the aquatic environment, making them vulnerable to various pollutants, including insecticides, which could affect their ability to learn predators. Neonicotinoids are one such widely used class of water-soluble insecticides. Their use has become a major environmental concern because both pests and non-target species are equally affected. We investigated the effects of exposure to various concentrations (0, 0.1, 1.0, or 10.0 $\mu g \cdot L^{-1}$) of a neonicotinoid insecticide (imidacloprid) on the learned recognition of predatory chemical stimuli by damselfly larvae (*Lestes* sp.). First, damselflies were conditioned to recognize risk by exposing them to zebrafish odour combined with conspecific damage-released alarm cues or a control of dechlorinated water. We subsequently assessed learning by quantifying the change in feeding bites performed after an injection of zebrafish odour alone. Larvae in the control group learned to respond to the predator odour based on their prior conditioning with alarm cues but not water. Such learning also occurred for larvae in the 0.1 $\mu g \cdot L^{-1}$ treatment group (i.e., the lowest concentration group) but failed for individuals exposed to the higher concentrations (1.0 and 10.0 $\mu g \cdot L^{-1}$). These results suggest that imidacloprid insecticide contaminations impair the chemosensory cognition of damselfly larvae, which may have important ecological implications.

Behavioral responses as indicators of toxicity resulting from exposure to microplastics in wood frog (*Rana sylvatica*) larvae (PL)

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[1]*University of Winnipeg,* [2]*Queen's University,* [3]*University of Waterloo,* [4]*Environment and Climate Change Canada,* [5]*University of Toronto*

Effects of microplastics on aquatic animal behaviour are unknown. This study examined behavioral variability in wood frog (*Rana sylvatica*) larvae exposed to a mixture of polystyrene, polypropylene, and polyethylene terephthalate fragments (ranging in size

from 40 to 1400 µm) at either a 1x concentration (50 000 microplastic particles·L^{-1}) or 10x concentration (500 000 microplastic particles·L^{-1}), or to no microplastics (i.e., negative control). Wood frogs were collected as embryos and exposed throughout development. After 49 days of exposure, larval frogs were placed into an arena and video-recorded for 10 minutes to test the hypothesis that exposure to microplastics would alter behaviour. Automated tracking (EthoVision® XT14) was then used to quantify various behaviours. Generally, there was moderate to weak evidence to suggest frog larvae exposed to microplastics behaved differently than larvae in the negative control group. Specifically, larvae exposed to 10x concentration of microplastics spent less time in a high activity state and did fewer bouts of high activity than control larvae. Additionally, larvae exposed to 1x concentration of microplastics had fewer bouts of moderate activity, and there was a moderate negative effect of microplastics on swimming velocity and distance travelled in the 1x concentration larvae compared to the control larvae. These findings shed light on the possible ecological effects of microplastics on aquatic animals, as behavioural changes have the potential to influence food capture, predator/prey dynamics, and habitat-use. Future research may focus on linking behavioural findings to changes in energetic and developmental status of the larvae to understand potential mechanisms for the behavioural changes.

Characterizing the effects of chronic conventional heavy crude oil exposure on behaviour of larval wood frog (*Lithobates sylvaticus*) and their links to growth, development, and histopathology (PL)

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The Floating Wetland Treatments to Enhance Remediation (FloWTER) study examined the efficacy of plant-microbe relationships for *in situ* degradation of oil-derived hydrocarbons at the International Institute for Sustainable Development-Experimental Lakes Area in 2021. Uptake and effects of *in situ* chronic exposure to conventional heavy crude oil (CHV) water-accommodated fraction on the growth, development, and behaviour of wood frog (*Lithobates sylvaticus*) tadpoles were characterized in the presence or absence of floating aquatic plants, and a no-oil control. Apical endpoints, specifically snout-vent and total length, and weight were not statistically different between control and oil exposed animals over the course of the study. Weekly behavioural assays were recorded on a subset of five tadpoles to examine parameters of swim speed, latency time, distance traveled, and nearest neighbour distance over the chronic exposure period, along with anxiolytic and anxiogenic assays (caffeine and ethanol). Behavioural assays were assessed using tracking software (Loligo® Systems) to evaluate and quantify compromised or enhanced behaviour in wood frog tadpoles as well as characterize predation risks as a result of CHV.

Concentrations of total polycyclic aromatic hydrocarbons (TPAH) in whole tadpoles were also not statistically different among treatments, though tadpoles in enclosures with planted floating treatment wetlands did have lower TPAH overall. Qualitative histology analysis also supports these results, as there were no apparent treatment effects. This research provides much needed data on the toxicological effects of CHV on amphibians to inform risk assessment related to the over-land transportation of crude oil and potential impacts of freshwater oil spills.

Consequences of chronic bisphenol-A exposure on adult fathead minnow behaviour and their capacity to recover following withdrawal from the exposure (VPL)

Arash Salahinejad[1], Ahmad Ghobeishavi[1], Anoosha Attaran[1], Som Niyogi[1], Maud Ferrari[1], Douglas Chivers[1]

[1]*University of Saskatchewan*

Bisphenol A (BPA) is a ubiquitous environmental contaminant. Due to its omnipresence in aquatic ecosystems, many aquatic vertebrates such as fish are often exposed to BPA. Previous studies demonstrated that BPA disrupts estrogenic signalling pathways in vertebrates, which negatively impacts their physiology and behaviour. Consequently, many manufacturers are in the process of replacing BPA with other substances. While this should reduce BPA concentrations in the environment over time, it remains largely unknown to what extent vertebrates are capable of recovering from previous BPA exposure. Hence, the present study examined to what extent the behaviour of fathead minnows (*Pimephales promelas*) is negatively impacted by BPA exposure and whether their behavioural responses improve following long-term withdrawal from the exposure of this toxicant. In our experiment, we exposed adult fathead minnows to three concentrations of BPA (0, 0.1, and 1 $\mu M \cdot L^{-1}$) as well as to 0.1 $\mu M \cdot L^{-1}$ of 17-β-estradiol (E2) as a positive control, in addition, to control water (no BPA or E2) for 90 days. Other minnows underwent the same treatments but were tested after a 30-day recovery period in clean water. We assessed shoal preferences as a proxy for social behaviour, individual responses in a novel tank test as a proxy for anxiety-related behaviours, and individual responses in an emergence assay as a proxy for boldness. The results from this study will improve our knowledge about the capabilities of vertebrates to recover from previous BPA exposure. They may also allow us to predict the long-term consequences of environmental contamination with BPA.

Chronic dietary arsenic exposure disrupts dopaminergic signaling pathways and impairs cognitive performance in adult zebrafish (*Danio rerio*) (VPL)

Mahesh Rachamalla[1], Som Niyogi[1]

[1]*University of Saskatchewan*

The present study was designed to investigate the neurobehavioral effects of chronic exposure to environmentally relevant concentrations of dietary arsenic (As) in zebrafish. Adult zebrafish were exposed to three different concentrations of dietary arsenic (30, 60, or 100 µg As per g dry weight; as arsenic) in addition to control for 60 days. The cognitive performance of fish was then examined using a latent learning paradigm in a complex maze, which exhibited a dose-dependent effect of As on the cognitive performance. As-treated fish demonstrated significant impairment of all of the learning parameters tested in the present study when compared to control fish. These behavioral effects were associated with a dose-dependent increase in As accumulation in zebrafish brain. Since oxidative stress is a key driver of neurotoxicity, the antioxidative balance in the brain of experimental fish was assessed. A significant decrease in thiol redox and a significant increase in lipid peroxidation were recorded in As-treated fish relative to control, along with the altered expression of enzymatic antioxidant genes. Dopaminergic neurotransmission in the brain regulates important fish behaviors, including learning, memory, and reward-motivated behaviors. In the present study, a significant increase in dopamine level and an altered expression of several dopaminergic genes were also observed in the brain of As-treated fish relative to the control fish. Overall, it appears that As causes cognitive impairment in zebrafish, likely by inducing oxidative stress and disrupting dopaminergic signaling in the brain.

Behaviours of yellow perch fry reared at optimal and non optimal incubation temperatures (PL)

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Behavioural traits are genetically determined but are plastic and influenced by the environment. Environmental temperature can affect behavioural performances through indirect effects on metabolism, locomotion, and growth, or through direct neurological and sensory effects, which impact stress response and sensory cues perception. In this study yellow perch embryos were incubated at three constant incubation temperatures (12, 15, and 18°C) from cell cleavage period to hatching. An additional incubation temperature group simulated ambient seasonal incubation temperatures (12 to 18°C, +1°C every 3 days). After hatching, fish were reared in the common garden temperature of 18°C. We studied activity visual motor response using light and dark conditions, sensory motor

coordination as escape velocity through startle reflex (using acoustic and visual cues), and locomotion through general swimming behaviour in ambient light conditions. The behaviours were assessed in fry (at 28, 32, 40, and 60 days post-hatch). The influence of incubation temperature on fry behaviour was complex; fish reared at 12°C had a higher mean swim velocity but lower angular velocity. Visual motor responses of perch fry reared at 15°C were influenced in both light (smaller distance moved) and dark (larger distance moved) cycles. Yet, maximal swim speed with the change to light was lower in the fish reared at 18°C. Lastly, startle to an acoustic tap was higher in fish reared at 18°C. Yellow perch show developmental plasticity in behaviour in response to incubation temperature and data suggest that warming waters may impact fitness related traits in this species.

Patterns of gill transcript abundance are related to behavioural responses to acute CO_2 exposure (PL)

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The degree to which dissolved CO_2 might impact fish populations is unclear. One major obstacle has been the wide variation in observed responses—both within and across populations. Thus, we completed a study to investigate the possible effects of chronic CO_2 exposure on fish with different behavioural responses (i.e., phenotypes) to acute CO_2 exposure. Using standard behavioural assays, lab-reared Japanese medaka (*Oryzias latipes*) were first divided into 'responsive' and 'non-responsive' phenotypic groups based on their performance during brief periods of CO_2 exposure. Fish of each behavioural phenotype were then exposed to either control (~480 ppm) or high CO_2 (~1250 ppm) conditions for a 6-week period. Body condition, behaviours, reproductive performance, and relative abundance of various mRNA transcripts in whole brain, gill, and liver tissues of fish were measured. Generally, CO_2 exposure had limited effects on fish, regardless of behavioural phenotype. However, the abundance of mRNA transcripts of genes associated with gill ion and acid-base regulation were predictive of the initial behavioural phenotype, indicating that there may be underlying differences in gill function that predict how fish respond behaviourally to elevated CO_2. Our findings have implications for ecotoxicology studies, as they suggest that interindividual variation in the behavioural responses to environmental conditions may relate to underlying differences in cellular function. As such, initial screening of behavioural phenotypes prior to experimental exposures may help decipher instances of large variation in individual responses.

Environmental Radioactivity: Characterization, Transport, Exposure and Effects

Radium-226 absorbed dose rates to North American freshwater species (VPL)

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Mining operations release radionuclides such as radium-226 (^{226}Ra) into the aquatic environment. Our objective was to gather activity concentrations of ^{226}Ra in water, sediment, and aquatic organisms native to North America; calculate absorbed dose rates; and link these exposure activity concentrations to absorbed dose rates to derive protective no-effect levels to aquatic organism. For this purpose, we gathered data for eight algal species, twelve benthic invertebrate species, and eight fish species. ^{226}Ra bioaccumulation along with water and sediment activity concentrations were used to calculate absorbed dose rates for each species. ^{226}Ra activity concentrations in receiving or tested waters ranged from 0.001 to 37 Bq·L^{-1}. Bivalve and crayfish species accumulated ^{226}Ra the most, being exposed to absorbed dose rates ranging from 100 to 200 µGy·h^{-1} when exposed to 0.06 Bq·L^{-1}, pH 8, and 8 mg·L^{-1} of dissolved organic carbon. The available data only allowed us to derive a no effect activity concentration of 260 Bq·L^{-1} that would be protective of lake trout. We could not derive no effect concentrations for algae nor for benthic invertebrates or other fish species. This study recommends toxicity testing on algae, benthic invertebrates, and fish to derive a water quality guideline that would meet the requirement of the Canadian Council of Ministers of Environment. The effect of pH, calcium, and dissolved organic carbon on toxicity should also be explored to determine if any of these factors could affect toxicity. This will be the objective of the new Natural Science and Engineering Research Council Alliance grant.

Radium-226 treatment at decommissioned uranium mine tailings management areas – Emerging challenges with improving water quality (PL)

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In Elliot Lake, Ontario, uranium mining occurred from the 1950's until the 1990's when decommissioning took place. There are 11 tailings management areas (TMAs) which have been either vegetated or flooded and are currently under post-closure care and maintenance consisting of water management, treatment, and environmental monitoring. Radium-226 is a parameter of concern associated with mine impacted water and monitored within the receiving environment. Raw water from the TMAs contains sulphate due to the historical use of sulphuric acid to extract uranium as well as sub-aerial pyrite

oxidation of tailings prior to flooding. Typical radium-226 treatment involves barium chloride addition to raw TMA water to induce barite (barium sulphate) precipitation, which co-precipitates and/or adsorbs radium-226. These treatment solids settle out prior to discharge to the receiving environment. The decommissioning environmental impact statement predicted that TMA radium-226 and sulphate concentrations would decrease over time. Presently, the TMAs have performed as predicted with decreased radium-226 and sulphate concentrations post-decommissioning. Thermodynamically, barite will precipitate at these decreased sulphate concentrations, however, barite precipitation and settling prior to discharge has become increasingly challenging. Radium-226 monitoring has shown that treatment efficacy decreases during spring and fall. This decrease has been the subject of multiple investigations. In this study, the hypothesis was tested that treatment challenges are caused by seasonal influx of organic acids, combined with the dilution of sulphate due to run-off. This phenomenon has the potential to affect other decommissioned TMAs where radium-226 is being treated using barite precipitation.

Modelling the environmental transport of uranium-series radionuclides (PL)

Stacey Fernandes[1], Katherine Woolhouse[1], Caroline Lucas[1], Harriet Phillips[1]

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Environmental modelling is a powerful tool that is used to assess the implication of potential changes in the environment due to external influences such as effluent releases or remediation at a site. There are compilations of information and databases available to assist with modelling as measured data are not always available for every compartment that is modelled. Common sources of information for ecological transfer are the ERICA database, and IAEA TRS and TECDOC documents. Within Canada, CSA-N288.1 provides factors that are used in the estimation of derived release limits, while this document focuses on human health there is some information relevant to ecological modelling. A significant amount of measured data are available for the uranium mining region in the boreal environment in northern Saskatchewan. This information can be used to derive regional specific transfer factors to improve the modelling exercise. This is consistent with CSA-N288.6, which supports the use of site or regional specific exposure factors. The implication of the use of site-specific transfer factors in environmental modelling will be discussed. In addition, the difference between the use of concentration ratios and transfer factors for modelling food chain pathways will be explored and the strengths and weaknesses of the approaches will be highlighted.

Human health and ecological risk assessment and targeted environmental monitoring to guide development of a remediation plan for the former Rix Athabasca and Smitty uranium mines in northern Saskatchewan (PL)

Caroline Lucas[1], Stacey Fernandes[1], David Sanscartier[2]

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A Human Health and Ecological Risk Assessment (HHERA) is being performed for the receiving environment associated with former Rix Athabasca and Smitty uranium mines, located near Lake Athabasca in northern Saskatchewan. Information from the site indicated elevated levels of uranium and radium-226 in a wetland and small lake directly adjacent to the mine site. Environmental data for the Rix-Smitty area were limited and therefore the results from a preliminary HHERA were used to guide targeted environmental monitoring to reduce uncertainties/conservatism built into the HHERA, and to investigate identified potential risks. The HHERA was updated to incorporate additional data, which included vegetation, benthic invertebrate, and wetland invertebrate community health as well as surface water, sediment, and peat samples. In addition, co-located vegetation and peat samples allowed for the development of site-specific transfer factors. The results of the monitoring showed that under current conditions there is limited uptake of uranium by biota. Overall, although there may be some decrease in the productivity of ecological receptors in the wetland, there are no signs of significant effects. The refined HHERA results are being used along with information from other studies to support the development of a final remediation plan for the site to allow transfer of the site to Saskatchewan's long-term monitoring and maintenance program.

Environmental and nutritional chemistry of wild harvested berries vs commercial berries: Depositional and uptake chemistry and human health assessment (PL)

Christy Maynard[1], Matthew Herod[1,2], Malcolm McKee[1], William Shotyk[3]

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In northern Saskatchewan, there are several Canadian Nuclear Safety Commission (CNSC)-licensed active and decommissioned uranium mines and mills. In these areas, Indigenous communities harvest traditional foods and Canadian diet studies have identified wild berries as an important part of their diets. Food ingestion is recognized as an exposure pathway of anthropogenic and naturally occurring radioactive materials. Accurately communicating potential human health risks from environmental contaminants in traditional foods is of vital importance since the information may affect the Indigenous community's mental and physical well-being. Uninformed concerns and miscommunication may drive dietary changes to replace traditional foods with less nutritionally dense market foods that can increase the risk of chronic diseases such as cardiovascular disease and

diabetes. Wild blueberries and the soil the plant roots grow in were sampled near CNSC-licensed facilities in northern Saskatchewan. Samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) in a metal-free, ultraclean laboratory to obtain trace metal concentrations, and radionuclide concentration data was obtained for important dose contributors to estimate ingestion dose. As a comparison with the Saskatchewan blueberry and soil results, commercially-available blueberries were collected from Ontario farms and grocery stores. This research project identifies geochemical relationships between radionuclides and trace elements in blueberries, helps inform CNSC's regulatory decision-making process, and supports future human health risk communication with Indigenous communities. Acknowledgements: Dr. Matt Herod (CNSC), Malcolm McKee (CNSC), Dr. Slobodan Jovanovic (CNSC), Niall Crawley (CNSC), and Dr. William Shotyk (University of Alberta). Thank you all for your incredible support!

The movement of cesium-137 through the lichen -> caribou/reindeer -> human food chain (VPL)

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Of anthropogenic radionuclides, the main radionuclide of concern is cesium-137, because it is the most important long-term contributor to the environmental radiation dose to humans from human activity (NCRP 154, 2007). It has a half-life of 30 years and is chemically similar to potassium, allowing it to easily accumulate in plants and animals. Cesium-137 has been released into the atmosphere during the nuclear weapons tests in the 1950s-60s and during nuclear accidents, such as Chernobyl and Fukushima. In 1986, the Chernobyl accident occurred. Due to the wind direction and rainfall, a part of Scandinavia had received a substantial amount of ^{137}Cs from that accident. This presentation will look at what was done to mitigate the problem with respect to reindeer. The lichen -> caribou -> human food chain is as follows. Lichens can absorb ^{137}Cs directly from the air and bio-accumulate it. Caribou and reindeer eat lichens as their winter food and caribou is an important food source for Northerners. The movement of ^{137}Cs through this food chain has been well studied in Canada and Scandinavia. However, a number of open questions remain. This presentation will look at measurements made in Canada and Scandinavia since the 1960s up until the Fukushima accident in 2011. NCRP Report No. 154 (2007). Cesium-137 in the Environment: Radioecology and Approaches to Assessment and Management.

Recovery of the Great Lakes basin following a nuclear accident (VPL)

David Rowan[1], Elizabeth Priebe[1], Emily Saurette[1], Dan Festarini[1]

[1]*Canadian Nuclear Laboratories*

Radionuclide fate and transport models for the Great Lakes system have not been revisited in almost 40 years. Estimates of fallout and sparse surface water data for the Great Lakes were previously used to develop linked-basin models for the five main lakes, where radionuclides from one basin flow sequentially through the system. No tributary data existed and watershed processes were largely ignored. One of the key lessons of Fukushima, is that contamination can be relatively restricted such that only one or a few watersheds may be affected by releases of fission products during a severe nuclear accident. Most of the Ontario population resides in the Great Lakes basin or the adjacent Ottawa River basin, and recovery phase predictions that do not provide tributary watershed-scale detail will make decisions on drinking water, agriculture, fish, and wildlife extremely problematic and prone to large uncertainties. Using new data on HTO, cesium-137 and radionuclide stable analogues in major Great Lakes watersheds (river water, soil, plants), we show that variability in surface geology exerts control on radionuclide concentrations in tributaries, uptake by aquatic biota, and transfer from soil to plants. We then simulate several Fukushima-type releases in the Great Lakes basin, and show how differences in surface geology and watershed hydrology can lead to very different outcomes during ecosystem recovery.

Simulating tritium transport in the Great Lakes basin: An essential first step in recovery phase model development (PL)

Elizabeth Priebe[1], David Rowan[1], Emily Saurette[1], Karen Sharp[1], Dan Festarini[1]

[1]*Canadian Nuclear Laboratories*

Tritium concentrations in the Great Lakes system are simulated through 45 tributaries and 13 sub-basins of the Great Lakes using a simple hydrologic and tritium flux model. The model estimates tritium concentrations for each Great Lake and tributary on a daily time step, advancing the solution through the Great Lakes system from Lake Superior to the outflow at the St. Lawrence River. Results show that the model reproduces flows and concentrations well, with an average ~20% difference between simulated and measured flows and an average ~10% difference between simulated and measured tritium values. Tritium data used to calibrate the model comprise a large historic dataset and many seasonal samples collected recently for this investigation. Seasonal tritium trends observed in tributaries indicate lake effect precipitation as a tritium transport mechanism, with tributary concentrations in spring reflecting those of the upwind Great Lake, and tributary concentrations in fall resembling “background” concentrations similar to those observed at the IAEA monitoring station in Ottawa, Ontario. Our model design incorporates the lake

effect by seasonally varying precipitation source fractions to reflect observed inputs. As a final step, the calibrated model is used to simulate an aquatic tritium spill from the Bruce nuclear generating station in Kincardine, Ontario. Results indicate that it would take several decades for lake and tributary concentrations to return to pre-spill levels. A Python implementation of the model was created to simplify implementation and for use as a regulatory and recovery phase modelling tool.

Application of the adverse outcome pathway to the radiological risk posed to aquatic environments (PL)

Danielle Beaton[1], Marilyne Stuart[1], Richard Goulet[2], Sean Langley[2], Anne Crémazy[3], Claude Fortin[3], Knut Erik Tollefsen[4,5], Vinita Chauhan[6]

[1]Canadian Nuclear Laboratories, [2]Natural Resources Canada, [3]Institut national de la recherche scientifique, [4]Norwegian Institute for Water Research (NIVA), [5]Norwegian University of Life Sciences (NMBU), [6]Health Canada

Currently in Canada, there are no water quality criteria for the protection of aquatic life related radionuclides within these environments. Separately, release limits for radionuclides from mining and milling activities are based on calculated releases from the best available technology, and not from environmental impacts by radionuclides entering a receiving water. While these limits are considered to be sufficient to be protective for humans, the scientific basis for these limits is not established for aquatic organisms. This paucity of relevant data offers an opportunity to apply the Organisation for Economic Cooperation and Development (OECD) Adverse Outcome Pathway (AOP) approach as a basis for setting effect thresholds. A specific AOP repository, the AOPwiki (https://aopwiki.org), is the entry point into a framework that organizes available effects data, and is the portal through which those AOPs submitted for endorsement to the OECD AOP developmental programme are tracked as they undergo transparent scientific peer review towards final endorsement and publication. Connections between individual AOP components are critically evaluated using modified Bradford Hill considerations for biological plausibility, essentiality, and empirical evidence; thus, incorporating causal reasoning connecting dose-response evidence with individual level effects, such as growth and development, or population level outcomes, such as reproductive success, susceptibility to disease, or lethality. The goals for an AOP can be either qualitative, semi-quantitative, or quantitative. This presentation provides a background to the AOP approach featuring two proposed pathways based on literature for the aquatic snail, *Lymnaea stagnalis* as examples for evidence mapping, gap identification, and experimental planning.

Developing a Canadian database of parameter values required for modelling non-human biota doses. An effort to make future Canadian nuclear power plant and mining site environmental risk assessments consistent (PO)

Sohan Chouhan[1], Kim Vu[1], Sarah Chester[1]

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Canadian Nuclear Laboratories is developing a database of parameter values for assessing doses to non-human biota. The project includes a review of pertinent environmental risk assessments (ERAs) and relevant literature, a compilation of applicable parameter values, the quality control of the data selected for inclusion in the database, and the evaluation of different modeling approaches for the assessment of doses to biota. A literature search was conducted with the aim to gather data regarding radioactivity in Canadian environments collected within the last 25 years. A total of 400 abstracts were obtained and amongst those, 215 were deemed likely to contain relevant parameter values. The full papers were therefore obtained and reviewed. Only 100 out of the selected 215 papers contained suitable data. The relevant data was then extracted from those 100 documents and entered into MS Excel spreadsheets. Each reference was also kept with the data. Additional relevant parameters were obtained from IAEA guidelines and 25 ERA reports, and from databases associated with existing models such as ERICA, RESRAD-BIOTA, and CSA-N288.1. For quality control, each entry was then independently verified by a second researcher. Model testing was done for seven different models and for 7850 different exposure scenarios. This allowed for the formulation of recommendations on the selection of a dose assessment methodology that would be most suitable for Canada. Findings have been shared with stakeholders and will be presented, bringing Canada closer to the goal of developing a consistent framework for predicting radiological doses to non-human biota at Canadian nuclear sites.

Development of a water quality guideline for radium (^{226}Ra) in Canadian Waters (PO)

Edgar Pérez[1], Charlotte Lacroix-Durand[1], Karsten Liber[1], David Janz[1]

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In Canada, the mining, milling, and refinement of uranium and other minerals, and the combustion of fossil fuels generate byproducts that contain radionuclides like ^{226}Ra. While strict discharge and monitoring regulations are in place for ^{226}Ra in mining effluents (i.e., a maximum authorized mean monthly concentration of 0.37 Bq·L^{-1}), current allowable concentrations of ^{226}Ra in surface water are 0.11 Bq·L^{-1} (Saskatchewan), and 1 Bq·L^{-1} (Ontario). Despite strict regulations, there is limited empirical evidence suggesting that current thresholds for ^{226}Ra are indeed protective for all aquatic organisms. The lack of a Canadian water quality guideline for the protection of aquatic life for ^{226}Ra highlights the

need for the establishment of a criterion that protects Canadian aquatic ecosystems. This need is illustrated by the potential ecosystem hazards ^{226}Ra poses since it has a half-life of ~1600 years, is highly mobile under typical environmental conditions, and is bioaccumulative. Using standard and more elaborate toxicological assays, we (in partnership with researchers from other institutions) propose to develop a water quality guideline for ^{226}Ra, by generating a species sensitivity distribution that includes 12 key aquatic species (fishes: *Oncorhynchus mykiss* and *Pimephales promelas*; snail: *Lymnaea stagnalis*; epibenthic invertebrate: *Hyalella azteca*; benthic invertebrates: *Chironomus dilutus*, *Hexagenia limbata*, and *Pisidium* sp.; pelagic invertebrate: *Daphnia magna*; unicellular green algae (*Chlamydomonas reinhardtii*, *Raphidocelis subcapitata*, and *Chlorella fusca*), as well as the aquatic plant *Lemna minor*). Ecotoxicity data generated from this project will be useful to Canadian environmental regulators, risk assessors, and nuclear industry professionals to improve current standards and management practices for Ra.

Exposure of Canadian Receiving Waters to Radium-226 from Metal and Diamond Mining Effluent (PO)

Catherine Proulx[1,2], Richard Goulet[3]

University of Ottawa, Kilgour & Associates, [3]Natural Resources Canada

Mining and milling operations in Canada release radionuclides such as radium-226 (^{226}Ra) into the receiving aquatic environment. Consequently, the *Metal and Diamond Mining Effluent Regulations* (MDMER) requires each facility to measure ^{226}Ra, along with all other prescribed deleterious substances, in final discharged effluent on a weekly basis. Releases of ^{226}Ra from mine effluent were characterized using available monthly mean compliance data from 2014 to 2018. Monthly mean concentrations of ^{226}Ra in final treated effluent ranged from 0.0004 to 0.8250 Bq·L^{-1}. Discharged mine effluent typically contained 0.005 Bq·L^{-1} ^{226}Ra. This value is within natural background concentrations estimated using ^{226}Ra data from reference areas, with an upper tolerance limit of 0.021 Bq·L^{-1}. Discharged mine effluent from uranium mines had the highest variation in ^{226}Ra and highest median concentration of 0.007 Bq·L^{-1}. The MDMER maximum authorized monthly mean concentration of 0.37 Bq·L^{-1} for ^{226}Ra was exceeded 16 times in over 4000 samples collected at six different facilities from 2014 to 2018. Exceedances occurred in the precious metal (n=1), base metal (n=7), iron (n=1), and niobium (n=2) mining sectors. While these exceedances of MDMER limits occur rarely, when these exceedances occur, they do not necessarily translate to impacts to aquatic biota. This highlights the need for a better understanding of the toxicity of ^{226}Ra to aquatic organisms necessary to derive a robust environmental quality criterion for the protection of aquatic life, which could then provide an improved understanding of the potential impacts of discharged effluent containing ^{226}Ra and identify any mitigation measures, if necessary.

CNL Capabilities and Facilities to Support Canadian Environmental Radioactivity Research (VPO)

Jennifer Olfert[1]

[1]*Canadian Nuclear Laboratories*

Canadian Nuclear Laboratories (CNL) has a broad spectrum of capabilities to support multi-disciplinary environmental research covering areas including radioecology, hydrogeology, environmental chemistry, molecular biology, and waste management. These capabilities include completion of baseline environmental site assessments, environmental monitoring, fate and transport modeling, and expertise in strategies for used fuel reprocessing, as well as the evaluation of waste streams, treatment, and disposal strategies. They are supported by a number of existing facilities including a low-level analytical laboratory, a toxicity laboratory and waste processing technologies laboratories, among others. In addition, we are now establishing an active greenhouse. Together, these capabilities and facilities allow CNL to respond to current and emerging research priorities, such as the environmental impacts of and waste strategies for small modular reactors (SMRs). This presentation will provide more details on CNL capabilities and facilities, and the research we deliver to our stakeholders.

Mapping selected metals and natural radioactivity in northern Ontario, Canada, using the caribou lichens *Cladonia rangiferina* and *C. mitis* (VPO)

Julia Anderson[1,2], François Caron[3], Peter Beckett[2], Graeme Spiers[2], Nykola Lévesque[2], Mark Charbonneau[1]

[1]*Testmark Laboratories Ltd,* [2]*Laurentian University,* [3]*Royal Military College of Canada*

We present an overview of a survey conducted in a closed uranium (U) mining camp in the Elliot Lake (northern Ontario, Canada). The area was subjected to U mining in the ‘50s to ‘90s. The area has since undergone environmental restoration and recovery, particularly after the permanent closure of the Elliot Lake mines in the mid 1990s. Our recent surveys (2010, 2020) re-investigated earlier assessments of metal pollution using lichens (1980’s, 1990's). The main elements of interest are U, Th (thorium), Pb (lead), and Ti (titanium), which are diagnostic of past mining operations, and selected toxic elements (As (arsenic), Cd (cadmium), Cs (cesium), ^{137}Cs). We found that, particularly, the U concentrations in lichens decreased by about two orders of magnitude, compared to the 1980s. In our recent survey, the levels of U, Th, Pb, Ti, in *C. rangiferina* were slightly elevated for the more northern sites compared to the levels from the southern part, but this was not statistically different. For the most part, the levels of the metals and radionuclides throughout the region likely reflect background. The concentrations of some elements (U, Th, Cs, Cd) were statistically different between the two lichen species, suggesting different capture abilities of the lichens. We believe this survey establishes a set of baseline data, which is potentially

important in the context of future industrial activities as well as demonstrating the effectiveness of recovery from the earlier U industry.

Determination of ^{210}Po in environmental samples by alpha spectrometry using CuS micro-precipitation: method development and application on environmental samples across Canada (VPO)

Stephanie Walsh[1], Matthew Bond[1], Nicolas Guérin[1], Jules Blais[2], David Rowan[1]

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Polonium-210 (^{210}Po) is a naturally occurring alpha-emitting radioisotope from the uranium-238 (^{238}U) decay series with a high activity per unit mass and a relatively short half-life, making it one of the most toxic radionuclides in the environment. Naturally, ^{210}Po may be elevated in various environments based on geology and land cover. Anthropogenic activities such as mining, fracking, and fossil fuel combustion, may enhance ^{210}Po in the environment by increasing the amount of source rock at the surface of the Earth and by volatizing and/or mobilizing ^{238}U and its decay products during industrial processes. Despite the high toxicity and significant contribution of ^{210}Po to the natural radiation dose received by living organisms, ^{210}Po remains the least studied natural radionuclide in the environment and large data gaps exist in the understanding of the ultimate fate and behavior of the ^{210}Po in aquatic systems. This is largely due to challenges associated with methodology as no rapid and highly sensitive method exists to measure low-level activity concentrations in complex environmental samples. We developed a highly sensitive and efficient method to determine ^{210}Po in a diversity of environmental samples using a copper sulfide micro-precipitation. The chemical recovery was high (≥75%) and a very low minimum detectable activity was obtained. We have collected environmental samples from aquatic environments across Canada, including uranium mining regions, and will present preliminary results. This data will be used to study the behavior of ^{210}Po in the environment and expand on biokinetic and risk assessment modelling capabilities.

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