Proceedings of the 46th Annual Canadian Ecotoxicity Workshop: October 6 – 9, 2019, Québec, Québec

Editors
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National Capital Region
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Ottawa, Ontario
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2020

Canadian Technical Report of Fisheries and Aquatic Sciences 3407

Fisheries and Oceans Canada | Pêches et Océans Canada

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Canadian Technical Report of Fisheries and Aquatic Sciences

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Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. II n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques.*

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

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.

Canadian Technical Report of

Fisheries and Aquatic Sciences 3407

2020

PROCEEDINGS OF THE 46TH ANNUAL CANADIAN ECOTOXICITY WORKSHOP: OCTOBER 6 – 9, 2019, QUÉBEC, QUÉBEC

Editors:

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Cat. No. Fs97-6/3407E-PDF ISBN 978-0-660-36366-0 ISSN 1488-5379 (online version)

Correct citation for this publication:

Lise Parent, Patrice Couture, Valérie S. Langlois, Monique Boily, Travers R. Pretorius and Lisa N. Taylor (Editors). 2019. Proceedings of the 46th Annual Canadian Ecotoxicity Workshop: October 6-9, 2019, Québec, Québec. Can. Tech. Rep. Fish. Aquat. Sci. 3407: xxiii + 116 p.

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ABSTRACT

Lise Parent, Patrice Couture, Valérie S. Langlois, Monique Boily, Travers R. Pretorius and Lisa N. Taylor (Editors). 2019. Proceedings of the 46th Annual Canadian Ecotoxicity Workshop: October 6-9, 2019, Québec, Québec. Can. Tech. Rep. Fish. Aquat. Sci. 3407: xxiii + 116 p.

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 46th annual CEW was held at the Centre des congrès de Québec. The workshop included 105 platform presentations and 74 poster presentations. Total attendance was 206.

This workshop was one of a continuing series of annual workshops in Canada on ecological toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of sediment and water quality criteria. These workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide 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. The Proceedings are published with the support of Fisheries and Oceans Canada.

RÉSUMÉ

Lise Parent, Patrice Couture, Valérie S. Langlois, Monique Boily, Travers R. Pretorius and Lisa N. Taylor (Editors). 2019. Proceedings of the 46th Annual Canadian Ecotoxicity Workshop: October 6-9, 2019, Québec, Québec. Can. Tech. Rep. Fish. Aquat. Sci. 3407: xxiii + 116 p.

Pendant 41 années, l'Atelier annuel sur la toxicité aquatique (ATW) a eu lieu à divers endroits autour du Canada. En 2015, l'atelier a été rebaptisé l'Atelier canadien annuel sur l'écotoxicité (CEW) pour tenir compte de l'étendue des intérêts environnementaux des participants à l'atelier.

Le 46^{ième} Atelier canadien annuel sur l'écotoxicité a eu lieu au Centre des congrès de Québec, du 3 au 6 octobre 2019. L'atelier a donné lieu aux 105 présentations orales et 74 présentations par affiche. Deux-cents six personnes ont assisté à l'atelier.

L'atelier a permis de poursuivre les discussions tenues annuellement au Canada sur l'écotoxicologie. Ces ateliers annuels organisés par un comité national constitué légalement réunissent des représentants des secteurs industriels, des administrations publiques et des universités que le domaine intéresse. Ces derniers y échangent des idées et des connaissances sur les notions fondamentales de la toxicologie aquatique, mais aussi sur son application pour la surveillance de l'environnement, l'élaboration de lignes directrices et de règlements, et la définition de critère pour les sédiments et pour la qualité de l'eau. Ils passent également en revue les principes de la spécialité, de même que les questions d'actualité et les méthodes adoptées dans le domaine. Les comptes rendus sont publiés avec le soutien du ministre des Pêches et Océans.

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 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 Dr. Jill Watson for her 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. On est prié de communiquer directement avec les auteurs pour faire des remarques sur les travaux. Toutes les déclarations et opinions paraissant dans le présent rapport sont 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 Dre. Jill Watson dans la préparation de ces comptes rendus.

46th Canadian Ecotoxicity Workshop Organizing Committee / Comité organisateur du 46e atelier canadien sur l'écotoxicité

Workshop Co-Chairs / Co-présidentes et coprésident de l'atelier:

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Patrice Couture	Institut national de la recherche scientifique
Lise Parent	Université TÉLUQ

Workshop Organizing Committee / Comité organisateur de l'atelier:

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Alternative Approaches to Animal Testing for Ecotoxicology

Development and uses of fish gill cell lines: An update with RTgill-W1 in toxicology (PL)

Lucy Lee[1], Niels Bols[2]

[1]University of the Fraser Valley, [2]University of Waterloo

The gills of fish perform multiple functions, including respiration, osmoregulation, and innate immunity provision, that together make possible a life aquatic. The tissue organization of the gill is complex, with numerous cell types. In order to simplify the organ for cellular studies, cell lines have been derived from the gill. These cell lines can be grown indefinitely, providing a stable, inexpensive source of cells for use in studies on gill physiology and diseases. They are especially useful in aquatic toxicology as they allow animal-free testing. Currently, close to 30 fish gill cell lines have been reported in the scientific literature, with RTgill-W1 from rainbow trout being one of the few available from the American Type Culture Collection (ATCC CRL 2523). For the last 25 years, RTgill-W1 has been used in over 100 publications in a wide range of disciplines from basic to applied research. However, perhaps the most widespread use in toxicology has been for such goals as ranking the toxic potencies of individual contaminants and of environmental samples. As of April 2019, RTgill-W1 has been incorporated as Water quality testing standard under the International Standards Organization, ISO 21115:2019. New gill cell lines continue to be developed from various fish species for a variety of experimental goals. This presentation will give an overview of methods for developing fish gill cell lines and characterizing their cellular lineage and species of origin, and will describe how they are being used in aquatic toxicology and in research more broadly.

Predicting the sensitivity of European eels to dioxin-like compounds based on *in vitro* activation of the AhR (PL)

Markus Brinkmann[1], Marko Freese[2], Jan-Dag Pohlmann[2], Jonathon Doering[1], Malte Damerau[2], Lasse Marohn[2], Reinhold Hanel[2], Markus Hecker[1]

[1]University of Saskatchewan, [2]Thühnen Institute for Fisheries Ecology

Populations of European eels (*Anguilla anguilla*) have seen a dramatic decline in recent decades. Recruitment failure as a result of maternally transferred contaminants has been proposed as one of several potential causes of the decline, and dioxin-like chemicals (DLCs) have been identified as a class of chemicals of concern as they tend to bioaccumulate, are highly embryotoxic, and are maternally transferred in eels. However, to date, researchers have been unable to locate reproducing adult eels or developing embryos in their natural spawning grounds, and embryotoxicity data to identify causative chemicals are unavailable. In this study, we isolated and sequenced isoforms of the aryl hydrocarbon receptor (AhR) from eels and constructed a species-specific *in vitro* luciferase reporter gene assay using transfected COS-7 cells. A previously developed quantitative adverse outcome pathway based on the relationship between AhR activation and embryo lethality across nine species of fishes exposed to DLCs

was used to predict eel-specific relative potencies of DLCs. Using this data, mortality of early life stages of eels was estimated based on measured internal concentrations. Based on these results, European eels appear to be among the most sensitive fish species to the exposure with DLCs, and exposure levels previously measured in this species are predicted to significantly contribute to the observed decline in recruitment of eels.

In vitro screening of bisphenol A replacement compounds: Cytotoxicity and mRNA expression in chicken embryonic hepatocytes (PL)

Tasnia Sharin[1, 2], Doug Crump[1], Jason M. O'Brien[1]

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

A market for bisphenol A (BPA) replacement compounds has emerged due to restrictions on the use and production of BPA. In the present study, an avian *in vitro* toxicogenomic model was used to compare the biological activity of five replacement compounds to BPA. Cell viability and mRNA expression were compared in primary chicken (*Gallus domesticus*) embryonic hepatocytes (CEH) exposed to BPA, bisphenol F (BPF), bis (3-allyl-4- hydroxyphenyl) sulfone (TGSH), 1,7-bis (4-hydroxyphenylthio)-3,5-dioxaheptane (DD-70), bisphenol AF (BPAF) or 4-hydroxyphenyl 4-isopropoxyphenyl sulfone (BPSIP). Transcriptomic effects were determined using two PCR arrays: 1) the ToxChip, which measures the expression of several toxicologically relevant genes; and 2) the AestroChip, which measures estrogen-responsive genes. Compared to BPA (LC_{50}=61.7µM± 43.1), DD-70 (LC_{50}=32.9µM ±7.3) and BPAF (LC_{50}=13.1µM± 5.0) were more cytotoxic in CEH. Both BPA and the 5 replacement compounds altered the expression of genes related to xenobiotic metabolism, DNA repair, and the thyroid hormone pathway on the ToxChip array. The rank order of gene dysregulation based on the ToxChip array was TGSH > BPF >DD-70>BPAF∼BPA >BPSIP. All of the replacement compounds and BPA altered at least one gene on the AestroChip array. BPA upregulated the expression of two genes, apovitellenin and carnitine palmitoyltransferase. BPSIP altered the most estrogen-responsive genes (7/10). The rank order of AestroChip gene dysregulation was BPSIP >BPF>BPAF∼BPA>DD-70∼TGSH. Overall, the results suggest that certain BPA replacement compounds elicit comparable or even greater toxicity than BPA and may act via different mechanisms.

Liver dethroned!? The caudal fin as a non-lethal alternative to transcriptomics-based evaluation of oil spill effects in Pacific salmon (PL)

Jacob Imbery[1], Emily Koide[1], Craig Buday[2], Rachel Miliano[2], Dayue Shang[2], Jessica Round[1], Honoria Kwok[2], Graham Van Aggelen[2], Caren Helbing[1]

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

Rapid and sensitive tools for assessing environmental toxicity can be crucial to monitoring oil spill impacts and remediation efforts. Transcriptomic biomonitoring of sentinel animals can be a powerful method for tracking and evaluating the impact of environmental toxicants; however, current methods require animal sacrifice. We have previously demonstrated that the salmonid caudal fin, which can be

rapidly sampled non-lethally, is a viable alternative to conventional liver tissue sampling. Our previous work using targeted qPCR revealed that *cyp1a1*, a "classic" hepatic indicator of polycyclic aromatic hydrocarbon exposure, was significantly increased in the caudal fin after exposure to marine diesel, demonstrating that this tissue is very sensitive to oil exposure. The purpose of the present work is to create a more comprehensive view of the transcriptome response in comparison with that of the liver and to identify additional biomarker candidates. Using RNA-Seq analysis, we show that the caudal fin more consistently responds to marine diesel exposure in a sex-dependent manner in comparison to the liver. We performed RNA-Seq on paired caudal fin and liver tissue from 20 genotypically-sexed male or female juvenile coho salmon following a 96-hour exposure to either 1000 $mg \cdot L^{-1}$ marine diesel seawater accommodated fraction (WAF) or seawater control. In the male and female caudal fin, marine diesel WAF exposure induced the differential expression of 620 and 501 transcripts respectively, consistently enriching cellular adhesion and morphogenesis pathways. Cell signalling pathways were uniquely enriched in males and metabolic, apoptotic, and embryonic pathways in females. Of the 176 and 352 transcripts differentially expressed in the male and female livers, some protein folding pathways were commonly enriched, with cellular localization and transport largely enriched in females and cellular metabolism in males. In the caudal fin, 36 marine diesel responsive transcripts were common in males and females, whereas only 12 transcripts were common in the liver. These results show that the caudal fin exhibited a more consistent response between sexes than the liver tissue. Furthermore, the results indicate that the caudal fin is more responsive to marine diesel WAF exposure than is conventional liver tissue. Taken together, sampling and transcript analysis of the caudal fin present an exciting non-lethal alternative for assessment and monitoring of oil spill effects.

Avian hepatic transcriptomic responses to ethinylestradiol: Are early life stage Japanese quail representative of adults? (PL)

Yeon Seon Jeon[1], Doug Crump[2], Niladri Basu[1], Emily Boulanger[1], Amani Farhat[2], Markus Hecker[3], Othman Soufan[1], Jeff Xia[1], Jessica Head[1]

[1]McGill University, [2]Environment and Climate Change Canada, [3]University of Saskatchewan

The use of toxicogenomic endpoints and increased reliance on early-life stage (ELS) animal exposures are two strategies that have been proposed to improve toxicity testing for regulatory risk assessment. However, it is unknown whether transcriptomic measures in ELS organisms are predictive of those measured in their adult counterparts. The present study aims to compare hepatic transcriptomic responses of ELS and adult Japanese quail (JQ) following exposure to sublethal concentrations of ethinylestradiol (EE2). EE2 was dissolved in dimethyl sulfoxide and injected into the air cell of JQ embryos prior to incubation at 0, 3.33, and 33.3 $\mu g \cdot g^{-1}$ egg. Adult JQ were fed a single dose of EE2 (dissolved in corn oil) at 0, 0.5, and 5 $mg \cdot kg^{-1}$ body weight by gavage. Liver tissue was collected from 5 JQ embryos per dose group at mid-incubation, and from 5-6 adult JQ per dose group 4 days after dosing. Transcript quantification, differential expression analysis, and enrichment analysis were performed using the Kallisto workflow in Galaxy (galaxy.ecotoxxplorer.ca), the EdgeR method in EcoToxXplorer (www.ecotoxxplorer.ca), and the ClusterProfiler R package, respectively. ELS and adult JQ showed 223 and 94 differentially expressed genes (DEGs) respectively, 11 of which were common.

Fold change values were significantly correlated for these 11 DEGs between the two life stages, but there was little overlap at the pathway level. The present study contributes to the evaluation of toxicogenomics and ELS approaches as alternative toxicity testing methods, and to a large-scale Genome Canada-funded project (EcoToxChip; www.ecotoxchip.ca) aimed at transforming ecological risk assessment.

Assessing risks of selenomethionine toxicity in the fathead minnow using semi-quantitative modeling (PL)

Derek Green[1], David Janz[1], Karsten Liber[1], Natacha Hogan[1], Alper J. Alcaraz[1], Taylor Lane[1], K. Raes[1], K. Bluhm[1], Markus Brinkmann[1], Doug Crump[2], Niladri Basu[3], Markus Hecker[1]

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Selenomethionine (SeMet) is an organic form of the atomic element and essential micronutrient, selenium (Se). Selenium is released to the environment from natural (e.g., volcanic) and anthropogenic (e.g., coal mining) activities, and is biotransformed to SeMet by primary producers. When selenium concentrations are elevated in the aquatic environment, SeMet is bioaccumulated through food webs due its dose-dependent substitution for the essential amino acid and biochemical SeMet analogue, methionine. Selenomethionine can then impair recruitment of oviparous animal populations as their embryos are exposed to elevated oxidative stress that results from SeMet metabolism during development. Current protective guidelines provide threshold values for total selenium concentrations in the whole-body, muscle, or eggs/ovaries of fish; however, these guidelines only indicate problems after they have occurred. This study exposed adult fathead minnow (*Pimephales promelas*) to sublethal control (1.18 µg $Se \cdot g^{-1}$), low (3.88 µg $Se \cdot g^{-1}$), medium (8.75 µg), and high (29.6 µg $Se \cdot g^{-1}$) dose experimental diets spiked with SeMet. Experimental data are currently being used to validate a quantitative toxicokinetic model of Se in the fathead minnow which can predict the initiation of toxicity as qualitatively described through omic, biochemical, and somatic analyses and indices. This latter approach is currently being used to identify dysregulated transcripts and pathways capable of predicting adverse outcomes in fathead minnow health and reproduction. These data are also being used to inform the development of the EcoToxChip (www.ecotoxchip.ca), a high-throughput qPCR screening tool designed to evaluate potential effects of uncharacterized chemicals released in quantities of concern to the aquatic environment.

Modifications to existing transcriptomics dose-response methods to improve performance in ecological species (PL)

Jessica Ewald[1], Othman Soufan[1], Doug Crump[2], Markus Hecker[3], Jeff Xia[1], Niladri Basu[1]

[1]McGill University, [2]Environment and Climate Change Canada, [3]University of Saskatchewan

One emerging strategy for linking changes in gene expression to adverse outcomes of regulatory concern is through dose-response modeling. It has been shown previously that benchmark doses (BMDs) computed using gene expression are consistently within one order of magnitude of BMDs from

apical outcomes. However, existing methods for transcriptomic dose-response analysis were developed using mammalian species and rely on gene set libraries such as KEGG, the Gene Ontology, or Reactome. This makes it difficult to use them for ecological species, which typically have limited genome annotation resources. The objective of this study was to modify the transcriptomic dose-response approach implemented in BMDExpress to improve its performance in ecological species. First, whole-transcriptome data were summarized using our own gene sets, a collection of 21 "toxicity modules" which were designed for the interpretation of toxicogenomics data and can be rapidly defined in species with no existing functional annotation. Next, BMDs were calculated by fitting statistical models to module-level responses. This significantly decreases the computational time because models are fit to 21 outcomes (toxicity modules) instead of to 1000s (individual genes). Finally, the BMDs calculated using the modified approach were compared to BMDs calculated using the BMDExpress software. These three steps were first applied to the original data used to develop the methods implemented in BMDExpress (various rat tissues; 24 exposure experiments; n=720). Next, they were repeated with data from fathead minnow (ovary tissue; 5 exposure experiments; n=132). This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Amphibian transcriptomic responses to chlorpyrifos: Are early life stage toxicity pathways predictive of adverse outcomes of exposure? (PO)

Nicole Baldwin[1], Alper James Alcaraz[1], Anita Massé[1], Doug Crump[2], Niladri Basu[3], Markus Hecker[1], Natacha Hogan[1]

[1]University of Saskatchewan, [2]Environment and Climate Change Canada, [3]McGill University

Chlorpyrifos (CPF) is one of the most widely used organophosphate pesticides worldwide, with extensive occurrence in aquatic ecosystems. Exposure to CPF is associated with adverse effects in fish, most notably neurotoxicity through inhibition of the enzyme acetylcholinesterase (AChE). However, effects of exposure to CPF in amphibians are relatively poorly studied. In particular, little information is available on the underlying molecular mechanisms that drive adverse outcomes. The main objective of this study was to identify key molecular response patterns in a model amphibian, *Xenopus laevis*, following early life-stage exposure to CPF, which may enable prediction of apical outcomes of ecological relevance. Individuals were exposed to CPF (0.4, 2, and 10 $\mu g \cdot L^{-1}$ nominal) from 24 hours post-hatch through to metamorphosis (50-55 days post hatch). A subset of exposed individuals was sampled at 96 hours, and whole-body transcriptome profiles were assessed using high throughput sequencing (RNA-Seq). Pathway analysis revealed a number of significantly dysregulated pathways including those associated with well-characterised outcomes of CPF exposure such as serine hydrolase activity (e.g. AChE) and immune pathways including immune function, inflammatory response, and cytokine receptor activity. Tadpoles exposed to CPF through to metamorphosis exhibited increased relative liver weight (30% increase in 10 $\mu g \cdot L^{-1}$ treatment) and a dose-dependent decrease in brain AChE activity, which agrees with disruption of pathways associated with serine hydrolase following CPF exposure during early life stages. Transcriptomic analysis also revealed a number of novel dysregulated pathways that were not directly linked to apical outcomes measured in this study (e.g., vasculature development, sensory perception of light stimulus, and blood coagulation), suggesting that CPF impacts

a wide range of biological pathways in amphibians that warrant further study. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

EcoToxChip: a toxicogenomics tool for chemical prioritization and environmental management (36 month update) (PO)

Emily Boulanger[1], Anita Massé[2], Niladri Basu[1], Markus Hecker[2], Doug Crump[3], Jessica Head[1], Natacha Hogan[2], Jeff Xia[1], Gordon Hickey[1], Steve Maguire[1]

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The EcoToxChip project aims to develop, test, validate, and commercialize quantitative polymerase chain reaction (qPCR) arrays and a data evaluation tool (EcoToxXplorer.ca) for the characterization, prioritization, and management of environmental chemicals and complex mixtures of regulatory concern. In Project Phase 1, EcoToxChips are being developed for laboratory model species representing the most important vertebrate groups in ecological risk assessment (fish-fathead minnow; bird-Japanese quail; amphibian-*Xenopus laevis*). Model species (adult and early-life stage) are being exposed via standardized tests to eight chemicals representative of natural resource/environment sectors of Canadian concern and also to ones that impact a wide biological space (17α-ethynylestradiol, chlorpyrifos, benzo(a)pyrene, lead, fluoxetine, selenomethionine, trenbolone, hexabromocyclododecane (Activity 1). An integrative systems approach based on functional '-omics (combined global transcriptomic and proteomic profiling, targeted metabolome) and physiological analyses across levels of biological organization is being applied to characterize relevant toxicity pathways, including adverse outcome pathways (Activity 2); from this and other resources, species-specific EcoToxChips consisting of 384 environmentally-responsive genes of regulatory concern are being informed, built, tested, and optimized (Activity 3). EcoToxChip performance will be further optimized through studies with our collaborators (Activity 4). Under Activities 5-7, knowledge from Phase 1 is being translated to three native species (i.e., fish: rainbow trout; bird: double-crested cormorant; amphibian: northern leopard frog). EcoToxXplorer.ca provides intuitive bioinformatics support. To position the team advantageously with regard to the commercialization and institutionalization of the deliverables, our GE3LS research will produce and leverage social science knowledge about the phenomenon of "institutional entrepreneurship". Here we provide a 36-month update of our project (www.ecotoxchip.ca).

Use of co-expression analysis to identify life stage-specific subnetworks in toxicogenomics data from Japanese quail (PO)

Jessica Ewald[1], Yeon Seon Jeon[1], Jessica Head[1], Doug Crump[2], Markus Hecker[3], Jeff Xia[1], Niladri Basu[1]

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Early life stage (ELS) exposures are an attractive alternative toxicity testing strategy for avian species because of time, cost, and ethical advantages compared to adult exposures. Differences in responses to contaminant exposure across life stages may be partially due to changes in the way genes

are regulated and how they interact with each other as an organism develops. Thus, understanding the structure of gene interaction networks in ELS and adults may help explain and predict differences in biochemical responses to contaminant exposure between organisms of different life stages. The objective of this study was to compare the topological structure of gene-gene interaction networks across multiple life stages in Japanese quail. The rationale is that understanding which biological pathway networks are more or less conserved across life stages will improve our ability to design effective molecular assays for ELS exposures. First, RNA sequencing data derived from liver tissue of ELS and adult Japanese quail exposed to a medium and high dose of 8 environmental contaminants (n=100 samples for ELS; n=96 samples for adult) were generated. Second, co-expression network analysis was used to "discover" the underlying interactions between genes by constructing a correlation-based network for each life stage. The subnetworks were annotated with KEGG pathways and GO terms using gene set overrepresentation analysis to investigate their functional relevance. Finally, subnetworks were compared across life stages using three metrics: number of overlapping genes, Jaccard index, and a subnetwork conservation score. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Characterizing molecular toxicity pathways associated with 17β-trenbolone exposure in adult fathead minnows to predict adverse apical outcomes (PO)

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17β-trenbolone is a biologically active metabolite of trenbolone acetate, an anabolic steroid used as a growth promoter in the beef cattle industry. 17β-trenbolone is found to be stable in cattle excreta and has been found in aquatic systems associated with concentrated animal-feeding operations through surface water runoff from manure-amended soil. It is a strong androgen receptor agonist and has been shown to cause masculinization of female fish where females, morphologically and chemically, exhibit male characteristics (i.e. dorsal pad and dorsal nuptial tubercle formation, as well as decreased vitellogenin and estrogen). While many studies have reported morphological and biochemical effects of 17β-trenbolone in fish, there are fewer studies focused on the underlying molecular pathways that contribute to these apical outcomes. To this end, this exposure was conducted to look at molecular response patterns in fathead minnows *(Pimephales promelas)* exposed to 17β-trenbolone and use these to characterize the toxicity pathways associated with exposure. Sexually mature fathead minnows were exposed to 17β-trenbolone for 21 days. After 4 days, fish were subsampled for omics endpoints (i.e., transcriptomics, proteomics, and metabolomics). After 21 days, the remaining fish were sampled for apical endpoints (i.e., histopathology, plasma steroid concentrations, as well as dorsal pad and dorsal nuptial tubercle formation in females). Fecundity was monitored throughout the exposure. Toxicity pathways will be characterized by analyzing the 4-day omics data and linking those to the 21-day apical endpoints. It is anticipated that this research will help identify molecular toxicity pathways for 17β-trenbolone in adult fathead minnows that could be used to predict adverse apical outcomes of exposure. This study is part of the EcoToxChip project (www.ecotoxchip.ca).

Fecal contaminant profile of polar bears living in the Northern Beaufort Sea and the Southern Hudson Bay areas (PO)

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As apex predators, polar bears are known to biomagnify contaminants such as mercury and organopesticides in their tissues. High levels of contaminant body burden in bears could yield negative reproductive and immune effects, amongst other effects. Our recent work has demonstrated as a proof-of-concept the usefulness of fecal samples as a new matrix to evaluate polar bears' contaminant loading. The main objective of this follow up work is to create a database of contaminant concentrations found in tissues of bear living in the Northern Beaufort Sea and the Southern Hudson Bay areas, including feces. Once compiled, data will be used to model contaminant fate within the animals. A total of 25 sets of 4 samples (liver, fat, muscle, and feces) per individual has been analyzed for a suite of contaminants. Preliminary data suggest that over 20 metals were found in polar bears, including essential and non-essential metals, with differential profile among the two regions of interest. Complementary polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAhs), polychlorinated biphenyls (PCBs), total mercury (THg), methylmercury (MeHg), and chlordane analysis is ongoing. Ultimately, this project aims to develop relationships between contaminant body burdens in tissues and feces, and use these relationships to develop non-invasive ways to monitor polar bear health.

Investigating the applicability of the fish embryo test (FET) to inform read-across for ecological hazard assessment (PO)

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Development and validation of new-approach methodologies is a global initiative to improve throughput and efficiency of toxicology tests. Application of these methods as alternatives to commonly used regulatory tests is being explored for chemical risk assessment. Two promising methods include the fish embryo toxicity test (FET) and general and behavioural toxicity test (GBT). The present study investigates the application of the FET and the GBT to inform read-across potential between three select phthalates and three structurally similar cyclohexane-based plasticizers. Zebrafish embryos/larvae (n=12) were exposed 6–120 hours post fertilization (hpf) for the FET and 72–120 hpf for the GBT. Three technical replicates per concentration were used for both tests. Concentration-effect graphs were produced as the definitive test for each substance. As phthalates have difficult-to-test properties and potential concerns regarding stability, an uptake analysis was conducted by measuring concentrations in both the external media and larval tissue using liquid chromatography-high resolution mass spectrometry. Preliminary results for lethality, developmental and behavioural effects, as well as the uptake analysis, will be presented. This study seeks to identify appropriate opportunities

to consider using non-traditional lines of evidence to inform read-across approaches in regulatory risk assessment.

Development of a site-specific water quality limit for total dissolved solids (PO)

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In late 2017, a North American mine began treating deep, saline water from the bottom layer of a pit lake with the goal of achieving effluent quality that met permit requirements for total dissolved solids (TDS) and acute and chronic toxicity, such that this effluent could subsequently be discharged. Mining and water treatment operations are predicted to add tailings and reverse osmosis brine to the bottom layer, resulting in elevated influent TDS, which will require additional treatment to meet discharge permit limits. Therefore, this study was undertaken to propose and validate an effluent TDS limit. Predicted effluent composition was profiled to identify key TDS constituents, and literature was reviewed to determine the threshold for toxicity with a focus on key TDS constituents and test organisms used in regulatory toxicity testing (i.e., *Ceriodaphnia dubia* and *Pimephales promelas*). A preliminary TDS limit (950 $mg \cdot L^{-1}$) was developed from the lowest relevant toxicity endpoint in the literature. Toxicity testing was then conducted to refine the proposed limit. *C. dubia* and fathead minnow were exposed to effluent blends with varying ratios of treated, ultra-filtered to reverse osmosis effluent, as baseline samples and TDS-amended blends. No adverse effects on *C. dubia* survival and reproduction were observed with TDS of 1,084 to <1,231 $mg \cdot L^{-1}$ and no adverse effects were observed in fathead minnow in any treatments. These findings confirmed the desktop review results, providing information on a TDS range over which toxicity is not predicted. Accordingly, a TDS limit of 1,000 $mg \cdot L^{-1}$ was proposed.

Metabolomics analysis of the Japanese quail liver after exposure to eight environmental contaminants of concern (PO)

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Environmental metabolomics focuses on detecting system-wide biochemical changes in organisms in response to environmental stressors. Metabolomics provides a direct measure of a tissue's or an organism's phenotype at the molecular level, which could be linked to adverse outcome pathways. This study aims to improve the understanding of the liver metabolome in the Japanese quail (JQ) following chemical exposure. Eight contaminants were chosen to represent diverse natural resource/environment sectors of Canadian concern and which could impact a wide biological space. Early life stage (ELS, n=81) and adult JQ (n=130) were exposed to solvent control (dimethyl sulfoxide (DMSO) or corn oil, respectively), ethinylestradiol (EE2), chlorpyrifos (CPF), benzo(a)pyrene (BaP), lead (Pb), selenomethionine (SeMe), fluoxetine hydrochloride (FLX), trenbolone (TB) or

hexabromocyclododecane (HBCD) at three different concentrations (low, medium and high dose) for 9 and 4 days, respectively. The high dose was designed to be $<LD_{20}$. Metabolites were extracted using a 3x methanol protocol and analyzed by LC-MS (SGS-AXYS). Multivariate and univariate statistical analysis demonstrated that CPF and EE2 elicited the greatest effect on the metabolome in ELS; 34 and 10 metabolites were significantly different from the control, respectively. CPF showed the highest impact in the adult with 22 metabolites significantly different from the control, followed by FLX, TB and HBCD with respectively 18, 15 and 13 impacted metabolites. In-depth analyses are ongoing to complete the identification of statistically significant metabolites and pathways. The present work provides new information about the effect of contaminants of concern on the JQ as a part of the EcoToxChip project (www.ecotoxchip.ca).

Agricultural Contaminants: Evaluation and Reduction of Environmental Risk

Amphibians and neonicotinoids: A potentially stressful situation (PL)

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Ponds and ditches used by frogs in agricultural environments can receive agricultural contaminants via runoff during snowmelt and rain events. Given the contribution of pesticides and disease to amphibian population declines, it seems prudent to assess the effects of high-use insecticides, such as neonicotinoids, on exposed amphibian species. The objective of our study was to assess the physiological and haematological effects of two neonicotinoids on two native Canadian frogs (i.e., wood frogs (*Lithobates sylvaticus*) and leopard frogs (*Lithobates pipiens*)). Tadpoles of each species were raised separately in outdoor mesocosms for up to 10 weeks in the presence of clothianidin (CLO) or thiamethoxam (THI) at 0, 2.5 and 250 $\mu g \cdot L^{-1}$. To evaluate stress responses, water-borne corticosterone was collected and blood cell profiles were assessed. We found differing physiological and haematological responses between the two frog species in relation to duration of exposure. Wood frog tadpoles had lower concentrations of corticosterone when acutely exposed to 250 $\mu g \cdot L^{-1}$ of THI compared to controls; however, there were no differences after chronic exposure to either compound. Wood frogs chronically exposed to CLO and THI were also anaemic (albeit not at 2.5 $\mu g \cdot L^{-1}$ of CLO), and after chronic exposure at 250 $\mu g \cdot L^{-1}$ of THI they were considered mildly stressed based on elevated neutrophil to lymphocyte ratios. Preliminary results for leopard frogs suggest no changes in corticosterone after acute exposure and no anaemia after chronic stress. Hence, neonicotinoids may influence stress responses and disease susceptibility of amphibians, but species sensitivity may vary.

Evaluating the environmental risks of neonicotinoids *in vitro*: Genotoxicity in fish cell lines (PL)

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Neonicotinoids (NN) are novel insecticides widely used in agricultural and domestic settings worldwide. Unlike previously developed insecticides that were surface sprayed and non-penetrating in plants, NNs are taken up by plants and become systemic within all tissues. NNs are thought to be stable and long lasting not only within plants and animals but also in the environment, where they persist in soil and water bodies as a result of leaching, runoff, and spray-drift. NNs affect neural function in target insect pests through specific nicotinic acetylcholine receptors. However, non-receptor-mediated effects have also been reported in target and non-target species via oxidative stress mechanisms. Furthermore, genomic damage through DNA intercalation in non-target species has been suggested. Cell lines offer an

effective means of studying environmental toxicants, and as of April 2019 a fish cell line has been adopted by the International Organization for Standardization (ISO 21115) for water quality determination. In this study, NNs such as imidacloprid and thiacloprid were evaluated for potential cytotoxic and genotoxic effects using various fish cell lines. Preliminary results suggest that NNs affect fish cell lines, and both imidacloprid and thiacloprid induced genotoxic damage in the tested fish cells, but usually at higher than environmentally-relevant dosages. The responses also varied by species and tissue origins of the cell lines. Further insights into the biological effects of NNs in fish could be obtained rapidly, reproducibly, and economically using fish cell lines. It is hoped that the results of our study will further encourage regulatory bodies to develop better guidelines for NN usage.

Does exposure to agriculture pesticides impact early life stages of an endangered fish that is endemic to Quebec? (PL)

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The copper redhorse (*Moxostoma hubbsi*) (CR) is an endangered fish that is endemic to Quebec. Many anthropogenic factors may be leading to the poor natural recruitment of CR, including contamination from pesticides of their only known spawning grounds, in the Richelieu River. Here, we investigate whether current levels of pesticides in the Richelieu River have a negative impact on early life stage CR. Fertilized embryos were obtained from an artificial breeding program and exposed to river water or temperature-matched laboratory water control. Embryos exposed to river water hatched prematurely, with a median hatch time 19 hours earlier than the control group, and had a lower survival rate (73% vs. 93%). Similar but less pronounced effects were observed with river redhorse (*Moxostoma carinatum*) (RR), a related species that is classified as 'special concern' according to the Species at Risk Act. RNA sequencing was performed on 9 pools of 5 CR larvae per treatment, and 137 genes were differentially expressed. Numerous genes involved in development, growth, stress, inflammatory and immune responses were dysregulated following incubation in river water. To pinpoint possible causes of the effects we observed, a series of laboratory exposures will be performed using chemicals previously identified in the river water. This study will provide data on the effects of agricultural contaminants present in the Richelieu River and increase our knowledge on the effects they have on endangered fish.

Toxicity of neonicotinoid alternatives to *Hyalella azteca* and *Hexagenia rigida* (PL)

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Concerns regarding the widespread use and environmental presence of neonicotinoids, as well as impacts on non-target species, have led to restrictions and bans on the use of some of these insecticides. As a result, the use of neonicotinoid alternatives is increasing and there is a need to understand their

toxicity to aquatic invertebrates, which can be exposed via runoff events. The objective of this study was to assess the toxicity of five neonicotinoid alternatives to *Hyalella azteca* (amphipod) and *Hexagenia rigida* (mayfly): flupyradifurone (butenolide), sulfoxaflor (sulfoximine), cyantraniliprole and chlorantraniliprole (ryanoids), and flonicamid (pyridine). Preliminary tests indicated no acute toxicity of cyantraniliprole or flonicamid to either species up to 1000 $\mu g \cdot L^{-1}$; therefore, subsequent testing focused on flupyradifurone, sulfoxaflor, and chlorantraniliprole. We conducted water-only tests, 28-day for amphipods and 96-hour for mayflies, and assessed survival and growth of amphipods, and survival and behaviour (number of animals inhabiting artificial burrows) of mayflies. Concentrations that reduced amphipod survival and growth ranged from 16 $\mu g \cdot L^{-1}$ (28-day EC_{50} for flupyradifurone) to 516 $\mu g \cdot L^{-1}$ (7-day LC_{50} for chlorantraniliprole). In preliminary tests with mayflies, sulfoxaflor significantly altered behaviour at 100 and 1000 $\mu g \cdot L^{-1}$, and chlorantraniliprole significantly reduced survival and altered behaviour at 1000 $\mu g \cdot L^{-1}$. Although there are few data on environmental concentrations of these neonicotinoid alternatives, the information that does exist indicates that levels in surface waters are in the low $\mu g \cdot L^{-1}$ range, lower than the effect concentrations observed in this study.

Ecotoxicological effects of pesticides: Analysis of the ecological significance of surpassing water quality criteria (PL)

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In Quebec, water quality monitoring programs have demonstrated for nearly 25 years the increase in the degradation of watercourses in agricultural environments dominated by maize and soybeans, among others. For example, a recent report (Giroux, 2019) shows an increase in glyphosate concentrations and its degradation product AMPA, imazethapyr and S-metolachlore, in four agricultural rivers of Montérégie (Québec). These four pesticides, as well as atrazine and neonicotinoids, were present in more than 90% of the samples. Although a very slow decrease in other pesticides (atrazine, dicamba and 2,4-D) has been measured, guidelines for the protection of aquatic life (chronic effects) (GPAL) are exceeded for thirteen pesticides (atrazine—forbidden in Europe since 2003), metribuzine, S-metolachlore, glyphosate, neonicotinoides (clothianidine, thiamethoxame, imidaclopride), chlorantraniliprole, chlorpyrifos, carbaryl, diazinon, malathion and perméthrine. In the St. Lawrence River, 99% of the samples contained pesticides, and 31% of the samples exceeded the GPAL for neonics (Montiel-León et al., 2019). However, what is the ecological significance of these excess levels given that the criteria for aquatic quality of life vary from jurisdiction to jurisdiction? This presentation will report on the systematic analysis of the criteria calculated using different methods for different pesticides, including glyphosate, atrazine and S-metolachlore, in order to analyze the scientific reasons for the differences. Some hypotheses will be explored to anticipate the ecotoxicological effects when water quality criteria are exceeded.

Retention ponds in the agroecosystem: Ecotoxicological implications on an amphibian species (PL)

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Retention ponds are becoming more frequently implemented in the agroecosystem as a mitigation strategy to reduce the impacts of agricultural runoff entering surface waters. One of the consequences of this technology is the attraction of wildlife to these constructed wetlands (i.e., as breeding grounds for amphibians). Therefore, the main objective of this research project is to study the effects of water from an agricultural retention pond on larval amphibians. Pond water quality has been assessed for 3 summers, and chemical analyses revealed the presence of a suite of pesticides, including organophosphorus pesticides (e.g., atrazine, glyphosate) and emergent pesticides (e.g., neonicotinoids). Chronic exposures to this retention pond water were performed on the American toad (*Anaxyrus americanus*) from the free-feeding stage throughout metamorphosis. Simultaneously, an *in situ* chronic exposure was conducted by housing tadpoles in mesh cages that were deployed at the field site. During the exposure, survival, developmental, and morphological endpoints were assessed. Preliminary data suggest that treated toads were significantly smaller in morphometric indices and completed metamorphosis earlier compared to control animals. At metamorphic climax and the completion of metamorphosis, liver tissue was collected and transcript level was measured for a suite of genes related to energy metabolism and thyroid hormone signaling. Urogenital-complex was also sampled and gonadal histology was assessed. Finally, anti-predatory behavioural assays were conducted at both tadpole and juvenile stages. This work provides ecologically relevant information about the potential effects of agricultural activities on a Canadian amphibian species.

Effects of ditch management in agroecosystems: From water quality to frog health (PL)

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Industrial agricultural land-use has caused extensive loss of natural wetlands in Ontario, such that vegetated ditches are some of the only remaining features in agroecosystems where wetland plants and wildlife can exist and proliferate. Ditch management regimes (i.e. removal of vegetation and dredging of sediments to improve water flow) disturb many ecological services, particularly habitat provision and water purification. My research aims to understand how such management practices may change water quality and how this may impact wildlife, by using the northern leopard frog (*Lithobates pipiens*) as a bioindicator. Ditches under different management (vegetated, treeless, and dredged) were studied over two years. In each ditch, water quality parameters and frog development were monitored using in-situ cages. Preliminary results indicate that 26 different pesticides were detected in the ditch waters, with atrazine, propazine, metolachlor, and atraton most frequently detected. Temperature was higher and turbidity lower at treeless sites compared to maturely vegetated sites. Interestingly, at the treeless site

there was higher tadpole survival, body size and development than at the mature site. This research expands the Watershed Evaluation of Beneficial Management Practices (Agriculture and Agri-Foods Canada) in the assessment of the economic and environmental benefits of naturalized waterways and reduced dredging. It will help guide policies to improve water quality and biodiversity without compromising crop yields.

The use of a retention basin to capture agricultural contaminants from surface waters (PL)

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The use of fertilizers and pesticides is increasing in an era when agriculture is intensifying to meet the demands of a constantly growing population. Fertilizers mainly include nitrogen and phosphorus, which can cause disruptions in the functioning of aquatic ecosystems and, in excess amounts, can deteriorate water quality. Pesticides that are found in aquatic environments can have toxic effects on non-target organisms. Aquatic systems are also exposed to increasing inputs of organic matter and suspended particles from eroding soils. The purpose of this research project is to evaluate the efficiency of an agricultural retention basin at Saint Samuel in reducing the supply of nutrients, pesticides and particles to the Nicolet River, which is a tributary of the St. Lawrence River and flows into Lake Saint Pierre. This project also aims to quantify the greenhouse gas emissions (CO_2, CH_4, N_2O) from the basin. The general hypothesis is that the retention basin behaves like a wetland capable of treating contaminants, and therefore that there is a considerable reduction in nutrients, pesticides and particles. Several chemical, biological, ecotoxicological and microbiological analyses are carried out on the water and sediments at the entrance and exit of the basin to calculate the abatement, and in the basin itself for understanding its functioning and dynamics along the open-water season. This project is the result of a collaboration between the Institut national de la recherche scientifique, Agriculture and Agri-Foods Canada, and Centre d'expertise en analyse environnementale du Québec.

Menacées en milieu rural, les abeilles domestiques (*Apis mellifera*) vivent-elles mieux en ville? (PL)

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Depuis de nombreuses années, la santé des abeilles est au cœur des débats. La surmortalité de l'abeille domestique (*Apis mellifera*) à l'échelle planétaire a fait l'objet de nombreuses recherches afin d'en déterminer les causes. Aujourd'hui, il est reconnu que ce phénomène est la conséquence d'un ensemble de facteurs. Avec des recherches principalement réalisées sur des abeilles en milieu ruraux, il a été démontré que ces dernières sont fragilisées par l'usage des pesticides (néonicotinoïdes) et par l'appauvrissement de la biodiversité florale liée à l'agriculture. Depuis peu, l'apiculture urbaine est en plein essor. Toutefois, nous ne savons pas si les abeilles sont en meilleure santé dans cet environnement

où l'usage des pesticides est moindre, mais où d'autres contaminants comme les métaux dominent. Ce projet vise à déterminer si le milieu dans lequel vivent les abeilles influence la réponse de biomarqueurs sélectionnés pour évaluer leur état de santé. À l'été 2018, des abeilles (adultes et larves) ont été échantillonnées dans des ruches de la région des Laurentides (milieu ruraux) et de l'Ile de Montréal (milieu urbain). Les métaux ont été mesurés dans les tissus et à l'aide des biomarqueurs (la métallothionéine, les caroténoïdes, les rétinoïdes, les tocophérols et la peroxydation des lipides), et nous avons comparé les « réponses » entre les butineuses et les larves des milieux urbains et ruraux. Nos résultats démontrent que la peroxydation des lipides et la concentration de métallothionéine sont significativement plus élevées chez les abeilles vivant en milieu urbain, en lien avec une plus grande concentration de métaux.

Strength of methods assessments for freshwater aquatic primary producer toxicity data: A case study with atrazine studies from the peer-reviewed literature (PO)

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Improving the quality of pesticide toxicity studies is a shared goal in ecotoxicology and a priority for risk assessors. Using the herbicide atrazine and freshwater primary producers as a case study, we developed a transparent scoring system for assessing the quality of peer-reviewed studies. While many studies (147) reported experimental data fitting basic inclusion criteria, only a small proportion provide sufficient information on the test substance, test organism, and test results to be considered of sufficient quality for decision-making (i.e., a minimum score of > 8 out of 16, with no critical study weaknesses). Optimal studies for use in first-tier risk assessment were identified for each taxonomic group as the highest-scoring study scoring > 8, that also used the technical-grade active ingredient, reported an EC_{50} for a population-level endpoint (e.g., cell density, dry weight), and an exposure period consistent with standard tests (≤ 96-hours for algae, ≤ 14-days for macrophytes). Ultimately, 22 studies (four periphyton, ten macrophytes, and eight phytoplankton) achieved scores > 8. Finally, registrant studies were evaluated and, in many cases, were most appropriate for risk assessment, with the greatest scores observed for their respective species. This exercise highlights the importance of defining and identifying well-performed toxicity tests, illuminating knowledge gaps, and reporting high quality data in support of the risk assessment process outside of the standard regulatory framework. We recommend that researchers thoroughly review available literature before initiating new toxicity testing with atrazine to ensure the need and the ability to improve on previous work.

Toward a Better Prediction of Metal Bioavailability and Toxicity to Aquatic Organisms

Influence of water chemistry on the bioavailability of technology-critical elements (PL)

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The growing use of rare earth elements and other technology-critical elements such as In, Ge and Ga in personal electronic devices, green technologies, and medical applications results in a developing concern for impacts in aquatic environments. However, there are no water quality guidelines/criteria for technology-critical elements (TCEs) and a poor understanding of their bioavailability. The objective of this research is to contribute data towards the establishment of assessment tools for the effects of TCEs. In this presentation we report on our studies with gallium and germanium and their effects on sensitive invertebrates (*Hyalella azteca* and *Daphnia magna*). Acute and chronic tests followed standard methods and were done in a reconstituted medium (0.5 mM Ca and Na, and 0.13 mM Mg at pH 7.3). The toxicity modifying influences of cationic competition (with additions of Ca) and complexation by dissolved organic matter (DOM) were assessed. Similar to the rare earth elements (REEs), Ge tests solutions were associated with precipitation which made determination of bioavailability more challenging compared to Ga. For both elements acute toxicity was in the low $mg \cdot L^{-1}$ range; about 3-5 $mg \cdot L^{-1}$ for Ga and 8-12 $mg \cdot L^{-1}$ for Ge. *H.azteca* were more sensitive than *D. magna*. DOM, tested at 10 mg $DOC \cdot L^{-1}$, provided a moderate protective effect against Ga toxicity but had no modifying influence on the effects of Ge.

Biotoxicity of the rare earth elements neodymium, thulium, and yttrium toward microalgae (PL)

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The use of rare earth elements (REEs) in electronic devices, clean energy technologies, and various other industrial applications is increasing. Canada currently hosts many mining projects at different stages of development. However, the use of these metals may have toxic effects on aquatic organisms and there are few studies available. In this project, we are investigating the ecotoxicological effects of three REEs on two unicellular microalgae, *Chlorella fusca* and *Chlamydomonas reinhardtii*: neodymium (Nd) as representative of light REEs, thulium (Tm, a heavy lanthanide), and yttrium (Y). Phosphates (PO_4^{3-}) are indispensable ions for microalgal growth but they cause a precipitation of the metal of interest. Thus, to circumvent this problem, the inorganic form of phosphorus is substituted with an organic source: β-glycerol phosphate. Microalgae were exposed to metals (0.1 to 5 µM) during 120 hours and monitored for growth. The software MINEQL + 5.0 was used to model the speciation of each metal. Experimental exposure concentrations and accumulated REEs were measured by Inductively

Coupled Plasma Mass Spectrometry (ICP-MS). Based on algal growth, dose response curves were obtained which allowed us to compare the respective REE toxicity values (EC_{50}). As expected, growth inhibition of *C. fusca* increased with increasing accumulation of Nd and Tm. Based on the average of metal concentration, we obtained 1.53 µM [95%CI = 0.69-2.65] and 1.51 µM for Nd expressed as a function of the total or free-ion concentration, respectively. With regards to Tm, these values were 0.69 µM [0.62-0.76] and 0.52 µM [0.46-0.57]. Previous work performed in the same conditions by our team showed that cerium (Ce) and lanthanum (La) are more toxic than Tm and Nd. Further experiments will be run to determine the toxicity of metals of interest on *Chlamydomonas reinhardtii.* Finally, we will examine microalgal growth in a medium containing both Nd and Tm.

Chronic toxicity of rare earth elements (PL)

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Very little ecotoxicological data is available for rare earth elements (REEs), and information on the sensitivity of aquatic organisms to chronic REE exposures is scarce. This presentation will summarize the findings of chronic toxicity tests with four REEs (lanthanum, neodymium, yttrium and cerium), including testing with invertebrates (a cladoceran [7-day *Ceriodaphnia dubia*], a mayfly [14-day *Neocloeon triangulifer*] and a clam [28-day *Sphaerium simile*]), with two salmonid fish (brown trout [28-day *Salmo trutta*] and coho salmon [35-day *Oncorhynchus kisutch*]) and a non-salmonid fish (fathead minnow [7-day *Pimephales promelas*]). In addition, testing with two benthic organisms, *Hyalella azteca* and *Chironomus dilutes,* in water-only and sediment exposures will be presented. Discussion will touch on REE solubility in toxicity testing, REE partitioning in the sediment exposures, comparisons of the toxicity of the four REEs tested, differences in organism sensitivity, and a possible connection to organism sensitivity and their ecosystem zone. In addition, a novel approach for a lowest effects concentration (LECx) is applied across the dataset to help define possible benchmarks for the REEs.

Role of humic acid on uptake and toxicity of platinum in freshwater microalgae (PL)

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There is a growing interest in platinum group elements (PGEs) in ecotoxicology. The demand for these rare metals is very high, especially for catalysts in the automobile industry, leading to environmental contamination owing to their release with exhaust fumes. PGEs are mostly emitted as particles, but as they reach aquatic ecosystems they can undergo transformations and interact with natural organic matter (NOM). According to the biotic ligand model, the formation of complexes reduces metal bioavailability to living cells. As a consequence, global toxicity should be minimized in presence of NOM, but several observations with PGEs suggest otherwise. This study focused on uptake and toxicity of platinum for three microalgae exposed to a range of platinum concentrations up to 200 $\mu g \cdot L^{-1}$. Two green algae, *Chlorella fusca* and *Chlamydomonas reinhardtii,* and a periphytic diatom,

Nitzschia palea, were studied as test organisms. Growth inhibition and platinum internalization were determined at three concentrations (0, 10 and 20 mg C·L^{-1} of humic acid ((HA); Suwannee River humic acid). In parallel, platinum speciation in the presence of HA was determined experimentally using a partial ultrafiltration method. To better understand the impact at the cellular level, transcriptomic analyses were also performed on *C. reinhardtii* and *N. palea*. Results show that the toxicity of platinum is enhanced in presence of HA for both green algae, while a protective effect was observed for the diatom. The role of the silica frustule surrounding the cell membrane will be investigated as a potential explanation for the difference in HA impact on PGEs uptake and toxicity compared to green algae. These results appear to be in conflict with the biotic ligand model, demonstrating the importance of continuing such studies in order to better predict bioavailability of metals in the presence of complex ligands such as NOM. Also it raises the issue of the impact of platinum on microalgae in realistic environmental conditions (with ubiquitous NOM), primary producers being of great ecological importance.

Using advanced analytical chemistry techniques to link metal speciation to toxicity: A case study (PL)

Andrew Nagel[1], Chad Cuss[1], Greg Goss[1], William Shotyk[1], Chris Glover[1,2]

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The toxic fraction of waterborne metals is functionally defined as that which passes through a 0.45 µm filter. However, it is understood that this "dissolved" fraction includes metals bound to colloids and other particles which may have limited bioavailability, and thus toxic impact. Using Asymmetric Flow Field Flow Fractionation (AF4) under ultraclean metal-free conditions, combined with acute and chronic toxicity assays, the relationship between thallium (Tl) speciation and toxicity was examined. Initially, standard acute (48-hour) toxicity assays conducted on neonate *Daphnia magna* were performed to establish a median lethal effect concentration (LC_{50} in mg C·L^{-1}). Thereafter, this LC_{50} value was used to assess how water chemistry factors influenced Tl toxicity. Despite changes in inorganic cation and anion levels and concentrations of dissolved organic matter, *D. magna* sensitivity to Tl was largely unaltered. These outcomes were, however, consistent with speciation analyses that showed little complexation of Tl with inorganic or organic ligands. This finding has potentially important regulatory implications, suggesting that measurements of site-specific water chemistry will add little to assessments of Tl risk in natural settings. To assess the relationship between speciation, bioavailability, and toxicity, chronic (21-day) Tl exposures were performed. These studies showed that exposure to Tl concentrations of 8 µg·L^{-1} resulted in inhibited growth, impaired reproduction and delayed time to first brood. These effects were related to whole animal Tl burden. These data indicate robust relationships between Tl speciation, bioavailability, and toxicity in a model toxicological organism.

Subcellular metal distribution in wild fish exposed to an environmental metal gradient: Relationships to biological effects to better predict toxicity to aquatic organisms (PL)

Nastassia Urien[1], Alice Urien[1], Lisa Ramilo[1], Helga Sonnenberg[1], Peter Campbell[1], Patrice Couture[1]

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Toxic responses to metal accumulation may vary depending on the nature of the subcellular ligands to which metals bind; interactions of metals with cytosolic molecules and organelles may cause deleterious effects. Alternatively, detoxification may take place when the metals are sequestered by biomolecules designed to limit their toxicity. The objective of the present study was to investigate the links between the binding of metals to particular subcellular fractions and the expression of metal-related effects. To this end, the subcellular partitioning of cadmium (Cd) and selenium (Se) among putative metal-sensitive fractions (MSF) and biologically detoxified fractions (BDM) in livers of white suckers (*Catostomus commersoni*) collected from lakes characterized by an environmental metal gradient was determined after differential centrifugation and heat-denaturation steps. A suite of biomarkers was also investigated, ranging from general indicators of energy accumulation to specific indicators at the biochemical level. Relationships were investigated with principal component analysis (PCA) and simple regressions. Subcellular metal partitioning was similar among areas but specific to elements; over 70% of the Cd burden was found to be detoxified. In contrast, 75% of liver Se burden was associated with MSF, suggesting that exposed fish were likely subject to stress. The PCA showed that increasing [Se] in all fractions was strongly correlated with lower fish condition and associated with higher oxidative stress, suggesting a trade-off between growth and control of oxidative stress. The potential of this approach to advance our understanding of the toxic modes of action of metals in aquatic organisms and to better predict toxicity will be discussed.

Effects of selenium on pelagic and zooplankton associated microbiotas: A lake mesocosm study (PL)

Yuwei Xie[1], Stephanie Graves[1], John Giesy[1], David Janz[1], Vince Palace[2], Markus Hecker[1], Paul Jones[1]

[1]*University of Saskatchewan,* [2]*International Institute for Sustainable Development–Experimental Lakes Area*

Microbiota play important roles in maintaining homeostasis of host organisms and ecosystems. Selenium (Se) is bio-accumulated in primary aquatic consumers mainly through their diet (e.g., microorganisms, detritus) and can be biomagnified in concentrations that can cause reproductive failure and teratogenicity effects in vertebrates. However, important knowledge gaps remain concerning the ecological effects of Se on both pelagic- and zooplankton-associated microbiota because of the massive complexity of microbiota. Here, we conducted a lake mesocosm study to characterize the effects of Selenium (Se) on pelagic- and zooplankton-associated microbiota. Se was added to freshwater boreal lake mesocosms as selenite (IISD-Experimental Lake Area, Manitoba, Canada) following a regression design (concentrations: 0, 0.5, 1, 2, 4, 7, and 10 μg $Se \cdot L^{-1}$). Water and zooplankton were sampled at multiple time points to study both short-term (3 and 7 days-post-exposure (dpe)) and medium-term (21 and 63 dpe) ecological responses to Se addition. Bacterial, algal and protozoan

communities were characterized by metagenomics. Composition, alpha- and beta- diversities of microbial communities were compared to tracking the disturbed changes following the exposure of Se. This study presents comprehensive microbial ecological responses of controlled mesocosms to Se and increase the understanding of how environmental stressors impact microbial food webs and interactions between zooplankton and their associated microbiota.

Trophic dynamics of selenium along a gradient of exposure concentrations in a boreal lake ecosystem (PO)

Stephanie Graves[1], Karsten Liber[1], Vince Palace[2], Markus Hecker[1], Lorne Doig[1], David Janz[1]

[1]University of Saskatchewan, [2]International Institute for Sustainable Development

Selenium (Se) is both an essential micronutrient and a contaminant of concern, and is of particular interest in mine-affected waterbodies in Canada. The objective of this research was to characterize the trophic dynamics of selenium along a gradient of exposure concentrations in a Canadian boreal lake ecosystem. From June 20 to August 22, 2018, six limnocorrals (littoral, ~3000 L enclosures) were spiked with mean measured concentrations of 0.4, 0.8, 1.6, 3.1, 5.2 and 7.9 μg $Se \cdot L^{-1}$ as selenite, with three untreated controls (background aqueous Se = 0.09 $\mu g \cdot L^{-1}$). Water, periphyton, phytoplankton, sediment, benthic macroinvertebrates, zooplankton and female finescale dace (added on day 21 of the experiment) were collected throughout and at the end of the experiment. Total selenium was measured in all matrices. Non-linear (polynomial) regressions and generalized additive models were used to determine the relationships between aqueous and biota Se. Preliminary results show significantly greater enrichment of Se by phytoplankton relative to periphyton, and taxonomic differences in accumulation of Se by invertebrates (Heptageniidae > Chironomidae > Zooplankton). Variability in bioaccumulation of Se by fish and relationship with shifts in diet along the gradient of Se exposure concentrations will be discussed. This research provides new information on patterns of enrichment and trophic transfer of Se over a gradient of exposure concentrations and will aid in the prediction of Se exposure and risk assessment under similar environmental conditions.

Use of remote sensors to monitor changes in water quality in a metal-contaminated stream (PO)

Ademola Adekunla[1], Carrie Rickwood[2], Boris Tartakovsky[1]

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There are many barriers to consistently monitoring water quality in freshwater ecosystems. For example, the logistical and financial constraints to deploying field teams on a regular basis results in only a snapshot of water quality at the time of sampling. The development of remote sensors is a growing area of research, which has tremendous benefits for continuous real-time water quality monitoring. The National Research Council of Canada is currently developing a microbial fuel cell (MFC)-based biosensor designed to indicate toxicity of water-containing metals and other inorganic and organic compounds. To further its development, field-deployment at Junction Creek in Sudbury,

ON, alongside lab-validation analysis and toxicity tests at CanmetMINING laboratories have been conducted. Junction Creek receives multiple sources of mine effluents throughout its course and is also subject to intermittent releases of metals from historically contaminated tributaries. Three locations in Junction Creek were identified for sensor deployment. Water was collected weekly by the Junction Creek Stewardship Committee and sent back to CanmetMINING in Ottawa for metal analysis and toxicity testing using *Daphnia magna*. The results from both the field deployment and lab validation studies will be presented. The development of the MFC-based biosensor shows promising results that can provide information on temporal changes occurring in this area that may not have been detected with conventional water sampling.

Behavioural Ecotoxicology

La valvométrie comme outil de mesure du comportement de fermeture des bivalves, en tant que trait biologique lié à la résistance à la pollution chimique: Expérience avec la moule bleue (*Mytilus edulis*) et le pétoncle géant (*Placopecten magellanicus*) exposés au dispersant chimique Corexit 9500, sous conditions hivernales (PL)

Richard Saint-Louis[1], Guillaume Durier[1], Jean-Bruno Nadalini[1], Réjean Tremblay[1], et Luc Comeau[2].

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L'intégration des traits biologiques (propriétés mesurables d'un organisme) dans l'évaluation des impacts de la pollution sur les écosystèmes marins est en développement et présente un intérêt grandissant. L'objectif de notre projet est de démontrer, par l'analyse par valvométrie du mouvement des valves, que le comportement de fermeture des bivalves, incluant l'herméticité, est un trait biologique important pour prédire la résistance d'un bivalve face à la pollution chimique. Nous avons choisi la moule bleue (*Mytilus edulis*) et le pétoncle géant (*Placopecten magellanicus*) comme bivalves modèles; la moule peut fermer hermétiquement ses valves, ce que ne peut faire le pétoncle. Nous avons exposé les bivalves au dispersant chimique Corexit 9500, utilisé pour disperser les nappes de pétrole, à des concentrations de 10, 50 et 250 $mg \cdot L^{-1}$ d'eau de mer filtré, pendant 48 heures, à une température de l'eau variant de 5°C (décembre) à 3°C (février). À la concentration la plus élevée de Corexit 9500, la mortalité des pétoncles a été de 13/16 individus (81%) alors qu'elle a été de 2/16 individus (12%) pour la moule. Les résultats des mesures par valvométrie montrent que les deux espèces réagissent rapidement, en quelques minutes, à la présence du contaminant par la fermeture des valves. Cependant, la moule se distingue du pétoncle par un nombre de fermetures plus élevé et une durée totale de fermeture plus longue. Les bivalves qui ont ce même trait biologique que *M. edulis* auraient une résistance accrue à la pollution chimique, que ceux qui partagent le trait biologique de *P. magellanicus*.

Effects of cadmium on the escape response, foraging, and shelter-use behaviours of juvenile American lobster, *Homarus americanus* (PL)

lla Maltby[1], Jim Williams[1], Russell C. Wyeth[1], Meg Davies[1]

[1]St. Francis Xavier University

Bioassays that use animal behavior can potentially be sensitive tools to detect the presence of industrial contaminants, allowing remedial actions to be taken before widespread impact. We are developing behavioural bioassays using the American lobster, *Homarus americanus*, which is an important economic resource in Eastern Canada. The juvenile lobster escape response (known as the tailflip) can be repeatedly and consistently stimulated, making it an ideal behavior to use in a bioassay. Foraging and shelter-use behaviours are also critical for the growth and survival of juvenile lobsters and a potentially valuable means to test for additional toxicological effects. By using quantitative video analysis of movements relative to both shelter and food in repeated laboratory trials, we are testing

whether these behaviours are also suitable for a bioassay. In May 2019, juvenile lobsters (15-45mm carapace length) were collected from Ballantynes Cove, NS, and housed individually in a cascading system. Lobsters were either held in 1 $mg \cdot L^{-1}$ cadmium-spiked seawater (n=11) or clean seawater (n=11) for eleven weeks. Analysis is currently being performed to determine if there are any significant behavioural changes after long-term exposure to cadmium. Some lobster mortality occurred in the cadmium treatment between weeks nine and eleven, likely due to cadmium interfering with ecdysis. If successful, these bioassays can be used to determine if lobsters from other areas have been exposed to cadmium. Moreover, such results would suggest it is possible that behavioural bioassays could be more widely applied to other animals to assess contaminant exposure.

Does temperature influence the impact of metals in northern and temperate salmonid fish species? (PL)

Derek Alsop[1], Cathy Lin[1], Joanna Wilson[1]

[1]McMaster University

A number of studies have shown that olfactory function in temperate fish is impacted by low concentrations of waterborne metals. Recent work in our lab has also demonstrated impacts of copper and cadmium on olfactory-mediated behaviours in Arctic charr (*Salvelinius alpinus*; northern species) and lake whitefish (*Coregonus clupeaformis*; temperate-to-northern species). In these experiments with food odours and a Y-maze, Arctic charr were more sensitive than lake whitefish to both metals. Impacts occurred at metal concentrations that were also the thresholds for increased tissue metal burdens. Current experiments with rainbow trout (*Oncorhynchus mykiss*; temperate species), Arctic charr and lake whitefish are exploring whether water temperature influences metal impacts on olfactory-mediated behaviours and tissue metal burdens. The goals of our project are twofold: 1) to determine the relative sensitivity of northern fish species, and 2) to understand the influence of climate change on the impacts of pollution to Arctic fish.

A burning issue: the effect of UV-filter exposure on the behavioral responses of vertebrate and invertebrate freshwater species (PL)

Danielle Philibert[1,2], Aaron Boyd[1], Tamzin Blewett[1], Keith Tierney[1]

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Sunscreen is used worldwide to mitigate the effects of ultraviolet (UV) radiation. Protective chemical agents and physical filters in cosmetic products are applied to the skin to decrease the prevalence of malignant melanoma and other sun exposure-related pathologies. The widespread use of UV filters has led to increased environmental concentrations of these compounds, and sunscreen use could have indirect effects on both vertebrate and invertebrate aquatic species. Previous studies have suggested that UV filters are endocrine disrupting compounds and could be neurotoxic to aquatic life. In this study, we compared the developmental and behavioral effects of exposure to common UV filters (oxybenzone, avobenzone and octocrylene) on both fish (*Danio rerio*) and Daphnia (*Daphnia magna*).

Exposure to UV filters had both chronic effects and persisting effects on the thigmotaxic behavior of neonate and adult Daphnia and altered the basal activity of zebrafish larvae. The effects of UV-filters on the behavioral responses of aquatic organisms is poorly understood, and further research is needed to elucidate the mechanism of action.

The olfactory-mediated behavioural dysfunction of copper nanoparticle-exposed rainbow trout integrates with internal biological impairments (PL)

Parastoo Razmara[1], Jacob Imbery[2], Emily Koide[2], Caren Helbing[2], Greg Pyle[1]

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

Similar to dissolved copper (particularly Cu^{2+}), copper nanoparticles (CuNPs) can disrupt fish olfactory-mediated behaviour. However, it is not clear whether the CuNP-induced behavioural change is linked to other levels of biological dysfunction. This study investigated the comparative olfactory-driven behavioural mechanism of CuNPs and Cu^{2+} in rainbow trout. Fish were exposed to equitoxic concentrations of Cu contaminants that induced 50% inhibition of olfactory sensitivity for 24 hours or 96 hours. Following the 96-hour exposure, differential gene expression was studied in the olfactory epithelium (OE) using RNA-Seq which showed approximately 2% of transcripts differentially expressed in the CuNP- and Cu^{2+}-exposed fish, with only a small amount in common between the two treatments. Transmission electron microscopy revealed CuNPs can be taken up by epithelial cells and stored in lysosomes and endosomes. Copper concentration analysis demonstrated that CuNPs, but not Cu^{2+}, were significantly accumulated within OE over both exposure periods. For both forms of Cu, the density of mucous cells was significantly increased over 24 hours, but not 96 hours. The results of the neurophysiological assay (measured by electro-olfactography) demonstrated similar olfactory impairment over 24 hours for both Cu^{2+} and CuNPs. After 96 hours of exposure, fish exposed to Cu^{2+} showed a partial recovery of olfactory function, whereas those exposed to CuNP showed a time-dependent deterioration. Overall, these results indicated that the CuNP-induced behavioural impairment was a result of transcriptional, histopathological, neurophysiological changes that occurred in response to Cu accumulation in the OE. Furthermore, CuNPs and Cu^{2+} exert a different mechanism of effects on the OE.

Amphibian vocalizations: Novel ecotoxicological bioindicators (PL)

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The vocalizations of male frogs and toads are critical for attracting mates and reproducing. Consequently, disruption of such vocalizations by pollutants have been of concern since Rachel Carson's 1962 book *Silent Spring*. Two case studies of disrupted amphibian vocalization will be presented. Adult male *Silurana (Xenopus) tropicalis* were exposed to control conditions or a commercial mixture of naphthenic acids (NAs), an industrial contaminant from petroleum. Mating calls were induced by injection of human chorionic gonadotropin (an analog of luteinizing hormone) and recorded using

underwater microphones. The NAs reduced or completely inhibited calling. We also studied premetamorphic tadpoles of *Ceratophrys ornata* (Argentinian horned frog), the first vertebrate known to vocalize as larvae. Sounds are emitted during conspecific interactions and when touched to experimentally simulate an interaction, perhaps to avoid siblicide in this highly aggressive, carnivorous, and cannibalistic species. Exposure to the acetylcholinesterase inhibitor chlorpyriphos (CPF; insecticide) disrupted this unique behaviour. In addition to standard ecotoxicological endpoints in *C. ornata* tadpoles (mortality, swimming, abnormalities and growth inhibition), we show that sound production is extremely sensitive to water-borne CPF. For both examples, potential mechanisms of disruption will be discussed.

Differential effects of bisphenol-S (BPS) on memory function and synaptic plasticity in adult female zebrafish (PL)

Mohammad Naderi[1], Arash Salahinjead[1], Anoosha Attaran[1], Som Niyogi[1], Douglas Chivers[1]

[1]*University of Saskatchewan*

Bisphenol-S (BPS) was deemed a safe alternative for the endocrine disruptor bisphenol A (BPA) and can be found in many products labeled as “BPA-free”. However, recent evidence indicates that BPS can also disrupt neuroendocrine systems in several organisms. In this study, we aimed to investigate the effects and underlying mechanisms of chronic exposure to BPS on learning and memory functions in adult female zebrafish. To this end, fish were exposed to either vehicle (DMSO), estradiol (1 $\mu g \cdot L^{-1}$), or BPS (1, 15 and 30 $\mu g \cdot L^{-1}$) for 120 days. Fish memory was tested in object recognition and object placement tasks. In order to get insights into the mechanisms underlying the effects of BPS on the central nervous system, we examined the changes in ERK/CREB/BDNF pathway, which mediates the effects of neurosteroids on synaptic plasticity and memory formation. While the exposure to estradiol and the lowest concentration of BPS improved learning performance in fish, learning impairment was observed in fish treated with the highest concentration of BPS. Improvement and impairment of learning were associated with an increase and decrease in the phosphorylation of ERK and CREB proteins, respectively. Moreover, an upregulation in the expression of BDNF gene in the brain was recorded with improved memory performance in fish. However, this gene was downregulated in fish treated with the highest concentration of BPS. Collectively, our results showed that BPS can affect the learning performance of zebrafish in a dose-dependent manner through alterations in the synaptic plasticity.

Summer vs. winter: How do fish respond physiologically and behaviourally to wastewater exposure under two seasonal regimes? (PO)

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[1]McMaster University, [2]University of Waterloo, [3]Environment and Climate Change Canada

Municipal wastewater treatment plant (WWTP) effluents are one of the largest (by volume) anthropogenic sources of aquatic pollution in Canada. Conventional WWTPs are not capable of fully removing particular water-borne contaminants, such as many pharmaceuticals and personal care products. As a result, WWTP outfalls often become major point sources of contaminants into effluent-receiving watersheds. Wastewater can impact fish and other aquatic organisms on multiple levels of biological organization, including but not limited to endocrine disruption, metabolic and behavioural dysfunction, as well as population- and community-level disturbances. However, much of the research conducted to date has not considered the effects of seasonal variation on fishes' responses to wastewater contamination, despite how relevant and overwhelming its effects can be. Our research aimed to address this issue by conducting a laboratory-based experiment in which fathead minnow (*Pimephales promelas*) were chronically exposed for 21 days to 0%, 25%, and 50% concentrations of wastewater under two thermal regimes (summer: 20°C; winter: 4°C). Various fitness-linked behavioural traits were examined following the exposures, including boldness, locomotor activity, sociability, foraging, and predator responses. We also assessed various whole-organism physiological traits that are strongly linked to our behavioural markers such as resting and maximal metabolic rates, aerobic scope, and critical thermal maximum. We examined behavioural and physiological impacts of wastewater exposure in tandem to gain a more holistic and ecologically-relevant understanding of how aquatic organisms respond to anthropogenically-induced stressors across different seasons. Our research will be able to further bridge the gap between controlled lab experiments and highly dynamic real-world field-based studies.

Use of valvometry (study of valve movements) to estimate toxins effect and to develop an early detection system (PO)

Guillaume Durier[2], Jean-Bruno Nadalini[1], Richard Saint-Louis[1], Damien Tran[2], José Babarro[3], Luc Comeau[4], Réjean Tremblay[1]

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A non-invasive valvometry apparatus that concurrently measures the magnitude of valve openness is a method of interest for evaluating the impact of toxins and detecting the presence of characteristic behaviours. This early detection system could allow for faster action in avoiding toxicity to consumers. As demonstrated during Richard Saint-Louis's presentation, valvometry may be a technology for testing the toxicity of different products, such as Corexit oil dispersant. However, this method generates thousands of data, and development of bioinformatics tools is essential to quickly identify particular behaviours of bivalves studied. This methodology is also applicable with toxic algae. The impact of saxitoxin, a paralytic shellfish poison (PSP) from *Alexandrium* dinoflagellates, is currently studied on

Mytilus edulis mussels. Data similar to that for Corexit are obtained and demonstrate gaping change when the saxitoxin concentration increases. Thus, we use the knowledge of these behaviours that are characteristic in the presence of toxins to develop an early detection system. A valvometry system that automatically transfers real-time data on valve movements of sentinel bivalves in the laboratory, through a mobile network, will allow rapid action in response to toxins.

Effects of Contaminants and Other Stressors on the Microbiome in the Environment and Organisms

Fathead minnow gut microbiota and the impacts of exposure to aqueous and dietary benzo[*a*]pyrene (PL)

Abigail DeBofsky[1], Yuwei Xie[1], Jonathan Challis[1], Chelsea Grimard[1], Markus Brinkmann[1], Markus Hecker[1], John Giesy[1]

[1]*University of Saskatchewan*

Gut microbial communities are responsible for regulating a number of beneficial functions, from development of an organism to maintaining energy homeostasis. However, little is known about the impact of chemical exposures on the structure and function of gut microbiota of fishes. To ascertain a link between contaminant exposure and microbial disruption, fathead minnows (*Pimephales promelas*) were aqueously exposed to low concentrations of benzo[*a*]pyrene (BaP) (concentrations ranged from 0.0226 $\mu g \cdot L^{-1}$ to 2.1 $\mu g \cdot L^{-1}$) and dietarily exposed to much higher concentrations of BaP (concentrations ranged from 1 $\mu g \cdot g^{-1}$ to 1000 $\mu g \cdot g^{-1}$). Gut microbiota were assessed using 16S rRNA metagenetics. Low-level aqueous exposure to BaP did not result in a significant shift in microbiota, but the results did elucidate that sex is a significant driver in community composition of bacteria ($p < 0.05$). Higher concentrations of BaP via a dietary exposure route resulted in reduction in alpha diversity at the highest exposure level ($p < 0.01$) as well as significant differences in beta diversity ($p < 0.05$). This research illustrates that in addition to the well-studied molecular endpoints, community composition of fish gut microbiota can also be impacted by chemical stressors, providing an additional pathway for the generation of adverse effects.

Assessing the impact of molecular stressors on microbiomes (PL)

Frédéric Raymond[1]

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The response of complex microbial communities to external factors such as xenobiotics or contaminants is multifaceted and intricate. Potential impacts of these molecules include modulation of microbiome composition, selection of specific functional features, and actual modification of phenotype, which can affect the health of organisms or ecosystems. Using the impact of the antibiotic cefprozil on the gut microbiome as a model for microbiome perturbations, we observed different types of responses of the microbiome to this antibiotic stress: effects that were reproducible between participants, effects associated to the initial composition of the microbiomes, and effects that related to the emergence of specific genes or alleles that were associated with antibiotic resistance. Linking these findings to actual phenotypes of the microbiome or of the host is still a challenge that needs addressing. In recent years, we have deployed different approaches to study the relationship between microbiomes and phenotypes. We used comparative genomics to associate genes with their propensity to horizontal gene transfer. We mapped the genomic context of genes to generate hypotheses on their mobility. We used

machine learning to interpret and predict antibiotic resistance. All these methods could be applied in contexts related to exposure to contaminants or xenobiotics. Future work should include direct measurement of the interaction(s) of the microbiome with its environment, be it a host or an ecosystem.

Repeated pesticide exposure changes the microbiome in prairie biobed systems towards a pesticide-degrading specialist community (PL)

Jennifer Russell[1], Jordyn Bergsveinson[1], Benjamin Perry[1], Christopher Yost[1]

[1]University of Regina

Biobeds are tools used to contain and microbially degrade on-farm pesticide waste and rinsate in order to reduce environmental impacts caused by pesticide pollution. While these engineered ecosystems show great efficiency in pesticide removal, how the microbial communities shift, respond, and adapt to extensive pesticide exposure remains largely unknown; a greater understanding of the microbial community dynamics in biobed systems could aid researchers in effective biobed management and maintenance. This study coupled metagenomic and metatranscriptomic techniques to characterize the microbial community in a Canadian prairie biobed system before and after a season of pesticide application. The subsequent data analysis identified an enriched community of xenobiotic degrading microbes, such as *Afipia*, *Sphingopyxis* and *Pseudomonas*, and a reduction in rhizosphere-associated microbes, such as *Caulobacter* and Hyphomicrobiaceae, indicating a shift towards a pesticide-degrading specialist community. Additionally, the results revealed an increase in abundance and upregulation of genes related to the degradation of aromatic compounds such as pesticides, antibiotics, and hydrocarbons, among others, showing a versatility in degradative function in these pesticide-degrading communities.

The effects of wastewater treatment plant effluent on the gut microbiome of invertebrates on the Grand River, ON (PL)

Elise Millar[1,2], Karen Kidd[1,2], Patricia Gillis[3], Michael Surette[1]

[1]McMaster University, [2]Canadian Rivers Institute, [3]Environment and Climate Change Canada

Research has shown that the composition of gut microbiota can affect a host organism's weight, immune function, and disease status, and is sensitive to factors such as diet, environment, and pharmaceutical use. The Waterloo and Kitchener wastewater treatment plants (WWTPs) in the Grand River in Ontario produce effluents containing antibiotics and other pharmaceuticals that may negatively affect the gut microbiome of downstream invertebrates. In this study, we collected mussels (*Lasmigona costata*), several species of macroinvertebrate larvae, and riparian spiders (*Tetragnathidae*) in the fall of 2018 from sites upstream and downstream of the Waterloo and Kitchener WWTPs. Bacterial genomic DNA was extracted from the gut content of mussels and invertebrates, as well as whole spiders, followed by the nested PCR amplification of the bacterial signal and sequencing using the V3-V4 hypervariable region of the 16S rRNA genetic barcode. Mussel gut samples contained 10,664 unique

bacterial taxa. Alpha diversity varied significantly among locations (one-way ANOVA: Simpson F=6.258, df=2, p=0.004) and decreased downstream of Kitchener (Tukey HSD: Simpson p=0.005), as well as from the downstream Waterloo to downstream Kitchener locations (Tukey HSD: Simpson p=0.026). Spider whole-body samples contained 3,553 unique bacterial taxa. Alpha diversity indices varied significantly among locations (one-way ANOVA: Simpson F=6.783, df=2, p=0.002) and increased from the downstream Waterloo to the downstream Kitchener locations (Tukey HSD: Simpson p=0.001). These initial results indicate some effects of effluent exposure on the microbial community of these organisms, adding to our understanding of the impact of wastewater exposure on the gut microbiome of aquatic invertebrates.

The effect of wastewater effluent exposure on the gut content microbiome of rainbow darter *(Etheostoma caeruleum)* (PL)

Victoria Restivo[1,2], Michael Surette[1], Joanna Wilson[1], Karen Kidd[1,2]

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The microbiome is critical in maintaining organismal health and is an emerging focus in environmental studies. In fish, recent research suggests that the gut microbiome is influenced by environmental stressors, diet, and habitat. This study aims to determine if exposure to wastewater treatment plant (WWTP) effluent affects the diversity and composition of the gut content microbiome of rainbow darter (*Etheostoma caeruleum*). In October 2018, gut content was sterilely collected from female fish at 10 sites (n=150) at varying distances upstream and downstream of the Waterloo and Kitchener WWTP outfalls along the Grand River (Ontario). Genomic DNA was extracted, PCR amplification of 16S rRNA V3-V4 region was conducted, and sequencing was performed using the Illumina MiSeq platform. Amplicon sequence variants were determined using DADA2 and matched to taxonomy using the SILVA database (version 1.3.2). Bray-Curtis beta diversity and principle coordinate analysis showed that upstream and downstream samples were significantly dissimilar (Permanova, $F=5.422$, $p<0.001$). Upstream samples had increased Berger-Parker dominance (Tukey, $p<0.05$) while diversity increased significantly in downstream samples (Shannon Diversity, ANOVA, $F=4.829$, $p<0.001$). Downstream samples had a greater relative abundance of *Proteobacteria* - which may indicate suboptimal fish health, increased *Cyanobacteria,* and decreased *Firmicutes* when compared to upstream fish. Results suggest that the composition and diversity of the fish gut content microbiome is affected by exposure to mixtures of emerging and legacy compounds found in WWTP effluent. Data from laboratory exposures to WWTP effluent is being collected to further elucidate its effects on the gut content microbiome of rainbow darter.

Mining and the Environment

An evaluation of terrestrial wildlife health risks as a result of the Faro Mine Remediation Project (PL)

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The Faro Mine Complex is located northeast of Whitehorse, YT and was once the largest open pit lead-zinc mine in the world. Today, it is the site of one of the most complex abandoned mine remediation projects in Canada. The Faro Mine Remediation Project was developed in 2018 to execute a remediation plan that mitigates against future conditions at the Faro Mine Site and to provide long-term protection for people and the environment. An Ecological Risk Assessment was conducted as part of an Environmental Assessment to characterize the potential effects of the Faro Mine Remediation Project on terrestrial wildlife during active remediation and long-term operations and maintenance phases. The potential risks of constituents of potential concern (i.e., metals) to fourteen wildlife receptors via multiple exposure pathways were evaluated using a multimedia food web model. Three different soil depth scenarios (surface soils, 1 metre depth, and 2 metre depth) were evaluated for each of the remediation phases to account for different soil exposure of wildlife species present in the study area. In addition, two different scenarios were evaluated for covering tailings at the original and secondary tailings impoundments. The residual effect of the Faro Mine Remediation Project resulted in an overall improvement to the health of terrestrial wildlife compared to existing conditions. Potential risks to terrestrial wildlife due to the Faro Mine Remediation Project were negligible to low.

Evaluation of sulphate toxicity to aquatic life in high hardness mine-influenced waters (PL)

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There are currently no federal water quality guidelines for sulphate or total dissolved solids, despite rapidly developing science in the evaluation of major ion toxicity. For sulphate, many projects have relied on screening to the British Columbia (BC) water quality guidelines, which specify hardness-based guidelines for conditions of 250 $mg \cdot L^{-1}$ or lower (in calcium carbonate equivalents). These guidelines were developed from multi-species testing programs conducted at multiple hardness levels. For higher hardness conditions, which predominate under some conditions of mining, site-specific testing has been recommended to develop benchmarks for environmental management of sulphate. Our presentation summarizes several multi-species toxicity testing programs conducted for mining clients in Western Canada from the last three years; these studies were used to evaluate chronic sulphate toxicity under high hardness. The data indicate that, for commonly encountered ionic profiles, the BC water quality guideline may be extrapolated to higher hardness conditions while still providing protection of freshwater aquatic life. The tests confirm that hardness-based amelioration of toxicity

occurs, and that water chemistry profiles with high calcium to magnesium ratios are favorable for mitigating against sulphate toxicity. Results of tests with invertebrates, fish, and amphibians are discussed and compared to other studies of sulphate toxicity in the published literature. Implications of the results for assessing toxicity of complex ionic mixtures, including species and endpoint selection, test protocol considerations, and priorities for future research, are discussed.

Determining effluent exposure and cumulative risk to macroinvertebrates using autonomous sensors at the McClean Lake Mine in northern Saskatchewan (PO)

Beatriz Esther Cupe Flores[1], Maira Mendes[1], Maëlys Nestoret[2], Karsten Liber[1]

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Treated effluent from the McClean Lake uranium milling operation in northern Saskatchewan is released into McClean Lake east basin. This could potentially represent a toxicological risk to aquatic organisms. The goal of this study is to determine the cumulative risk from effluent exposure to macroinvertebrates and identify risk variations in different parts of McClean Lake. Ten monitoring locations were established in and near McClean Lake (Aug-Sept 2018) using Libelium® real-time water quality monitoring sensors. Each sensor was deployed to measure routine water quality (pH, conductivity (EC), turbidity, dissolved oxygen, and temperature). Water and macroinvertebrate samples were collected at the same locations. The toxicological risk was estimated by calculating hazard quotients (HQs) based on water quality benchmarks for the long-term protection of freshwater aquatic life. Real-time conductivity data and metal concentrations were used to describe changes in contaminant exposure and estimate the variability in toxicological risk. Preliminary results indicate that there were significant spatial and temporal variations in effluent exposure in the study sites. The individual HQs for zinc, arsenic, selenium, sulfate, and fluoride were higher than water quality benchmarks at some locations. The cumulative risk for surface water was >1 in Vulture Lake and McClean Lake east basin. However, the taxon richness and abundance of macroinvertebrates were relatively low, and generally, community composition indices showed no correlation with field measurements of EC, which was used as a surrogate for effluent exposure. Thus, results to date suggest that there are no measurable adverse effects on macroinvertebrate communities at these locations.

Toxicity of selenium to a boreal lake food web: Direct and indirect organism responses (PO)

Stephanie Graves[1], Karsten Liber[1], Vince Palace[2], Markus Hecker[1], Lorne Doig[1], David Janz[1]

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Selenium (Se) is a contaminant of concern in Canada mainly due to its teratogenic effects on egg-laying vertebrates in aquatic ecosystems, with effects on invertebrates relatively under-studied. The objective of the present study was to assess the community-level effects of selenium on benthic macroinvertebrates and zooplankton following 63 days of exposure to Se using limnocorrals in Lake 239 at the International Institute for Sustainable Development – Experimental Lakes Area. From June 20 to August 22, 2018, limnocorrals were spiked with mean measured concentrations of 0.4, 0.8, 1.6,

3.1, 5.2 and 7.9 µg $Se \cdot L^{-1}$ as selenite, with three untreated controls (background aqueous Se = 0.09 $\mu g \cdot L^{-1}$). Quantitative periphyton, phytoplankton, zooplankton and benthic macroinvertebrate samples were collected throughout and at the end of the 63-day exposure period. Zooplankton will be identified to species and counted, and benthic macroinvertebrates were identified to family, counted and weighed. Taxa richness, density, and biomass will be determined for all invertebrates. Multivariate analysis of community-level impacts is in progress, but preliminary results show that density and biomass of Heptageniidae decreased with increasing exposure concentrations, but Chironomidae were not affected by Se exposure. Phytoplankton and periphyton growth increased with elevated Se and was inversely correlated with invertebrate biomass. The results suggest that some invertebrates have greater sensitivity to Se than previously thought, and that Se can have both direct and indirect impacts on freshwater food webs.

Early Detection and Rapid Response: The Role of Environmental DNA (eDNA) as a 'Molecular Canary' to Enhance the Quality of Risk Assessments and to Promote Aquatic Ecosystem Health

Documenting the ongoing decline of the American eel (*Anguilla rostrata*) in Lake Ontario: How eDNA may help bring the species back from the brink of extinction (PL)

Louis Gasparini[1], Steven Crookes[1], Kevin Romanick[1] Mario Thomas[1]

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American eels (*Anguilla rostrata*) were once among the most abundant fishes in the Laurentian Great Lakes, possessing significant socio-economic value to indigenous peoples and European settlers alike. Highly migratory, these catadromous fish undergo a singular journey, after upwards of 20 years' maturation in freshwaters, to communal spawning grounds in the Sargasso Sea of the Atlantic Ocean. Juvenile elvers make the return journey upstream to maturation grounds. At each life-history stage, the migration—once unimpeded by anthropogenic influences—is beset by manmade hazards that may have significant deleterious impacts on eel survival. These threats encompass physical (e.g. dams), chemical (e.g. pollution), and biotic (e.g. food-web restructuring) categories. As a result, the eel has suffered a significant decline in numbers in the Upper Laurentian River and Lake Ontario over the past thirty years, so much so that the species is currently listed as "endangered" in Ontario, despite the closure of the eel fishery in Lake Ontario in 2004. As the species has suffered a near-catastrophic demographic contraction it has become increasingly difficult to determine the species presence because of static thresholds of detection in conventional monitoring methods. Environmental DNA detection has a clear advantage over many current methods of detection because of its extreme sensitivity and non-invasive application in detecting otherwise hard-to-find benthic eels. We demonstrate the utility of eDNA in detecting the eel in Lake Ontario, to enable focused and direct management of this species. We elaborate on how continuous eDNA monitoring may also help in eel restoration efforts.

Archived environmental DNA (eDNA) samples: An evaluation of sample performance over time (PL)

Aron Weir[1], Caren Helbing[2], Jared Hobbs[3], Doug Bright[4], Wayne Murray[1], Michael Allison[2], Jessica Round[2], Ali Mirabzadeh[1], Heather Allen[1], Lauren Bergman[2], Erin McCormick[1], Kristiina Ovaska[5], Lennart Sopuck[5]

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Environmental DNA (eDNA) is a significant and valuable scientific tool for reliable detection of target species. Based on the premise that animals shed their DNA into their environment, key advantages of using eDNA compared with conventional detection methods include: enhanced sensitivity and accuracy; lower sampling effort and cost; an expanded time-frame for sample collection; and reduced invasiveness to the habitat and organism. eDNA samples may also be archived for future

testing for additional species of interest without the need to return to the field site to collect additional samples, provided that samples are properly preserved and stored.Confidence in eDNA results can be achieved by following best eDNA practices in the field and laboratory. It is important that the quality of eDNA samples be assessed prior to analysis to control for false negatives (type II errors). eDNA samples that are too degraded or that contain assay inhibitors, found naturally occurring in the environment, may not generate a positive signal during analysis and could be falsely interpreted as a "non-detect" for the target species, when in fact the sample quality is unsuitable for analysis. This applies both to newly-collected samples and historical samples that are retrieved from archive for testing. Little is known about the performance of archived eDNA samples over time and the ability to generate meaningful data on species presence when stored samples are analyzed at a future date. Providing metrics to this question will enable users to appropriately incorporate eDNA sample archiving into their eDNA programs with the intent that samples can be interrogated in the future with confidence. Two filter preservation methods are commonly used: ethanol immersion and silica preservation. We provide perspectives on the utility of each of these preservation methods. Of the two, silica preservation has many advantages over ethanol, without compromising on effectiveness. We also discuss the results of a performance experiment of archived eDNA filter samples preserved with silica over time under different storage conditions.

Assessing the presence of a sensitive species (round whitefish) in a Great Lake, using environmental DNA Analysis (PL)

Fei Luo[1], Joe Tetreault[1], Paul Patrick[1], Elizabeth Haack[1], Jason Dietrich[1], Steven Crookes[2], Kevin Romanick[2]

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Techniques traditionally used for assessment or monitoring of sensitive fish species and communities in the Great Lakes can be resource- intensive and invasive. However, the need to characterize the temporal and spatial movements of these fish remains crucial, especially along shorelines where spawning occurs and an association with industrial outfalls exists. Novel tools that can reduce field time/cost and safety risk while generating comparable results are always in demand. We present the first study, to our knowledge, that evaluates eDNA for spawning surveys in the Great Lakes. We investigated the use of eDNA to detect round whitefish (*Prosopium cylindraceum*), a thermally sensitive species, during their spawning period in Lake Ontario.Water samples were collected at a known round whitefish spawning location in Lake Ontario, followed by on-site DNA extraction. Sampling for eDNA was conducted in tandem with conventional gillnetting. We developed a highly sensitive and specific quantitative polymerase chain reaction (qPCR) assay to detect round whitefish DNA. Results from both qPCR and gillnetting confirmed the presence of round whitefish in the sampling area. Our results suggest a useful role for eDNA related to spawning surveys. In support of the primary study, we applied metabarcoding to survey the fish population in Lake Ontario, and qPCR to examine DNA shedding by round whitefish at early life stages (embryos and larvae) to the surrounding water during laboratory incubations. eDNA from round whitefish was detected in both experiments, and several other species were identified with metabarcoding. These results suggest broader utility of eDNA in aquatic systems.

Taking environmental DNA (eDNA) to the field: Methods, applications, and lessons learned (PL)

Cayla Naumann[1], Stacie Kalyn[1], Jacqueline Huard[1], Carrie Kwok[1], Laura Dilley[1]

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Environmental DNA (eDNA) refers to DNA that is shed by a species into the environment through its life history processes. These eDNA materials can be collected and analyzed using quantitative polymerase chain reaction (qPCR) techniques to identify the targeted species. In the past decade, research on eDNA has grown significantly, as evidenced by the number of peer-reviewed publications that have accumulated. The technology of analyzing eDNA is now more accessible and available on a commercial scale. However, the use of eDNA as a means to detect species can be limited by lack of acceptance by regulators, lack of qualified practitioners to appropriately collect and process eDNA samples, and general lack of awareness of what eDNA can be used for. This presentation is a case study of two projects in British Columbia where eDNA was used in addition to traditional sampling methods to enhance detectability of several conspicuous species. We will discuss the applicability of eDNA on projects of varying scales and how eDNA can be incorporated into the field of environmental consulting.

Environmental DNA for ecotoxicologists: Promise and needs (PL)

Caren Helbing[1], Jared Hobbs[2]

[1]University of Victoria, [2]J Hobbs Ecological Consulting Ltd.

Environmental risk assessments require accurate and timely information regarding occurrence and distribution of 'at risk' and invasive species. Conventional survey methods generally rely on direct observation that can be stressful to the target species and damaging to their habitat. For species that are cryptic or low density, the likelihood of detection is low. A new approach involves sampling DNA in sediments, soil, or water that is sloughed off by target organisms. Environmental DNA (eDNA) detection is accomplished through the identification of unique sequence segments of genetic material without the necessity of coming into direct contact with the target organisms. The high sensitivity of eDNA methods can dramatically increase detection rates, particularly for species that occur at low densities, have secretive ecologies, feature discontinuous distributions, or share morphological traits that confound accurate identification. Furthermore, eDNA methods are more cost-effective, non-invasive, and more accurate than conventional survey methods. eDNA methods require an effective synergy between field and DNA detection methodologies with several points of consideration that require attention during development, adoption, and implementation. Analytical techniques and technologies are rapidly evolving and the methods chosen depend upon the desired purpose: 1) to detect taxa in a targeted fashion or 2) assess community biodiversity. Environmental samples are complex and the quality and quantity of DNA can vary substantially. As such, field and analytical components of eDNA methods face particular challenges that require a heightened awareness of, and attention to, methodological requirements. Best practices strive to mitigate sources of false positive and false negatives, utilize eDNA methodology appropriate for study design objectives with appropriate regard to statistical power, include attention to quality control, and provide transparency in assessment of test performance. We

present the highlights of a recent consensus-based synthesis of the current needs in eDNA practice in order to remove barriers to the adoption of eDNA methods for environmental risk assessment. These include addressing quality issues with accuracy and reliability, lack of accredited national standards for both sample collection and analysis, and the need for demonstrated competency and proficiency testing by practitioners.

Can we use environmental DNA to detect sensitive species on an oil sand lease? (PL)

Greg Schatz[1], Joanne Hogg[2], Mary Murdoch[1], Pam Reece[1], Robert Hanner[3], Tzitziki Loeza-Quintana[3], Steven Crookes[4], Mario Thomas[4]

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Environmental DNA (eDNA) methods provide a promising approach for improved detection of protected species and more confidence in making decisions about developments, without causing harm. eDNA tools developed for Canadian Natural's Horizon Oil Sands operation in northern Alberta were used to detect two fish species in a man-made lake and a protected toad species in an area of mine advance. Horizon Lake was designed and constructed to include spawning, rearing, and overwintering habitat for several fish species. Conventional capture methods are being used to monitor success of this lake in meeting fisheries productivity goals. For the toad relocation, conventional visual and auditory monitoring is being used over a few weeks in spring to detect breeding adults of a protected toad species so that they can be relocated away from mine development. For detecting fish in Horizon Lake, eDNA assays were developed for two species; water samples were collected and analyzed in 2018. Additional analysis of archived sample extracts was conducted using metabarcoding to confirm the presence of additional fish species in Horizon Lake. A parallel study is developing an eDNA assay for the protected Canadian toad to detect eDNA from any life stage, including eggs and tadpoles, and thereby to extend the survey window for detecting this species and improving the effectiveness of the relocation program. This presentation will present the preliminary results of these two studies, providing recommendations for next steps.

Achieving cumulative effects monitoring and management objectives: Need for new approaches and tools (PL)

Doug Bright[1,2], Caren Helbing[3], Aron Weir[4], Jared Hobbs[5,6]

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The role of eDNA studies in early detection of invasive species and in more transparent, improved approaches for defining distributions of hard to find at-risk species is very well established. Early detection of such taxa of interest in a localized area, however, may not be the most compelling reason to embrace eDNA approaches. For both targeted taxa eDNA observational approaches and metabarcoding/metagenomics approaches, among the most important features is the improved ability to obtain biotic distributional and community-level (presence – absence) data far more efficiently, using

sampling approaches that are less invasive of the ecosystems we are attempting to protect (less habitat disturbance; less opportunity for further transmission of pathogens and invasives). The new abilities offer promise for acquiring larger amounts of data per unit effort. This should enable a step-change increase in our ability to acquire various types of biotic data, with a greater spatial extent, spatial intensity, and temporal intensity. Ecotoxicologists have witnessed, and indeed driven, many important advances in practical knowledge about human interactions with ecosystems. The collective ability, however, to detect, adequately understand, and limit adverse cumulative effects at watershed- and landscape-level scales, as well as larger scales, remains rudimentary. Environmental toxicologists and environmental managers operate in an era when data pooled from multiple entities, big data approaches, data mining and meta-analytical approaches (including eco-epidemioloogical studies) have the potential to advance cumulative effects monitoring and management. We discuss how eDNA may advance us down this path. We further discuss the associated hurdles—particularly the need for eDNA data reliability, transparency, and inter-comparability across studies and researchers—that need to be overcome if the relevant data are to be combined and queried for the purpose of better detecting various biological changes over large scales and long time lines, and for developing useful scientific model of the important drivers and co-variates.

Toward an integrative environmental DNA approach to monitor ecosystem well-being (PL)

Steve Crookes[1], Louis Gasparini[1], Kevin Romanick[1], Mario Thomas[1]

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Aquatic ecosystems are vastly complex entities whose outward appearance of harmonic equilibrium is illusory. These hugely important repositories of biodiversity are better characterized as being in a constant state of tension embodied by the manifold levels of organism interdependencies across all trophic levels. Extraneous anthropogenically-mediated forces can have severe, unpredictable, long-lasting and cascading effects on ecological equilibria and, by extension, ecosystem health. Although ecosystems are highly complex, simplified models are necessary from which one can make predictions to plan for the future. To better manage aquatic habitats, models of responses to environmental stressors are required so that managers and stakeholders can take action to maximize the prevention of threats, provide mitigation against those pressures, or enact emergency rapid response(s) to them. The response of model taxa to changes in their immediate environment may act as a bellwether to conservation managers, informing them that other organisms may also be affected. A rapid alarm system is required – a suite of 'molecular canaries', whose songs represent the first warning against potential impending disaster. eDNA detection's extreme sensitivity is highly appropriate for use in aquatic ecosystems. High sensitivity allows for expeditious action to be taken against a panoply of potential ecosystemic threats, including aquatic invasive species (AIS), climate change, pathogens or parasites, and pollution. We discuss how the many facets of eDNA detection can be integrated into a coherent, predictable, and actionable framework that incorporates active, passive, and novel methods of nucleic acid detection to better protect the most vulnerable habitats on Earth.

In pursuit of Pasokos: Weaving environmental DNA monitoring with traditional Wolastoqey knowledge to understand changes in habitat use of shortnose sturgeon within the lower Wolastoq River (PO)

Mary Murdoch[1], Kaleb Zelman[2], Tim Vickers[1], Aruna Jayawardane[2], Robert Hanner[3], Matthew Litvak[4], Steven Crookes[5], Mario Thomas[5]

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The Wolastoqwiyik (Maliseet) people rely upon fish in the Wolastoq (St. John River) for food and ceremony, including the shortnose sturgeon, *Acipenser brevirostrum*, a federally listed species at risk. Construction of a large hydroelectric facility in the 1960s has effectively limited this species to the lowermost 120 km of the 673 km river and has altered habitat downstream of the dam. Maliseet traditional knowledge from First Nations elders, knowledge holders and resource users is vital in understanding changes in downstream habitat use by shortnose sturgeon. Conventional scientific monitoring involves fish capture and is challenging due to cost, logistics, and potential for negative effects to sensitive populations. Environmental DNA (eDNA) provides a new approach to detecting species presence without capture (thereby avoiding harm) and the opportunity to increase spatial area and frequency of sampling. This project has been initiated by the Maliseet Nation Conservation Council to pilot eDNA sampling paired with Maliseet traditional knowledge as a step towards the goal of understanding changes in seasonal habitat use of shortnose sturgeon along the lower Wolastoq in New Brunswick. A workshop was hosted to inform Maliseet community members on the application of eDNA for this project and potential future projects. Academic and commercial laboratory partners with Stantec developed the eDNA assay and will analyze eDNA samples collected. An additional academic partner is providing information on movements of this species using acoustic telemetry and video footage. This presentation will provide results to date and recommendations for next steps.

Subtleties and Subterfuge in Analyzing and Interpreting Fish Health and Chemistry Data in Aquatic Environmental Effects Monitoring

The reservoir effect five decades later: A bull trout mercury story (PL)

Laura Bekar[1], Gary Mann[1], Norm Healey[1], Randle Baker[1], Richard (Drew) Bodaly[1], Chelsea Coady[2]

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Williston Reservoir, created in 1968, is the largest reservoir in British Columbia (BC) and the second largest in Canada by volume. Fish mercury monitoring in 1980 and 1988 showed bull trout (*Salvelinus confluentus*) concentrations sufficiently elevated to warrant establishment of one of the few mercury advisories in BC in the early 1990s. It has been more than 25 years since the advisory was issued for the reservoir, and the perception of local peoples remains that fish mercury concentrations are elevated. To address these concerns, the Fish and Wildlife Compensation Program for the Peace Region commissioned a multi-year study to characterize fish mercury concentrations in the Williston-Dinosaur Watershed and in several regional waterbodies (to provide a regional context) and worked with the relevant health authorities to revisit the advisory. Spatial and temporal analyses were conducted to assess current bull trout mercury concentrations. This provided context with other lakes (spatial assessment) and with the historical data (temporal assessment) upon which the advisory was based. In light of the findings, options for revisiting the fish consumption advisory for Williston Reservoir are presented.

Subtleties in Environmental Effects Monitoring responses: Developing normal ranges for fish health indicators from a 30-year study of impacts of bleached kraft pulp mill effluent at Jackfish Bay, Ontario (PL)

Kelly Munkittrick[1], Mark McMaster[2], Shemar Williams[1], Erin Ussery[2], Thomas Clark[2], Jessie Cunningham[2], Abby Wynnia[2]

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Jackfish Bay is located on the north shore of Lake Superior (48°50'N, 86°58'W) and was identified as a Great Lakes Area of Concern in 1987 due to impacts associated with effluent from a bleached kraft pulp mill in Terrace Bay, Ontario. Extensive studies on white sucker (*Catostomus commersoni*) populations documented reduced gonadal sizes, delayed sexual maturation, and altered levels of circulating sex steroid hormones in the late 1980s. Studies in the early 1990s demonstrated that meeting the 1992 Pulp and Paper regulatory changes would not be sufficient to remove all environmental responses in fish. Studies have monitored the impacts following installation of secondary waste treatment (1989), changes in the bleaching process (1990s), and a series of closures associated with changing ownership (2000s). Collections in 2018 and 2019 extended the time series (previous collections stopped in 2013) of spring and fall collections. Differences in body size, liver size, gonad size and condition persist, although changes in liver and gonad are much smaller than in the

early years. Reference site variability over the 31-year time period approaches 2 SD, and the long-term data has been used to develop normal ranges for responses. The use of monitoring triggers can extend the utility of Environmental Effects Monitoring (EEM) as a regulatory tool in an integrated, tiered and triggered, adaptive monitoring framework.

Establishing common distributions for environmental and biological datasets that contain censored data (PL)

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Parametric statistical analyses assume that sample data are drawn from populations that can be modelled by a theoretical probability distribution (e.g., normal, lognormal). Given that parametric analyses are the most commonly employed approach of statistical inference in applied sciences, and sample sizes often do not allow for formal tests of underlying distributions, an understanding of the distributions for common types of environmental and biological data can be extremely beneficial. This is particularly true for data that frequently contain censored data (containing one or more values reported below a detection limit) because the left tail of the sample distribution is censored, making it more challenging to determine their underlying distributions. Thus, in practice, a normal or lognormal distribution is generally assumed for environmental data that are censored. We assess the validity of this assumption by identifying the best-fitting data distributions for several environmental and biological chemistry data sets that include water quality, sediment quality, benthic invertebrate tissue, and fish tissue. The best distributions were identified by testing the likelihood of five potential probability distributions (normal, lognormal, beta, gamma, and Weibull). The results of this study can be used to support decisions on the probability distributions that are assumed and the approaches that should be used to analyze various censored environmental and biological datasets.

Advances in Environmental Quality Guidelines Objectives and Benchmarks

Considering bioavailability modeling approaches in guideline development: Pros and cons of simple versus complex models (PL)

Robert Santore[1], Kelly Croteau[1], David DeForest[1]

[1]*Windward Environmental*

A considerable amount of research has been directed at advancing our understanding of why certain water quality characteristics impact the bioavailability and toxicity of metals and other toxicants. A benefit of this work has been the development of mechanistic predictive models such as the Biotic Ligand Model (BLM) that can explain and predict how water quality determines metal bioavailability and toxicity. The BLM has also been used to incorporate these bioavailability effects into water quality guidelines in jurisdictions around the world, including Canada and the U.S. Because it is a mechanistic model that attempts to use both chemical and physiological information, the BLM is fairly complex. Meanwhile, simple empirical equations have also been used to incorporate bioavailability information into water quality guidelines. These simple equations can represent how toxicity modifying factors affect metal toxicity, but may be lacking in realism or comprehensiveness compared with mechanistic models. Despite these potential limitations, empirical equations may still be useful, and their simplicity and transparency have other advantages for use in a regulatory setting. In this presentation, we will compare and contrast how mechanistic and empirical models can be used to incorporate bioavailability effects in water quality guidelines. We will describe several approaches for evaluating the effectiveness and predictability of these approaches and discuss settings in which one or the other approach may be preferable. Case studies with recent guidelines for copper and aluminum will be discussed.

Delivering practical environmental protection: The experience of implementing the bioavailability-based nickel standard in Europe (PL)

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Bioavailability-based approaches for metal environmental quality standards are being considered around the world. In the European Union (EU), the nickel Environmental Quality Standard (EQS) under the Water Framework Directive (WFD) is bioavailability-based. Recently, the European Commission (EC) developed a guidance document for implementing bioavailability-based EQS for metals. We will review the guidance and present case studies that illustrate lessons that can be applied to other jurisdictions where bioavailability-based approaches are being considered. Nickel toxicity is influenced by pH, hardness (Ca and Mg) and dissolved organic carbon (DOC). The Ni EQS under the WFD was determined by normalizing the extensive chronic ecotoxicity dataset (n=31 species) to a common combination of water chemistry parameters using nickel bioavailability models. A reference EQS was

calculated by determining the HC5 from a Species Sensitivity Distribution of EC10 values that were normalized using the pH, hardness, and DOC for a high bioavailability water from the most sensitive ecoregion occurring in Europe (pH=8.2, hardness=120 $mg \cdot L^{-1}$ $CaCO_3$, DOC=2 $mg \cdot L^{-1}$). The resulting HC5 is 4 $\mu g \cdot L^{-1}$ Ni. The EC guidance leads EU member states through the compliance-checking process using a tiered approach, where increasingly complex estimates of bioavailability and natural background concentrations are used to determine compliance. Experience demonstrates that the vast majority of sites show compliance with the Ni EQS after bioavailability correction. We will also demonstrate how the tiered approach can be used for different situations, including incomplete water chemistry, censored datasets, and out-of-boundary water chemistry.

The use of multiple linear regressions and biotic ligand models for incorporating bioavailability into water quality guidelines for metals (PL)

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Federal Water Quality Guidelines (FWQGs) are benchmarks for the quality of the ambient environment. They support various federal activities including risk assessment, risk management, and environmental quality monitoring. FWQGs are based on toxicity data and are set at a concentration below which there is low likelihood of adverse effects from a chemical on aquatic life. Published and draft FWQGs exist for several metals including zinc, iron, lead, copper, and aluminium. Toxicity modifying factors (TMFs), such as pH, dissolved organic carbon (DOC), water hardness, and temperature can alter the bioavailability of metals and hence the toxicity to aquatic organisms. Therefore, bioavailability is often addressed in FWQGs by incorporating TMFs so that users receive a final guideline value that is reflective of their site conditions. This is often done by using either a single or multiple linear regression (MLR) approach, or using a biotic ligand model (BLM). This presentation will summarize both approaches and how they are used in FWQG development. Examples of published and draft FWQGs that use these approaches will be presented.

Deriving soil quality guidelines for elements with and without sufficient toxicity data (PL)

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There are a number of elements for which regulatory authorities do not provide generic soil quality criteria; however, some activities may release these elements into the environment and the potential for adverse effects may need to be evaluated. Commonly, elements without criteria are either not assessed at all or are compared to background concentrations. An approach for developing criteria for these elements is discussed using ruthenium as an example. Ruthenium is a rare transition metal belonging to the platinum group of the periodic table. The potential for toxic effects to humans is considered negligible, although this is based on limited information. In terms of the environmental impact, although widely considered non-toxic, a few studies have identified concentrations of

ruthenium with the potential for harmful effects. In this study, criteria for ruthenium in soil for different land uses were derived based on the available toxicity data compiled from a literature search. The Canadian Council of Ministers of the Environment (CCME) provides a rationale for the development of soil guidelines; however, this approach was modified due to the fact that only a small database exists on toxicity for elements such as ruthenium. To gain more confidence in the derived guideline, Aquatox, a laboratory specializing in environmental testing, was contracted to conduct toxicity tests (plants, soil invertebrates) to supplement the data obtained in the literature. The approach to developing soil quality guidelines using this modified approach, including the overall uncertainties, will be discussed along with other aspects such as consideration of the use of surrogates.

Exploring statistical power in the *Oppia nitens* standardized test method (PL)

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The t-test is so familiar to us, it has become common practice to set alpha at 0.05, declare "no effect" when the p-value is greater than 0.05, and then congratulate ourselves on our unbiased conclusions. Using a cut-off p-value of 0.05 protects us from drawing wrong conclusions because of false positives—that is, declaring there is an effect when, in fact, there is none. But there is another error we can make—just as important, but largely hidden and unexplored: the false negative. Most of us have experienced this when we have high variability between our replicates, and then get a jaw-clenching p=0.06; we feel obliged to declare "no effect" when our scientific Spidey-senses would tell us otherwise. There is a relationship between variability, effect size, number of replicates, and false negatives, and this relationship can be understood through statistical power. Power can be particularly important in contaminated site assessments, because p-values are a convenient tool for risk assessors to categorize sites as "impacted" or "not impacted". To put power into practice, we will see how power analysis can be applied to the test design of a new soil toxicity test method. As part of the rigorous process to standardize this method, the oribatid mite reproduction test using *Oppia nitens* has been repeated in our lab many times, and has undergone three rounds of inter-laboratory validation. This has created a rich data set to explore the nature of variability in the test. From there, we have woven into the method a decision on how sensitive we would like the test to be (effect size) and how much effort is needed (number of replicates). We will examine all these threads graphically, and describe how we arrive at a final test design that considers both false positives and false negatives.

How to address the lack of toxicological data for technology-critical metals (PL)

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The development of new technologies has led to the use of metals for which impacts on aquatic ecosystems are poorly known, such as gold (Au), indium (In), germanium (Ge), and platinum group elements. These technology-critical metals or TCMs are "data-poor" elements, where both speciation

data and toxicological data are limited or missing. We explored the use of quantitative ion character activity relationships (QICARs) to predict the toxicity of the TCMs toward aquatic organisms based on intrinsic metal characteristics. Modelled organism responses were the measured acute EC_{50} values of 12 data-rich metals (Ag, Ca, Cd, Co, Cu, K, Mg, Mn, Na, Ni, Pb, Zn) for algae, daphnids and fish. Both total dissolved metal concentrations and the free metal ion activities (M^{z+}) were used to build the QICARs using simple and multiple linear regressions. Finally, the QICARs developed for the data-rich metals were then used to predict EC_{50} values for the data-poor TCMs. Among the 15 "best" QICARs obtained for the data-rich metals (adjusted $r^2 > 0.8$), 12 were simple linear regressions with $\chi_m^2 r$ as the predictor (χ_m = metal electronegativity; r = metal ionic radius). Unlike the case for the data-rich metals, the toxicity of the TCMs was much better predicted by the QICAR models based on the total dissolved metal concentration rather than M^{z+}. We suspect that this unusual result reflects the distinctive speciation of these metals where M^{z+} is present only at vanishingly low concentrations ($< 10^{-15}$ M). We conclude that QICARs show potential as a screening tool that could be used to review existing toxicity data and identify "outliers".

Addressing the issue of ambiguous taxa in benthic invertebrate community data (PL)

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Benthic invertebrate community assessment is often included in environmental monitoring programs to evaluate potential anthropogenic effects on ecosystems. Family-level identifications are common for these programs, but finer taxonomic resolution may be needed to detect more subtle environmental impacts or to better assess site-specific conditions. A common challenge with the lowest practical level (LPL) of taxonomic resolution is the presence of "ambiguous taxa" – when taxa are identified to multiple levels within a hierarchy (e.g., Baetidae, *Baetis*, and *Baetis tricaudatus* may all be present in a sample). This can occur when complete taxonomic keys only exist for certain life stages, or damaged specimen are present. The presence of these ambiguous taxa may result in inflated estimates of richness or distorted patterns of diversity. Common solutions include "rolling-up" ambiguous taxa to the highest shared taxonomic level, or "rolling-down" abundances into their lower level children taxa. Rolling-up results in a loss of valuable taxonomic resolution, whereas rolling down can further distort patterns of biodiversity. We developed a decision key that applies richness and abundance criteria to roll-ups or roll-downs in the presence of ambiguous taxa. In developing this decision key, our goal was to preserve as much detailed taxonomic information as possible, while addressing issues of taxonomic ambiguities. We evaluated the performance of the decision key relative to consistent roll-up and roll-downs using simulated community data. We found the decision key performed equally well or better than roll-ups and roll-downs, with the relative difference between methods depending on the overall pervasiveness of ambiguous taxa.

Shifting patterns of marine biotoxins on the west coast of British Columbia (PL)

Reza Afsahri[1]

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Saxitoxin (STX)-group toxins are a group of closely related tetrahydropurines that are produced mainly by dinoflagellates belonging to the genus Alexandrium, such as *Alexandrium tamarensis*. STXs cause paralytic shellfish poisoning (PSP) in humans, which is characterised by a range of symptoms from a slight tingling sensation or numbness around the lips to fatal respiratory paralysis. In fatal cases, respiratory arrest occurs two to 12 hours following consumption of contaminated shellfish. Data on all saxitoxin measurements were obtained for 2016-2018 from the Canadian Food Inspection Agency (CFIA) from different shellfish monitoring sites along the coast of British Columbia. The limit of detection was 25 µg STXeq /100 g in tissue. Results in a total of 10,287 tissue samples were studied, including tissue from mussels (common and blue), clam (geoduck, razor, butter, Manila, and cockle), oyster (Pacific and lam), unspecified species, and others. Saxitoxin was detected in more than 25 percent of cases. An above-regulatory limit of 80 ug/100g saxitoxin was observed in 9 percent. Below-regulatory limit saxitoxin levels occurred throughout each year from 2016 to 2018, with peak concentrations most associated with early summer and late fall (trends will be discussed in detail). Conclusion: high levels of saxitoxin in shellfish are much more common both in frequency and in high concentration than what is generally believed; however, human clinical cases of PSP are very scarce. The discrepancy could be related to effective closing of shellfish harvesting sites, as happens whenever sample concentrations measures are above regulatory limits, or to lack of efficient case findings among the population of British Columbia. Self-harvesters may need to pay more attention.

Benthic invertebrate community assessment using Kick versus Hess sampling: Potential implications for Environmental Effects Monitoring studies (PL)

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Environmental Effects Monitoring (EEM) studies conducted under the *Fisheries Act* are required to report on total benthic invertebrate density, which would appear to necessitate area-based sampling using devices such as Hess, Surber, or Ponar samplers. Technical guidance for EEM studies also allows for implementation of studies following a reference condition approach; however, this type of design has more typically involved sample collection using the travelling three-minute kick method of the Canadian Aquatic Biomonitoring Network (CABIN). Kick sampling has some advantages over Hess and Surber methods, namely that sampling can be done faster and in streams with greater water depth and flow. Also, training and certification are available for field personnel through CABIN. The current study compared benthic invertebrate community characteristics for closely located Hess and travelling kick samples in a watershed in southeastern British Columbia (BC) influenced by coal mining and other activities. There was good agreement between three-minute kick sample abundances and Hess sample densities among areas. Within-area coefficients of variation for benthic invertebrate abundance/density, family richness, Simpson's Evenness, percent EPT (Ephemeroptera, Plecoptera, and

Trichoptera), and percent Ephemeroptera for replicate kick samples were similar to, or lower than, those for Hess samples, suggesting that differences between disturbed and reference areas, or changes over time, are more likely to be statistically detected with kick than with Hess samples in this watershed.

Exploratory methodology for hexavalent chromium determination in fish tissues (PO)

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Recently, the Ontario government issued consumption limitations in boreal riverine fish due to concentrations of chromium (Cr). However, these limits are based on monitoring total Cr concentrations, even though not all forms of this metal are toxic. The current advisories are, therefore, likely overestimating the risk to consumers, a major issue for remote aboriginal communities across northern Ontario because of their reliance on these fish for subsistence. In the environment, Cr exists either as the benign form (trivalent Cr or Cr3) or in potentially toxic form (hexavalent Cr or Cr6), but no method exists to differentiate these forms in biotic samples. The goal of this project is to test two Cr speciation extractions and validate findings using speciated isotope dilution mass spectrometry (SIDMS; EPA 6800). Lab work is anticipated to begin in June 2019 and will test two extractions: (1) a microwave assisted extraction using methanol and water (20, 50, 80, and 100% v/v tests), and (2) direct measurement of Cr6 using an alkaline digestion (i.e., EPA 3060A) and subsequent Cr3 measurement on residual tissue using total extraction procedures (i.e., EPA 3052). Extracts from both approaches will be analyzed using inductively coupled plasma mass spectrometry interfaced with ion chromatography (IC-ICP-MS). We plan to validate the methods with SIDMS, during which each Cr species is "labeled" with an isotopically enriched spike in the corresponding species form. Results from this work will be help refine consumption guidelines and future monitoring programs based in the "Ring of Fire" mining development region and beyond.

Bioassays for routine detection of endocrine disrupting chemicals in complex effluents (PO)

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Endocrine disrupting chemicals (EDCs) are xenobiotics which have the ability to mimic or inhibit hormones. Their presence in aquatic ecosystems can affect the development and reproduction of aquatic wildlife. However, EDCs are not yet regulated in municipal and industrial wastewater in any country, including Canada. Current EDC frameworks, such as those from the United States' Environmental Protection Agency and Japan's Ministry of Environment, aim to identify EDCs using a single compound approach. However, the reality of cities (as well as hospitals and industries) is to deal with complex mixtures in effluents. These effluents could yield an overall endocrine disrupting (ED)

activity different from the sum of individual ED activity of each compound. This project aims to develop a two-tier approach for routine testing of complex wastewater mixtures, which will focus on reproductive endpoints. Based on an exhaustive literature review of existing EDC frameworks, we selected three *in vitro* bioassays for Tier 1: the transactivation assay of the human estrogen and androgen receptors, and the assay of steroidogenesis in H295R cells. For Tier 2, the fish short-term reproduction assay in fathead minnow was selected to validate any positive scores obtained in Tier 1. The optimization and validation of each bioassay is currently ongoing. When fully operational, these bioassays could assist municipal, provincial, and federal governments in a first phase of testing EDCs in municipal and industrial effluents. Altogether, this long-term research program aims to better manage the quality of wastewater being released into Quebec's and Canada's ecosystems.

Defining bioaccumulative substances for use in contaminated sites work in British Columbia (PO)

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This presentation explores the definition of bioaccumulative substances, although the content is not yet ministry-approved. The proposed definition for bioaccumulative substances is based on physical and chemical criteria (logKow >4.5; Bioaccumulation Factor (BAF) >2000; Bioconcentration Factor (BCF) >2000). However, these criteria have limited applicability to ionic organic (i.e., per- and polyfluoroalkyl substances) and inorganic substances (i.e., methylmercury) and thus do not capture all potentially-bioaccumulative substances. Therefore, a list of priority bioaccumulative substances could be established to be used in conjunction with the criteria. In contaminated sites applications, when a complete exposure pathway exists between receptors and contaminants of concern identified as bioaccumulative substances, subsequent evaluation of the potential for food chain effects should be completed. The ministry expects a rationale to be provided when BAF and BCF values from literature are used to ensure they are appropriate for site conditions; lists of recommended sources of Kow, BAF and BCF data will be discussed. It is acknowledged that site-specific BAF and BCF values are preferred when the scope and complexity of the contaminated site allows for it. As well, the uptake of substances is not always constant and may be better described using log-linear or linear uptake models as opposed to the BAF or BCF approach. The ministry prefers tissue sampling where bioaccumulative substances are encountered; however, alternative methods such as bioaccumulation testing and food chain modelling may be considered. The ministry hopes this presentation will facilitate improved bioaccumulative substance evaluations within risk assessments at contaminated sites in British Columbia.

Post-deposit monitoring approaches for measuring environmental concentrations and effects of drugs, pesticides, and antibiotics used in the Canadian aquaculture industry (PO)

Carrie Bentley[1], Lisa MacDonald[1], Colin McVarish [1], Nicole MacDonald [1], Cory Dubetz, Ian Bryson, [2], Jason Bernier [1]

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Aquaculture is an established practice world-wide and is becoming a large-scale commercial industry in Canada, with approximately 45 different species commercially cultivated. As our reliance on aquaculture grows, it is essential that we identify and understand the potential risks and implications to the health of the environment. The use of various therapeutants (i.e., drugs, pesticides and antibiotics) is sometimes necessary to treat fish afflicted with pests or pathogens. All pesticides and veterinary drugs require pre-market authorization by Health Canada, either through its Pest Management Regulatory Agency or Veterinary Drugs Directorate. There are approximately a dozen therapeutants currently approved for use in Canada and several that are emerging candidates for future regulatory review and registration for use. Within Canada, Fisheries and Oceans Canada has recently issued the Aquaculture Activities Regulations (AAR) and the Aquaculture Monitoring Standard under the Fisheries Act, which primarily focuses on biochemical oxygen demand (BOD) monitoring. In Canada, research is currently underway to better understand non-target species sensitivities to therapeutants in order to inform considerations for monitoring. We conducted a literature review on the toxicity of approved and emerging therapeutants to non-target organisms and international therapeutant monitoring programs with the objective of identifying scientifically-appropriate approaches for monitoring the fate of these therapeutants once released into the environment. This research will serve as a resource to inform decisions on the safe use and monitoring of therapeutants used by the Canadian aquaculture industry.

Inter-species extrapolation of uptake and biotransformation of benzo[a]pyrene in early life stages of the fathead minnow and white sturgeon (PO)

Chelsea Grimard[1], Annika Mangold-Döring[2], Markus Schmitz[2], Hattan Alharbi[3], Paul Jones[1], John Giesy[1], Markus Hecker[1], Markus Brinkmann[1]

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Understanding the species-specific toxicokinetic (TK) properties of a chemical is critical for characterizing cross-species differences in sensitivity to contaminants. Comprehension of these differences is particularly important when conducting ecological risks assessments (ERA) of environmental contaminants across species. TK models are an in silico approach that predicts the chemical concentration at the target site of interest. While this approach has proven effective for commonly used model species, it is limited in scope because current models only focus on a few species, life stages, and chemicals. The objectives of this study were to characterize transformation kinetics and to develop TK models that will enable extrapolation of biotransformation of the priority polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP) in early life stages of two evolutionarily distinct species of fish, fathead minnow and white sturgeon, and to validate model predictions using data from *in vivo* exposures. Analysis of BaP metabolites showed inter-species differences in metabolite abundance,

while significant dose- and time-dependent increases in BaP metabolites occurred within each species. No difference in biotransformation was observed through analysis of ethoxyresorufin-O-deethylase (EROD), glutathione-S-transferase (GST) and UDP-glucuronosyltransferase (UGT) activity within each species. Using this information, an OMEGA (Optimal Modelling for EcoloGical Applications) model was established to predict the abundance of BaP metabolites in early life stages of the test species. Based on data acquired to date, we conclude that TK models are a powerful tool for use in ERA, which can also account for life stage and species-specific differences in biotransformation.

Emerging Environmental Challenges Linked to the Mercury Cycle

Formation of methylmercury in boreal aquatic ecosystem: The role of organic matter composition (PL)

Andrea Bravo[1]

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One major challenge in contemporary environmental science is to identify factors controlling the formation of methylmercury (MeHg). The formation of MeHg is biotically mediated in aquatic systems. Although it is well known that organic matter (OM) interacts very strongly with Hg, the influence of the molecular composition of OM in MeHg formation remains poorly understood. Here we show the impact of terrigenous and planktonic derived OM on Hg methylation in different aquatic ecosystems. Concretely, we studied Hg methylation in sediments of 10 lakes and nine beaver pond ecosystems. While the selected lakes receive inputs of both terrigenous and *in situ* OM, sediments collected in beaver ponds are primarily dominated by terrigenous OM with different degradation status. We used inorganic mercury isotope tracers to determine mercury methylation and pyrolysis–gas chromatography mass spectrometry and/or optical measurements (spectrometry and fluorescence) to characterize the composition OM in the sediment or at their overlying waters. The characterization of OM suggests that, in lake sediments, algal-derived compounds primarily control Hg methylation by enhancing the activity of the whole microbial community. In beaver ponds, the qualitative analysis of the dissolved organic matter water with excitation-emission-matrix fluorescence spectroscopy at the overlying indicates that, besides the role of algal-derived organic matter, unprocessed terrestrial organic matter also enhances Hg methylation. We thus conclude that algal-derived compounds are the main driver of Hg methylation, but in systems limited by autochthonous sources, fresh terrigenous OM play an important role in the process.

Mobilization and transformation of mercury across a dammed boreal river are linked to carbon processing and hydrology (PL)

Jérémy de Bonville[1], Marc Amyot[1], Paul del Giorgio[2], Alain Tremblay[3], François Bilodeau[3], Dominic Ponton[1], Jean-François Lapierre[1]

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Hydroelectric reservoirs are known to affect the cycling of mercury and carbon following the flooding of large amounts of terrestrial organic matter. We explored the co-variation of mercury (Hg) and carbon (C) functional pools in natural and dammed aquatic ecosystems of La Romaine River watershed, a recently built hydro-electric reservoir complex in northern Quebec, Canada, to understand how the fate of these elements varies across systems with contrasting hydrology and environmental conditions. We found that total Hg (THg) concentrations in the water were relatively stable, while methylmercury (MeHg) concentrations and proportions tended to increase along the reservoir

sequence. While THg was related to total and terrestrial pools of dissolved organic carbon (DOC), MeHg was not strongly related to DOC but rather linked to concentrations of carbon dioxide (CO2), which we interpret as a proxy of cumulative carbon processing in prior seasons (e.g., under ice) and in shallow bays and deep portions of the river's main channel. This suggests that the studied reservoir acted more as a transformation site for already present Hg than as a mobilizer or new Hg, and that reservoirs are highly connected systems through space and time as processes occurring hundreds of kilometers upstream and months prior can affect Hg and C dynamics across the whole system.

Total mercury distribution in hydric soil, biomass, and ground-dwelling organisms in an upper St. Lawrence River wetland (PL)

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The Upper St. Lawrence River (USLR), shared binationally between the US and Canada, has regulated water levels managed by the International Joint Commission (IJC). Over the past 60 years, water levels remained stable until the regulatory guide "Plan 2014", enacted in 2017, began allowing for more natural water level fluctuations. Under stable water levels, there was a history of mercury (Hg) deposition and a simultaneous expansion of *Typha* wetlands (29%). As Plan 2014 reverts these wetlands back to pre-stable (natural) water level conditions, it is predicted that the 29% expansion of *Typha* wetland will be lost. It was hypothesized that fluctuating water levels will make wetland Hg bioavailable and mobilize it into the food chain. To determine the risk for Hg mobilization, total Hg was quantified in soil, biomass, and ground-dwelling organisms (arthropods and gastropods). Snails (*Succineidae*) had on average 41 ± 16 ng·g^{-1} dry weight total Hg and were compared to other ground-dwelling organisms based on trophic status and wetland proximity. The bioconcentration factor between hydric soil and *T. angustifolia* roots was 1.61 ± 1.46 and the translocation factor between roots and leaves was 0.22 ± 0.12. Detritus contained the highest total Hg (108 ± 53 ng·g^{-1} dry weight), although soil contributed the greatest overall Hg to the wetland due to sheer volume and weight. Results suggest that an erosion of 29% of wetland area in the USLR equates to 83 kg of Hg, with soil contributing 86%, detritus 10%, dry leaf 2%, green leaf 2%, and the remaining *Typha* organs contributing <1%.

Use of thiol-containing amino acids to understand variability in methylmercury among aquatic species (PL)

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Methylmercury (MeHg) concentrations in aquatic invertebrates and fishes are known to be highly variable within and among systems. While some of this variability can be explained by among-system

differences in physicochemical conditions or the dietary habits of the organisms, unexplained variability exists. Given the strong links between MeHg and sulphur cycling, our objective was to determine whether the content of thiol-containing amino acids (cysteine and methionine) may be used to model MeHg variability among taxa and systems. Macroinvertebrates from different functional feeding groups, bulk zooplankton and several species of fishes were collected from six lakes in Kejimkujik National Park, Nova Scotia, that are known to vary in physical and chemical characteristics and biotic MeHg. MeHg concentrations were measured and compared against protein-bound cysteine and methionine, as well as measures of dietary habits (delta15N, a measure of relative trophic position; delta13C, a measure of carbon source). Cysteine and methionine content (per mg total protein) differed among taxa, and were included (along with delta15N and sometimes delta13C) in within-taxa models predicting MeHg levels. In addition, log MeHg ($mg \cdot kg^{-1}$ dry weight) was significantly and positively related to log cysteine (nmols per mg total protein) (r^2 =0.74-0.90; $p < 0.001$) within food webs across all lakes; no among-system differences in these slopes were observed. For these same systems, relationships between MeHg and delta15N resulted in a better goodness of fit (r^2 =0.90-0.95; $p < 0.001$) and similar slopes (ANCOVA interaction term, p=0.5059). These results suggest that while cysteine content was a significant predictor of MeHg in aquatic food webs and within taxa, delta15N tended to explain more of the among-taxa variability within each system.

Using amino acid-specific stable isotope analysis to track mercury through the food web (PL)

Kyle Elliott[1], Birgit Braune[2], John Elliott[2]

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Top predators are often used as indicators of contaminant trends across space and time. However, signals are integrated over complex food webs that may confound such indicators. Although trophic position, assessed by bulk $\delta^{15}N$, is widely used to account for variation in diet, a single variable cannot completely describe complex food webs. Thus, we examined the relationship between up to 25 stable isotope values (including amino-acid compound-specific stable isotopes) and a variety of contaminants across three aquatic ecosystems: lacustrine birds of prey, a seabird guild, and an Arctic fish community. Variation in baseline $\delta^{15}N$ explained over half of the variation in bulk $\delta^{15}N$, and stable isotope values that reflected the base of the food web ($\delta^{13}C$, $\delta^{18}O$, $\delta^{34}S$) predicted some contaminants better than $\delta^{15}N$ alone—which was supported by a meta-analysis of other studies. In seabirds, changes in diet over time, only apparent once habitat was considered, obscured temporal variation in mercury over five decades. In Arctic fish, more accurate trophic magnification factors were calculated using $\delta^{15}N$ corrected for baselines. In conclusion, diet was an important determinant of contaminant levels, with habitat playing an important role. Accounting for complex food webs was essential to understand patterns in contaminants across space, time and biological communities.

Community-driven research in understanding and predicting fish mercury levels in northern lakes: Two case studies (PL)

Heidi Swanson[1], George Low[2], Nelson Zabel[1], Michael Low[2], Marlene Evans[3], Brian Branfireun[4], Mathieya Alatini[5]

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Many northerners are concerned about levels of mercury in fishes such as northern pike (*Esox lucius*), walleye (*Sander vitreus*), and lake trout (*Salvelinus namaycush*). In some traditional fishing lakes, mercury levels are high enough to warrant consumption advisories. Fishers, community members, regulators, monitors, and scientists want to understand causes of among-lake variability in fish mercury levels, and why levels are increasing in some lakes but not others. In this presentation, I will discuss challenges, successes, and lessons learned (so far) during two community-driven research projects on understanding and predicting fish mercury levels in northern lakes. In one study, a joint University and community research team completed an intensive one-year study on Kluane Lake, YT. This project formally included traditional knowledge interviews with elders, collaboration with subsistence fishers, and an intensive youth training component. Results of the study indicated that mercury levels in food fishes were remarkably low, and thus risk communication to the community was straightforward. In the second study, eight lakes in Dehcho region of the Northwest Territories were sampled over three years to determine drivers of spatial variability in food fish mercury concentrations. This project involved several different First Nations, and a joint University-Indigenous environmental monitor team. Results of this study have led to consideration of interesting mercury mitigation initiatives, such as fish-downs, and indicate that monitoring variables such as chlorophyll-*a* will help us predict the trajectory of mercury in some food fish species.

Mercury contamination in the Canadian Arctic marine ecosystems in a changing climate: How long will it take to recover? (PL)

Feiyue Wang[1]

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The observations of elevated mercury concentrations in Arctic marine mammals since the 1970s have raised much concern over the health of these animals and of Indigenous peoples who rely on them as part of their traditional diet. Major efforts have been undertaken in past decades to understand the sources and processes responsible for mercury contamination in the Canadian Arctic marine ecosystems. Here I will provide a synopsis of mercury research in the Canadian Arctic marine ecosystems, highlighting major findings over the past decade. We show that mercury contamination in the Canadian Arctic marine ecosystems no longer follows the general trend in global or regional mercury emissions; instead, it is increasingly driven by climate change-induced changes in biogeochemical and ecological processes that control the production and biological uptake of methylmercury. This has major implications for how the marine ecosystems will recover now that anthropogenic mercury emissions are being controlled under the Minamata Convention on Mercury. While mercury concentrations in the Canadian Arctic marine mammals are projected to decrease over

the long term, the "processes-driven" bioaccumulation dictates that it will take much longer, in the order of several decades or more, to establish a new steady-state in biotic mercury. In the shorter term, however, mercury concentrations in many marine animals the Canadian Arctic Ocean will likely continue to increase. Given the long and "bumpy" recovery road ahead, effective remediation and adaptation strategies are needed to assist the local communities that are facing mercury contamination in their ecosystems and food sources.

Mercury cycling in the saltmarsh of the Parker River Wildlife Estuary in Massachusetts, USA (PL)

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Salt marshes are among the most productive aquatic ecosystems and play an important role at the interface of land and sea ecosystems. In this study, we aim to address the dynamics of mercury (Hg), an important global contaminant, in the Great Marsh in the Parker River Wildlife Estuary, where previously high Hg levels have been observed in the blood of birds and other wildlife, although reasons for high Hg exposures are not fully understood. Our work aims to address sources and sinks of Hg in the Parker River salt marsh ecosystems. We collected samples of plants, soils and water to study the dynamics of Hg in the salt marsh ecosystems and its potential role as source to the estuary. Our hypothesis is that salt marsh plants absorb substantial Hg from the atmosphere and hence serve as an important deposition source of Hg to this estuary, via litterfall and plant senescence. Salt marsh Hg concentrations from the dominant plant species is in the range of three to 16 $\mu g \cdot kg^{-1}$, lower than vegetation in adjacent forests and showing substantial differences among different plant types. Salt marsh soils, collected to a depth of two meters, show high Hg concentrations up to 850 $\mu g \cdot kg^{-1}$, which is several folds higher compared to levels observed in adjacent forest soils. Gradient sampling of tidal water in the saltmarsh show a source of Hg from salt marsh soils to tidal waters, and relationships to salinity and auxiliary parameters such as organic carbon (DOC).

Mercury in aquatic food webs impacted by two run-of-the-river hydroelectric plants, forest fire, logging and constructed wetlands (PL)

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Methylmercury concentrations ([MeHg]) were unexpectedly high in fish from low-impoundment run-of-river reservoirs located in the St-Maurice River (Quebec, Canada). Apart from the flooded zones of a few square kilometers, other perturbations occurred in the watershed that could have led to conditions that favoured mercury methylation by microorganisms. To observe the dynamic of mercury transfer through food webs in different sectors, we collected 532 invertebrate samples and 432 fish of different sizes and species. We analyzed [MeHg], isotopic signatures of carbon ($\delta^{13}C$) and nitrogen

($\delta^{15}N$) as well as Northern Pike and Walleye growth rate. We calculated the trophic magnification slopes (TMS) for each sector using regressions of Log[MeHg] as a function of adjusted $\delta^{15}N$ of all organisms of each food web. TMSs were significantly different between sectors and were function of the intercepts of the regressions (Log[MeHg] at $\delta^{15}N = 0$). Those latter were similar to the measured [MeHg] in primary consumers. In comparison to top predatory fish, low trophic level fish (TL < 3.5) had higher [MeHg] than expected from their $\delta^{15}N$ values. Analysis of their $\delta^{13}C$ showed that [MeHg] were not function of trophic status but rather of their source of carbon ($\delta^{13}C$). A biodynamic model of accumulation successfully modeled [MeHg] in Northern Pike and Walleye and those estimates suggest that growth did not significantly influenced accumulation. In conclusion, entire food web [MeHg] was mostly affected by changes in methylation rates by microorganisms in flooded areas that could have been traced with carbon isotopes along the food chain.

Mercury in fish from the Far North of Ontario: Implications of climate change and the Ring of Fire development (PL)

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The Far North of Ontario, Canada, contains some of the largest intact boreal freshwater watersheds in the world, surrounded by undisturbed expanses of boreal forest and vast peatlands. The main goal of this work was to assess how various watershed-level processes affect mercury (Hg) bioaccumulation and biomagnification through freshwater food webs across the pristine Attawapiskat Drainage Basin (ADB) in the Far North. This watershed overlaps with the mineral-rich region known as the "Ring of Fire" which is expected to be heavily mined in the coming decades, potentially altering the physico-chemical environments of surrounding lakes and rivers. Water, macroinvertebrates, and fish were sampled from 58 lake and river sites across the ADB from 2012 to 2015. Water samples were analyzed for 39 chemical parameters including total Hg ([THg]) and methylmercury (MeHg) concentrations. Biotic samples were analyzed for THg and/or MeHg, as well as carbon and nitrogen stable isotopes. The results of three studies using these data will be discussed, examining: (1) the effect of changing physicochemical and ecological factors across the watershed on Hg bioaccumulation, (2) the optical properties of dissolved organic matter and their relation to Hg concentrations, (3) the proportion of MeHg (i.e., [MeHg]/[THg]) in fish muscle tissue relative to ontogeny and trophic ecology. Our work provides baseline data for a region that is expecting substantial alterations due to both industrial development and climate change. We conclude by making recommendations regarding the future monitoring of Hg in Far North aquatic systems and the fish which reside in them.

The role of gaseous mercury fluxes as deposition and sources of mercury loads in arctic and temperate ecosystems (PL)

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Several lines of evidence suggest that the dominant source of mercury (Hg) in terrestrial ecosystems is deposition of gaseous atmospheric elemental Hg(0). Yet, direct deposition measurements of this dominant Hg(0) source are largely lacking. Here, we present two studies that quantify atmospheric deposition of Hg(0) at the ecosystem level, both in a tundra ecosystem in Alaska and a temperate forest in Massachusetts (MA). In both ecosystems, we deployed micrometeorological flux-gradient methods to directly characterize surface-atmosphere exchange fluxes of Hg(0). In an arctic tundra in northern Alaska, 2-years of Hg(0) flux measurements showed that the tundra ecosystems serves as an important receptor area for atmospheric Hg with deposition dominated (~70%) by atmospheric Hg(0). Wet deposition of Hg was almost negligible in the interior arctic tundra. We found large pools of Hg sequestered in tundra active-layer and permafrost soils, which we attribute to thousands of years of atmospheric deposition along with strong retention of deposited Hg within arctic soils. In a temperate mixed deciduous at Harvard forest near Petersham, MA, ongoing Hg(0) flux measurements both above the canopy and above the forest floor show deposition of Hg(0) largely during nights, and emissions of Hg(0) during midday. Although the source of Hg in forests also is expected to be dominated by deposition of Hg(0), measurements of Hg(0) deposition prove to be extremely challenging by gradient-based techniques because the vertical differences are so small on account of efficient vertical mixing in the surface layer just above the forest canopy.

Inorganic Hg, methylmercury and selenium subcellular partitioning in yellow perch (PL)

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Mercury (Hg) is an element of particular concern since it is ubiquitous in the environment and because its methylated form (MeHg) readily bioaccumulates and biomagnifies in food webs. This latter process leads to elevated Hg concentrations in fish and may induce toxicity. One emerging approach to investigate potential Hg toxicity in the wild is via the use of subcellular fractionation of Hg and of its antagonist, selenium (Se). This approach allows the separation of subcellular fractions sensitive to metals (e.g., mitochondria) and others that are involved in detoxification (e.g,. metal-rich granules). Here, we present this approach on fish liver and test the necessity of validating the method. We show that thorough validation using enzymatic assays is essential. We then apply the approach to investigate the decline of yellow perch (*Perca flavescens*) in Lake St. Pierre, a fluvial lake. Results show that, in the liver, MeHg was primarily (51%) associated to the subcellular fraction containing cytosolic enzymes. Furthermore, 23% and 15% of MeHg was found in hepatic and gonadal mitochondria respectively, suggesting that yellow perch is not effectively detoxifying this metal. There was also a strong

relationship (R^2= 0.73) between MeHg bioaccumulation in the liver and MeHg concentrations in gonadal mitochondria, which corroborates the potential risk linked to MeHg maternal transfer. On the other hand, we also found that selenium might have a protective effect on Hg toxicity at a subcellular level. Overall, this study indicates that yellow perch accumulates Hg/MeHg in sensitive fractions, but that this apparent inadequate handling is potentially compensated by Se accumulation.

Fish intra-organ covariation of methylmercury, selenium and arsenic can be explained by lipid and protein content in striped bass (*Morone saxatilis*) but not in pike (*Esox lucius*) (PL)

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Current guidelines tend to limit fish consumption based on mercury (Hg) content in fish flesh, without considering the presence of antagonist chemical elements that could modulate Hg toxicity. However, it is difficult to assess the potential antagonistic interactions of these elements since their covariation within muscle tissues is poorly known. We mapped for the first time the intra-muscular covariation of Hg, selenium (Se, a Hg antagonist) and arsenic (As, a Se antagonist) in two fish species with contrasting muscular structure with respect to the presence of white and red muscles: striped bass (*Morone saxatilis*) possesses a thick layer of red muscle at the lateral line and pike (*Esox lucius*) has almost no red muscle. Red and white muscle are biochemically different: red muscle is richer in fat than white muscle. In individual striped bass muscle tissues, MeHg and Se covaried strongly and were related to protein content as assessed by %N; As was inversely related to these elements and was associated with the lipid fraction of the muscle. In pike, no such relationships were found because the content in protein and lipids was less variable. These results suggest that As is unlikely to act as an antagonist of Se in these fish since it is not located in the same tissue fraction, whereas Se can potentially inhibit Hg toxicity. Further, the elemental associations found with intra-muscular lipids and proteins could help explain changes in bioaccumulation patterns within and between individuals. Finally, those results allow us to better understand antagonistic relations regarding these three elements, with potential highlight for fish consumption guidelines.

Effects of non-native fish on food web structure and mercury biomagnification along a dissolved organic carbon gradient (PO)

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Both mercury (Hg) contamination and the introduction of non-native fish species threaten aquatic ecosystems worldwide. Although much research has been devoted to the two subjects separately, less has evaluated the impacts that non-native fish introductions have on the transfer of methylmercury (MeHg) in aquatic food webs. To better understand how non-native fish species influence the

biomagnification of MeHg, we sampled surface water and biota from eight lakes in La Mauricie National Park (Quebec, Canada), spanning a range of dissolved organic carbon (DOC) concentrations. Four of the lakes (Caribou, Maréchal, Baie Cobb, Onze Îles) were only inhabited by native brook trout (*Salvelinus fontinalis*) but the remaining lakes (Alphonse, Écarté, Besace, du Fou) contained populations of brook trout as well as non-native fish species Allegheny pearl dace (*Margariscus margarita*) and creek chub (*Semotilus atromaculatus*). Water samples from the eight lakes were analyzed to determine concentrations of DOC, total Hg (THg), and MeHg. Periphyton, zooplankton, benthic macroinvertebrates, and fish were analyzed for THg and MeHg, as well as stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios to delineate food webs and calculate various food web metrics. DOC concentrations ranged from 2.9 to 8.4 $mg \cdot L^{-1}$ with reference lakes having slightly lower DOC concentrations than invaded lakes. THg and MeHg in water increased linearly with DOC concentration across the study lakes. Similar to THg levels in dragonflies (baseline), THg levels in brook trout from invaded lakes (1.50 ± 0.94 $\mu g \cdot g^{-1}$ dry weight) were significantly higher than those from reference lakes (0.81 ± 0.12 $\mu g \cdot g^{-1}$ dry weight). Overall, DOC and trophic magnification slopes were the only significant predictors of brook trout THg among the reference and invaded lakes. Notably, $\delta^{13}C$ values, trophic position, and food chain length did not significantly affect THg levels of native brook trout. We noted that brook trout from invaded lakes tended to have much narrower isotopic niches than those from reference systems. Collectively, our results indicate that although non-native fish altered food webs, the level of DOC in lake water was most important in determining the Hg levels of native brook trout.

Mercury in the soft tissue of mussels from the St Lawrence Estuary and Gaspé Peninsula: Concentrations measured in 2016 are similar to those measured 40 years ago (PO)

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Various species of marine mussels have been used in the past 50 years as sentinel organisms for monitoring metal contamination along marine coasts. In practice, trace metal concentrations in mussel soft tissue reveal (after some adjustments for biotic effects) the contamination level of their surrounding environment. We present here the results of a mercury (Hg) survey in mussels (*Mytilus* sp.) collected in summer 2016 along the coasts of the St. Lawrence Estuary and the Gaspé Peninsula. The Hg concentrations in mussels ranged from 0.05 to 0.52 $\mu g \cdot g^{-1}$ (dry weight), averaging 0.16 ± 0.09 $\mu g \cdot g^{-1}$ (dry weight) for the 157 individuals analyzed. Concentrations were significantly ($p<0.01$) related to shell length and dry tissue weight, with the smaller individuals having highest Hg concentrations. In order to take into account these biotic effects, we normalized Hg concentrations of the mussel soft tissue for constant shell length and soft tissue weight. The normalized Hg concentrations are in the same range as those obtained with the same technique, for the same species, from the same stations visited in 1976 (Cossa and Rondeau, 1979, *Mar. Biol*., 88, 43-49). This observation suggests that the Hg bioavailable for mussels did not change after 40 years. In addition, the results exhibit a decreasing trend of Hg concentrations downstream of the St Lawrence Estuary, as already noticed in the previous survey, suggesting that St Lawrence River remains a significant Hg source for estuarine waters.

Using ice and sediment cores to quantify climate-warming induced inputs of legacy mercury to Lake Hazen, Nunavut (PO)

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Mercury (Hg) emitted from anthropogenic sources can undergo long-range atmospheric transport to the Arctic, where it may cause health concerns for Arctic wildlife and indigenous people. Many sediment records do not show a clear decline in Hg deposition despite estimates that Hg emissions from anthropogenic sources have recently stabilized or declined, suggesting that Hg is being remobilized in catchments and delaying the recovery of lakes following emission reductions. To date there is little data on whether the input of legacy Hg increases Hg accumulation in lakes. We hypothesize that the climate-warming induced melting of glaciers may be remobilizing legacy Hg from glaciated watersheds into downstream lakes, providing an important subsidy of Hg in addition to modern Hg inputs. To test this hypothesis, we will compare Hg accumulation rates through time measured in an ice core and sediments cores collected from the Lake Hazen watershed, Nunavut, Canada. By comparing ice and sediment core data, we can determine how post-depositional processes in the watershed controls delivery of legacy and modern Hg into Lake Hazen and whether legacy Hg inputs are increasing the rate of Hg accumulation in the lake. The temporal trends in atmospheric Hg deposition will be compared to known changes in anthropogenic production and/or emission of Hg. Our research will elucidate whether climate change may delay the benefit of decreasing Hg emissions in glaciated and Arctic watersheds, and help improve models of global Hg cycling, develop policies on Hg management, and better manage contaminant exposure for Arctic people and wildlife.

Mercury methylation in sediments of a river affected by forest fire, logging, and the construction of a run-of-the-river power plant (PO)

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Methylmercury (MeHg), an organic form of mercury (Hg), is a worrying contaminant in terms of human and ecosystem health due to its high toxicity and its ability to biomagnify along food webs. In a subsection of the Saint-Maurice River (Québec, Canada) in 2013, elevated concentrations of MeHg were measured in fish flesh, making it a source of contamination for local populations. This river section has been impacted by several landscape changes: (1) the construction of two run-of-river hydroelectric dams, (2) well-established logging activities in the region, and (3) a wildfire. This set of stressors could explain the high levels of MeHg found in the fish flesh because they act as indirect factors increasing Hg methylation. Hg methylation is mostly catalyzed biotically by anaerobic methylating microbial communities. As these communities are mainly present in sediments, this compartment is known as a hotspot for methylation and could thus act as a source of MeHg for the biota. Using both biogeochemical and metagenomic approaches, sediment cores have been analyzed on a biogeographic gradient along the river. Preliminary results highlight important variability in the MeHg/THg ratio (proxy for

methylation) between the different sites, along with high variability within the profiles. Future analyses will include assessment of the origin of organic matter using different indicators. Further, on each profile, several depths have been selected to sequence environmental DNA using NovaSeq technology. The combined results will provide new insights regarding mercury cycle in boreal river sediments impacted by both anthropogenic activities and natural perturbations.

Influence of nutrient stoichiometry on mercury bioaccumulation in Arctic freshwater zooplankton (PO)

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To predict long-term shifts in mercury dynamics with climate change, we need to better understand how aquatic productivity influences methylmercury (MeHg) bioaccumulation in freshwater food webs. Although studies have shown strong interactions between nutrient stoichiometry (C:N:P) and metal accumulation in the laboratory, relationships between these two variables have not been adequately tested in northern lakes. In this study, we sampled a gradient in ecosystem productivity in the eastern Canadian Arctic and measured MeHg bioaccumulation and carbon:nutrient ratios in seston and zooplankton from 47 lakes. Observations spanning 20° of latitude were used to compare MeHg bioaccumulation with water chemistry, food resource quality, growth rates, and food web structure. Contrary to previous findings at temperate latitudes, seston biomass, chlorophyll concentrations and seston nutritional quality (seston %P, and C:N, C:P ratios) were not good predictors of either seston or zooplankton MeHg concentrations. Rather, bulk zooplankton (57 ± 45 $ng \cdot g^{-1}$) and *Daphnia* MeHg concentrations (101 ± 86 $ng \cdot g^{-1}$) were positively correlated with water MeHg concentrations and only weakly negatively correlated to water nutrient concentrations (TN, TP). The highest zooplankton MeHg concentrations were found in lakes with high MeHg and low TN in surface waters. Overall, we found that indicators of lake productivity, seston nutritional quality and organism growth rates did not explain MeHg bioaccumulation, as predicted by biodilution theory. We suggest that aqueous MeHg exposure is the dominant factor controlling MeHg uptake at the base of these freshwater food webs, and that seston biomass and nutritional quality are not key drivers of MeHg bioaccumulation in these northern systems.

Mercury and selenium bioaccummulation and subcellular partitioning in northern pike (*Esox lucius*) in an impacted stretch of the Saint-Maurice River (PO)

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Mercury (Hg) is a ubiquitous trace element known to be neurotoxic in its methylated form (MeHg) which also has the ability to bioaccumulate and biomagnify along aquatic food webs, leading to elevated Hg concentrations in fish and thus potentially inducing toxicity. In order to explore the toxicity potential of Hg and MeHg in northern pike (*Esox lucius*) from a stretch of the Saint-Maurice River (Quebec,

Canada), we established a gradient of observed concentrations in liver and are currently assessing the subcellular fractionation of these species. We observed a strong gradient in Hg concentrations between individuals: Hg and MeHg in the liver varied by a factor of 63 (322 to 20321 $ng \cdot g^{-1}$ dry weight) and 40 (178 to 7272 $ng \cdot g^{-1}$ dry weight), respectively. With such large gradients, we hypothesize that there will be a change in subcellular partitioning of Hg and MeHg in response to increasing exposure. Also, selenium (Se) might have a protective effect against Hg toxicity. Indeed, Se:Hg molar ratios in livers were above 1, corresponding to the suggested protective threshold. Further, we will distinguish between Hg, MeHg, and Se accumulation in sensitive (e.g., mitochondria) vs non sensitive (e.g., granules, metallothionein-like proteins) cell compartments. As the subcellular partitioning approach is highly species- and organ-specific, a customized protocol was currently assessed and its efficiency was tested using enzyme biomarkers lactate dehydrogenase (LDH), citrate synthase (CS), and cytochrome c oxidase (CCO). The next step of this study will be to measure Hg, MeHg and Se in each fraction and determine if sensitive fractions are significant sites of accumulation at the higher end of the gradient.

Periphyton as methylmercury source in a river impacted by run-of-the-river hydroelectric plants (PO)

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The production of methylmercury (MeHg) by periphyton biofilm has been increasingly accepted in recent years. This methylation is mediated by species that contain hgcAB, genes responsible for the production of MeHg. In order to investigate periphyton's role in the boreal ecozone, the Saint-Maurice River was studied in a sector impacted by two run-of-the-river dams. This area has not only been influenced by the construction and operation of hydroelectric power plants, but also by recent logging and a forest wildfire, which might explain the high concentrations of MeHg found in predator fish. Littoral benthic invertebrates, periphyton and water samples from different sites were analysed for mercury and MeHg. The methylation (k_m) and demethylation rates (k_d) of mercury by periphyton were measured *in situ* using mercury stable isotopes. Periphyton biofilms genomic analysis was performed using 16S rRNA and hgcAB genes to assess the natural diversity of methylating microorganisms. MeHg concentrations in periphyton were the best predictors of the benthic predators (Odonata order) contamination, suggesting a periphyton role in the uptake of MeHg. The highest k_m was measured in constructed channels and greater k_d were found in these channels and in a flooded section of the river. These rates match the periphyton microbial composition of methylators in the channels. Our results indicate that periphyton from the boreal region is likely to play a key role in the mercury cycle through the production and degradation of MeHg, and these aquatic biofilms would be a major gateway for mercury in different food webs and habitats.

Impacts of forestry on methylmercury in water and aquatic invertebrates within three forested catchments in New Brunswick (PO)

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Studies have found that forest harvesting can increase methlymercury (MeHg) in streams by increasing the mobilization of mercury from the soil and increasing sediment loading that creates anoxic stream environments where mercury methylation occurs. Little information exists on how this resource sector affects MeHg fate in streams in Canada. For this project, water, particulates, different functional feeding groups of aquatic invertebrates, and the fish slimy sculpin (Cottus cognatus) were collected in the fall of 2018 from six sites along a longitudinal gradient in each of three different catchments that represented low (NBR), medium (NBE) and high (NBI) harvesting intensities. The main objective is to explore whether different harvesting intensities affect abiotic and biotic MeHg levels. MeHg was determined using inductively coupled plasma mass spectrometry (ICP-MS). Results to date indicate that average MeHg in stream waters were 27 pg·L-1, (5.5 pg·L-1-120 pg·L-1; n=16), 95 pg·L-1 (32 pg·L-1-150 pg·L-1; n=17), and 39 pg·L-1 (6.0 pg·L-1- 110 pg·L-1; n=15) for NBR, NBE, and NBI, respectively. The invertebrates Baetids, Ceratopsyche, Glossosoma, Pteronarcys, and Sweltsa represent collector/gatherer, collector/filterer, scraper/grazer, shredders and predators and analyses of these taxa and the fish are ongoing. Overall, these results will be used to assess whether MeHg levels in streams and their biota are affected by different intensities of forestry management and to show spatial trends within the watersheds.

Effects of Conventional and Unconventional Oils in Freshwater Ecosystems

Evaluating acute, chronic, and pulse exposures of hydraulic fracturing flowback and produced water (FPW) in the aquatic invertebrate *Lumbriculus variegatus* (PL)

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With continued high demand for oil and gas, more cost-effective means of extraction are being frequently used. One of these techniques is horizontal hydraulic fracturing. Hydraulic fracturing produces large amounts of flowback and produced water waste (FPW). The FPW associated with hydraulic fracturing has been shown to be a complex mixture containing high salinity and a variety of anthropogenic and geogenic compounds. In the present study, the risk of FPW releases to aquatic systems was studied using the benthic invertebrate, *Lumbriculus variegatus.* Acute, chronic, and pulse toxicity were evaluated to better understand the implications of FPW releases. Bioassays used FPW acquired from a local well in Alberta. Although *L. variegatus* have a high tolerance to aquatic contamination, acute toxicity was significant at low dilutions of FPW (48 hour LC_{50}: 4-5%). Chronic toxicity of FPW in this species was more pronounced with LC_{50}s (survival/reproduction) and EC_{50}s (total mass) as low as 0.53% FPW. Investigations evaluating pulse toxicity (6 hour and 48 hour) also showed a significant amount of latent mortality occurring when compared to the acute results. Causality in acute and chronic bioassays differed, as acute toxicity appeared to be driven mainly by salinity but was not the sole driver in chronic toxicity. The findings of this study show the importance of evaluating multiple exposure regimes, but perhaps more importantly the complexity of FPW.

Profiling volatile organic compounds from naphthenic acids, acid extractable organic mixtures, and oil sands process-affected water by solid-phase microextraction-gas chromatography-electron impact mass spectrometry and chemometric analysis (PL)

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Naphthenic acids (NAs) are naturally occurring complex mixtures of carboxylic acids from petroleum that are released to the environment after oil spills. There is significant research on the chemical composition and toxicity of water-soluble NAs because high levels are found in tailings ponds in the oil sands mining region of Alberta, Canada. Yet little is known about volatile organic compounds (VOCs) from these sources and their potential risks to humans and wildlife. Headspace solid-phase microextraction coupled to gas chromatography-electron impact mass spectrometry was used for VOC profiling of commercial NA blends, and an acid-extractable organics (AEOs) mixture from a tailings pond. From Sigma1, Sigma 2, Merichem NAs and the AEO extract, 54, 56, 40 and 4 compounds were

identified, respectively. These include aliphatic and cyclic hydrocarbons, carboxylic acids, alkylbenzenes, phenols, naphthalene and alkyl naphthalene, and decalin compounds. A sample of oil sands process-affected water (OSPW) from a tailings pond and aqueous solutions of the NA blends were also analyzed to evaluate the matrix effect on VOC profiles. Principal component and clustering analyses revealed that VOC profiles of commercial extracts were closely related but distinct from the AEO and OSPW samples. Some of the identified VOCs may be genotoxic or carcinogenic and should be evaluated for safety and environmental hazard potential.

Exposure to conventional heavy crude oil affects metabolism and swim endurance in fathead minnows (*Pimephales promelas*) (PL)

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Transport of fossil fuels is increasingly becoming a public and political concern, in part due to the potential for unintended releases to aquatic systems. Despite this, the current understanding of petroleum hydrocarbon toxicity in freshwater systems is limited. Much of the existing research has focused on early life stage developmental toxicity, however there has been little investigation into sublethal effects that may occur at other life stages. The focus of this study was to assess effects of conventional heavy crude exposure on the metabolism and swim endurance of sub-adult fathead minnows (*Pimephales promelas*). Fish were exposed to concentrations of either 0% (Control) 3%, 10%, or 30% of a conventional heavy crude water accommodated fraction (WAF) for seven days. Following the exposure period, one group of fish (n=16/treatment) was assessed for cardiac structure & function using ultra high-frequency cardiac ultrasound. A second group (n=12/treatment) was assessed for critical swim speed (U_{crit}) and active metabolic rate (AMR) using swim tunnel respirometry. A third group (n=5-6/treatment) was assessed for standard metabolic rate (SMR) using intermittent respirometry. Fish exposed to the highest WAF loading rate showed statistically significant increases in heart rate and cardiac output. During swim trials, the 30% WAF exposure group exhibited greater AMR in response to increasing swim speeds, as well as lesser mean U_{crit}. In the future, these results will be used to compare with results of a similar experiment in which diluted bitumen toxicity is assessed.

Effect assessment of offshore crude oil using different dispersants to American lobster larvae (*Homarus americanus*) (PL)

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The increasing development in Canada of oil and gas industries and transportation of their products draw the attention of both scientists and policy makers to potential for increased occurrence of oil spills and the consequent contamination of Canadian aquatic ecosystems. In this regard, a Multi-Partner Research Initiative (MPRI) was established as part of the Ocean Protection Plan to improve the understanding of impacts of oil spills to aquatic biota and to develop alternative response measures for

Canadian coastlines. To fulfill the objectives of MPRI in toxicity assessment, this study was conducted to 1) evaluate the toxic effects of water accommodated fraction (WAF) and chemically-enhanced water accommodated fraction (CEWAF) crude oil on the survival and development of planktonic American lobster larvae, *Homarus americanus*; and 2) compare the lethal and sub-lethal effects of the CEWAFs using three dispersants, Corexit 9500a, Finnasol OSR52, and Dasic SlickGone NS. Static 24-hour toxicity bioassays were conducted using stage I larval lobsters in the test medium with the offshore crude oil to UV sterilized 0.22 µm filtered seawater with a loading of 1 g oil per 1 L of water for all solutions, and the dispersant to crude oil ratio of 1:20 for the CEWAF solutions. The first objective was investigated using the total concentration of polycyclic aromatic hydrocarbons (PAHs) and total petroleum hydrocarbons (TPHs) measured in both the WAF and the CEWAF solutions. Using the measured concentrations of the TPHs there was no significant difference between the toxicity of the WAF and the CEWAFs to lobster larvae (i.e., the EC_{50} values of 4.51, 5.87, 5.89, and 4.23 $mg \cdot L^{-1}$ for the WAF, CEWAF-Corexit, CEWAF-Finnasol, and CEWAF-SlickGone, respectively). Measuring the dissolved oxygen before and after the experiments showed a decline in the percent saturation of dissolved oxygen in the WAF and CEWAFs treatments after finishing the experiments, whereas it did not change in the control seawater during the experiments. Challenges with interpreting oil toxicity data and the importance of measuring exposure solution concentrations will be discussed.

Environmental monitoring and impact assessment guidance for marine oil spills on the Pacific coast of Canada (PL)

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An oil spill in the marine environment has the potential to cause significant environmental impacts. The timely and efficient collection and evaluation of relevant monitoring data following spills at sea are essential to understanding and mitigating these impacts. Fisheries and Oceans Canada (DFO) commissioned the development of draft technical guidance for environmental monitoring and impact assessment (EMIA) of marine oil spills on the coast of British Columbia (BC). The technical guidance, which builds on recent national and international work, incorporates a risk-based approach and provides users with operational procedures for developing and implementing a spill-specific monitoring plan. Guidance on seafood assessment following an oil spill to determine when to close and re-open a fishery is also included. This presentation describes key components of the guidance. The first component consists of information necessary for completing the problem-formulation stage of a risk assessment, including the development of a conceptual site model. The second component guides decision-making during each phase of spill monitoring. The next two components of the technical guidance present the key aspects of a scientifically-defensible monitoring program and outline operational procedures and methods for the collection and analysis of data related to relevant media and receptors.

Comparative toxicity of diluted bitumen and conventional oil to two Canadian salmonids during early life stages (PL)

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Canada's crude oil production is growing and requires an increase of transport capacity. Pipeline construction projects are being developed to transport diluted bitumen (dilbit) from Alberta, Canada, to the coasts. However, limited information is available on the toxicity of dilbit in freshwater ecosystems. This project aims to evaluate the toxicity of dilbit in two salmonids found in Canadian freshwater ecosystems. Chronic exposures were performed on rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) at different developmental stages. One dilbit (Clearwater McMurray) and one conventional crude oil (Lloydminster) were tested. At exposure completion, mortality, growth, and malformations were assessed. CYP1A activity, an indicator of the detoxification capacity, was quantified along with the expression of its gene. Preliminary data suggest Clearwater McMurray dilbit is the most toxic oil to both salmonid species as it yielded higher mortality rates than the conventional oil. CYP1A activity and *cyp1a* mRNA levels increased in rainbow trout larvae following an exposure from the fertilized egg stage to hatching (approx. 30 days), but both decreased once these exposed fish were returned to clean water (depuration duration of approx. 20 days). Notably, CYP1A activity was not significantly induced when the exposures started after the eyed stage. When comparing salmonid species' responses to the dilbit treatment, mortality rates were similar for both species exposed to the two oils. This research project will improve understanding of the impact and toxicity of conventional and unconventional oils on the early life stages of economically important freshwater fish species for Canada.

Oil spill scenario comparison using pilot-scale tank tests (PL)

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In Canada, there is a strong need to get Alberta oil to "tide water" by either pipeline or rail, which has led to public concerns regarding the impacts of potential oil spills. Our research has focused on the fate and behaviour of oil spills, especially of diluted bitumen (DB), since little is known about how it would behave, and it is the major oil commodity transported through pipelines from Alberta. In this work, a pair of pilot-scale tank tests have been performed to systematically compare the physical and chemical behaviours of Cold Lake DB and an Alberta conventional crude (CC) spilled into freshwater, as well as to assess and compare the biological impacts of petroleum-contaminated water. For both trials, 10 L of oil was spilled onto the surface of 1200 L of fresh water (obtained from the North Saskatchewan River and containing 2.4 kg of river sediment) in the spill tank, and left for a month under controlled air

and water temperatures of 15 ± 1°C. The energy input used was a breaking wave pattern of two days on and two days off. Methods were developed to close the oil mass balance based on characterization of recovered weathered oils at the end of the tests. The changing water hydrocarbon profiles, which included benzene/ toluene/ ethylbenzene/ xylene (BTEX), total organic carbon (TOC), and polycyclic aromatic hydrocarbons (PAHs), were determined to better understand the oil weathering. Water-accommodated fractions (WAFs) were also sampled at five specific time points during both month-long experiments and were used to perform early life stage exposures using fathead minnows (*Pimephales promelas*) to assess differential toxicity associated with oil weathering. Comparisons of crude oil properties, oil fate and behaviour during the runs, final weathered oil distribution, water hydrocarbon profiles, and associated fish embryo toxicity data will be presented. Data such as these can be used to inform net environmental benefit analyses (NEBA) in order to help decision-makers in choosing the best response methods for DB and CC spills.

Weathering of diluted bitumen leads to a differential fish embryotoxicity (PL)

Linda Lara-Jacobo[1], Charles Gauthier[1], Qin Xin[2], Heather Dettman[2], Félix Dupont[3], Patrice Couture[1], Gaëlle Triffault-Bouchet[3], Valérie Langlois[1]

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For several years, the Devon Research Center of CanmetENERGY (NRCan) has been conducting experiments in a pilot scale spill tank that examines the physical and chemical behavior of several oils in freshwater. The tests started in 2018 using the dilbit Cold Lake Blend (CLB) with water temperatures of 15 °C (warm temperature) and 2 °C (cold temperature). Water samples were taken from the spill tank 5 times during the 35-day experiment (days 1, 6, 14, 28, and 35) and were used to perform early life stage exposures using fathead minnows. During each sampling time, newly fertilized embryos were exposed to a serial dilution of the water samples from the original tank, with uncontaminated river water (the same used in the tank), along with a reconstituted water control. The larvae were exposed until hatching. Preliminary results showed that exposure to CLB produced significant negative impacts on fish larvae, including an increase in the rate of mortality and malformations, a decrease in the heartbeat frequency, and an alteration of *in vivo* ethoxyresorufin-O-deethylase (EROD) activity. The volatile organic compounds were significantly different in the two temperatures, and this increased the number of malformations in the larvae and the *in vivo* EROD activity. Real-time polymerase chain reaction analysis is ongoing. This research project demonstrates the importance of testing the inherent toxicity associated with most natural climatic conditions of the oils as fish embryotoxicity can be affected by oil weathering (i.e., time) and environmental conditions (i.e., temperature).

Understanding the relationship between the geochemical evolution of a dilbit spill and associated toxicology in shallow groundwater systems (PL)

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Current monopoly on Canadian bitumen export by the USA has fueled global trade interest and future pipeline development initiatives, such as the Trans Mountain Expansion project. Pipelines efficiently transport diluted bitumen (dilbit); ~70% bitumen containing lighter hydrocarbon fractions to reduce viscosity and facilitate flow. Although dilbit spills seldom occur, substantial environmental contamination has resulted from them, such as the Enbridge pipeline rupture near the Kalamazoo River in 2010. Canada's bitumen pipeline network currently has the capacity to transport greater than 3.5 million barrels of oil per day, yet the environmental fate and effect of spilled dilbit compared to conventional crude is not well understood, particularly in shallow groundwater systems. Many of these pipelines travel in close proximity to protected ecosystems and some of the most densely populated regions in Canada and the USA. Detailed investigations into the characteristics and evolution of subsurface dilbit plumes within the vadose zone, however, are limited. Thus, it is imperative to determine the geochemical characteristics and resultant toxicity of spilled dilbit over time and compare it to conventional crude. To this end, separate controlled spill experiments using both dilbit and conventional crude were conducted in large (1 × 0.6 m) unsaturated soil columns over a period of several months. Leachate samples were routinely collected to determine a suite of parent and alkylated polycyclic aromatic hydrocarbons (PAHs), naphthenic acids, and BTEX (benzene, toluene, ethylbenzene and xylenes) over time. Leachate samples were also collected for fathead minnow embryotoxicity analyses. Preliminary data will be presented.

Polycyclic aromatic hydrocarbon (PAH) exposure and health indicators in a riverine fish community following a diluted crude oil spill (PL)

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A spill of heavy crude and distillate into the North Saskatchewan River in 2016 led to immediate wildlife mortalities, but the long-term effects of exposure to oil constituents were uncertain. One and two years later, we sampled fish and invertebrate communities at two locations upstream and seven locations downstream of the spill. We examined health indicators at the individual (condition, liver somatic index, ethoxy-resorufin o-deethylase activity) and community (catch rates, proportion of tolerant species) levels and evaluated exposure to, and trophic transfer of, polycyclic aromatic hydrocarbons (PAHs) using a food web approach. There was no evidence for biomagnification of any PAH compounds, consistent with their rapid biotransformation and excretion by fish. Concentrations in muscle of large fish and corresponding bile PAH metabolites were both highest at downstream sites, but there was considerable variation among sites and years. Similar fish communities were present at

all sites, dominated by predatory walleye and small-bodied shiners, and catch rates were comparable with the exception of high densities of shiners at sites immediately below the spill. Fish body condition and liver somatic index at downstream sites generally fell within the expected range that was defined based on the upstream reference sites. These results suggest ongoing exposure to compounds in oil resulting from the spill, but an absence of long-term effects at highest levels of biological organization. They help contextualize laboratory studies by illustrating real-world consequences of unintended oil releases.

The effects of diluted bitumen on sediment organisms: chironomids and amphipods (PL)

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New oil exploration and exploitation projects are developed around the world to meet the growing demand for petroleum. One of them is the oil sands industry in Canada, which produces unconventional oils called diluted bitumen. However, despite advances in our knowledge of these products, their effects on benthic organisms following a spill are still largely unknown. In order to fill these gaps, this study aims to determine the lethal and sublethal effects of two diluted bitumens (Bluesky and Clearwater McMurray) and one conventional oil (Lloydminster) for freshwater benthic microorganisms and two benthic invertebrates: *Chironomus riparius*, a non-biting midge, and *Hyalella azteca*, an amphipod. One of the specific goals of this study is to assess the effects of dissolved hydrocarbons resulting from the physical dispersion of oil immediately after a spill. To this end, organisms were exposed for 48 hours to a fraction containing soluble hydrocarbons (WAF: water accommodated fraction; 10 $g \cdot L^{-1}$, 18 hours of agitation, 6 hours of sedimentation). After exposure, the genotoxicity of hydrocarbons was determined using the COMET test as well as after a short detoxification period (6 hours, 12 hours, 48 hours, 96 hours). Preliminary results indicate that dissolved hydrocarbons induce no clear effects on the growth, survival, and antioxidant capacities of *Hyalella azteca*. Growth and mortality assays for *Chironomus* and analysis of genotoxicity data for both species is ongoing and will be discussed. This study will contribute to improving our knowledge of diluted bitumen toxicity to freshwater invertebrates, and for decision-making related to spill hazard management in the aquatic environment.

Developing transcriptional biomarkers for *in ovo* exposure to diluted bitumen in embryonic Canadian avian species (PL)

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With oil transport intensifying in Canada, it is important to develop appropriate biomarkers of polycyclic aromatic compound (PAC) exposure if a spill occurs. Embryotoxicity is a prevalent effect of PAC exposure but the mechanisms of action have not been well elucidated in birds. The research

objectives of this study were (1) to establish the baseline expression profiles of several key genes, and (2) to assess the effects of PACs during avian embryonic development. Double-crested cormorant (*Phalacrocorax auritus*) eggs were collected from relatively uncontaminated sites in Ontario and artificially incubated from collection until 24 days. For baseline expression, five eggs were sampled every 4 days for a total of six time points. The remaining eggs were injected with a dilution ranging from 1:10 to 1:10,000 of one of the diluted bitumens produced in Canada (Clearwater or Cold Lake Blend) or with corn oil (vehicle control) prior to incubation. Embryos were sampled at day 12 when the liver is maturing and tissues were preserved for transcriptomic analysis. In addition, Northern gannet (*Morus bassanus*) eggs were collected from Bonaventure Island, Quebec, and were injected with 1:10 dilution of Cold Lake Blend, corn oil, or non-injected before artificial incubation until the liver matures. Preliminary results suggest that early developing avian embryos exposed to PACs have an upregulation of genes associated with xenobiotic metabolism and oxidative stress defense compared to controls. Overall, the comparison of transcriptional profiles can identify molecular mechanisms of action and develop appropriate biomarkers for oil exposure in avian species.

Toxicity of gasoline-, diesel- and weathered diesel-related petroleum hydrocarbons to freshwater and marine organisms (PO)

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The purpose of this study was to determine environmental effects-based concentrations of total petroleum hydrocarbons for assessing the impacts of fresh gasoline and fresh or weathered diesel in the freshwater and marine environments. The study was conducted by the Washington State Department of Ecology (Ecology) and Nautilus Environmental to determine the NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration) of gasoline, diesel and weathered diesel, in addition to IC_{25} and IC_{50} endpoints, using aquatic toxicity bioassays. In separate experiments, hydrocarbons within either the diesel or gasoline range were spiked into toxicity test solutions and weathered diesel in contaminated groundwater was obtained from a well-characterised site in Washington State. Freshwater organisms used were the fathead minnow (*Pimephales promelas*) and a cladoceran (*Ceriodaphnia dubia*). Topsmelt (*Atherinops affinis*) and the echinoderm, purple sea urchin (*Strongylocentrotus purpuratus*), were the marine species tested. Tests were conducted according to United States Environmental Protection Agency (US EPA) test methods and Ecology's whole effluent toxicity (WET) guidance document. Toxicity tests were conducted at Nautilus Environmental in Burnaby, British Columbia. Hydrocarbon concentrations in test solutions were determined at the Manchester Environmental Laboratory, Port Orchard, Washington. Gasoline caused similar toxicity between topsmelt, fathead minnow and *Ceriodaphnia*, which were more sensitive than the echinoderm. Diesel was generally more toxic than gasoline to all test organisms. With weathered diesel tests, this pattern changed and fish became the more sensitive organisms. All test organisms

were generally less sensitive to the weathered diesel compared with fresh diesel. Volatile compounds present in the fresh diesel may be responsible for greater toxicity to the invertebrates in particular.

Photodegradation of naphthenic acids and other organic classes in oil sands process-affected water extracts (PO)

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Naphthenic acids (NAs) are persistent and toxic natural constituents of bitumen that are solubilized and concentrated in oil sands process-affected water (OSPW) during oil sands extraction. Few studies have investigated environmentally-relevant photochemical transformation of NAs. We hypothesized that NA-like compounds are susceptible to phototransformation, and this process may be a significant removal pathway. To examine direct photolysis via wavelengths in solar radiation (i.e., >290 nm), changes in NA profiles (i.e., C and *z* numbers) were characterized following bench-top scale studies using OSPW, and acid- (AF) and base-extracted fractions (BF). Intensities were measured by high-performance liquid chromatography (HPLC) and ultra-high resolution mass spectrometry in negative and positive modes. Photolysis reduced all constituent groups in $OSPW^-$, $OSPW^+$, AF^-, and BF^+ over the course of the 18-day irradiation period. Total NAs in $OSPW^-$ were reduced by 99% and by 87% in AF^-. Highly photo-labile species included O_2^-, O_3^-, O_4^-, O_2S^-, O_4S^-, and O_5S^--containing structures, which were degraded by 93% to 100% after 5 days and by 94% to 100% after 18 days. Following photolysis, there was a noticeable shift towards smaller species with less cyclicity, double bonds, or aromaticity. On Day 18, mean carbon number and *z*-values in $OSPW^-$ shifted to 12 and -4, respectively, from 15 and -8 on Day 0. First-order kinetic models were used to investigate removal trends. Half-lives of heteroatomic classes ranged from 2.7 (O_4S^-) to 14.9 (O_3^-) days for $OSPW^-$, and from 3.9 (O_3S^+) to 20.9 (O_5^+) days in $OSPW^+$, indicating differences across structures, but overall efficient NA removal via photolysis.

Non-invasive methodology for assessing fish and amphibian recovery after an oil spill and different remediation strategies (PO)

Juan Manuel Gutierrez-Villagomez[1], Anita Thambirajah[2], Sonya Michaleski[3], Lauren Timlick[3], Caren Helbing[2], Gregg Tomy[3], Vince Palace[4], Valérie Langlois[1]

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The oil industry is economically important, and Canada was the world's fourth top producer in 2018. With high volumes of oil transported across the country, the environmental risk and impact of potential oil spills need to be considered. Therefore, validating efficient clean-up strategies is of importance in protecting Canadian ecosystems, including wildlife. The aim of this research project is to develop a non-lethal sampling approach to monitor ecosystem recovery after oil spills in freshwater

lakes. For this, three different remediation techniques were tested using a series of lake enclosures at the International Institute for Sustainable Development - Experimental Lakes Areas (IISD-ELA): 1) nutrient addition, 2) Corexit EC9580A (shoreline cleaner normally used in marine environment), and 3) engineered floating wetlands (EFW). EFW are substrates that support plants that develop root systems for biofilms where bioremediating bacteria can grow. Additional reference enclosures with no spill events were also examined. Free-swimming adult fathead minnows (*Pimephales promelas*) and wood frog (*Lithobates sylvaticus*) tadpoles native to the study lake were acclimated for one month before the application of a 0.1 cm-thick coating of weathered Cold Lake Blend diluted bitumen to the shoreline. Four days after bitumen application, absorptive and low-pressure water flushing was used to recover oil, followed by the selected remediation techniques. Fin clips and mucus were collected for transcriptomic analyses in order to understand the molecular responses to oil spills and to identify biomarkers of oil recovery in fish and frogs. This project will provide insights into the rehabilitation responses of freshwater aquatic species to diluted bitumen spills.

The state of polycyclic aromatic compounds in Canada: From emissions to biological effects (PO)

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Twenty-five years after the first comprehensive assessment of polycyclic aromatic compounds (PACs) in Canada, this paper presents the current state of knowledge of PAC emission sources, deposition, hot spots of PAC contamination (air, water and sediment levels), and effects on wildlife. Analyses are based on several national databases (e.g., the National Pollutant Release Inventory, the Air Pollutant Emissions Inventory, Canadian wildfire database) and long-term datasets for environmental levels. Preliminary results show that, on a national scale, wild forest fires continue to be the dominant contributor to PAC emissions in Canada. At a finer scale, emissions and releases from the oil and gas industry and motor vehicles are more important than forest fires at many locations. Many challenges remain in properly sampling environmental matrices and accurately measuring PACs, especially alkylated and heterocyclic PACs. Locations historically impacted by industrial emissions tend to exhibit decreasing trends in ambient air that are not being observed in the Athabasca Oil Sands or many cities. Occurrence and distribution of PACs indicate expansion of urban areas' influence on contamination of sediments and surface water. In Canadian wildlife, most PAC measurements exist in invertebrates, fish, and birds, and fewer in amphibians and mammals. Health effects in wildlife include cancer, embryotoxicity, cellular damage, and physiological impairments following PAC exposure. Assessing the ecological risks of individual PACs is hindered by their occurrence in complex mixtures with other PACs and contaminants, the unknown sensitivity of most Canadian species, and potential interactions of PAC exposure and toxicity with natural stressors, including climate change.

Potential interactive effects of diluted bitumen and UV on juvenile fathead minnows from the IISD-ELA Freshwater Oil Spill Remediation Study (FOReSt) (PO)

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Potential environmental impacts of oil spills are a concern for the Canadian public and the oil industry. Optimizing methods to treat residual oil that remains after an oil spill cleanup and to assess the potential impacts of residual oil in impacted freshwater systems are both high priorities. In 2018, the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) began a collaborative program to examine the efficacy of minimally-invasive remediation methods for residual bitumen and conventional heavy crude oil in freshwater shoreline environments. In 2018, model oil spills in contained shoreline environments (15 x 2.5m) were used to examine the efficiency of immediate oil recovery and then to compare degradation of residual oil via monitored natural recovery (MNR). Results from the 2018 pilot study were used to design a larger study being performed in 2019 that focuses on comparing the efficacy of oil removal using nutrient additions, a shoreline cleaner, and engineered floating wetlands relative to MNR. As part of these studies, the effects of residual diluted bitumen on juvenile fathead minnow development and deformity rates were evaluated. Because exposure to UV radiation can potentiate the toxicity of certain compounds in residual oil by up to 100-fold, we conducted a paired test exposing juvenile fathead minnows to water from enclosures treated with model spills of diluted bitumen with and without UV radiation.

Limnocorral study to examine potential impacts of diluted bitumen on wild small-bodied freshwater fish at IISD-Experimental Lakes Area, northwestern Ontario (PO)

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The Boreal lake Oil Release Experiment by Additions to Limnocorrals (BOREAL) project began in 2018 at the IISD-Experimental Lakes Area (Ontario, Canada) to study the fate, behaviour, and potential toxicological impacts of diluted bitumen (dilbit) in fresh water. Model spills were contained within seven 10 m-diameter littoral limnocorrals (~2 m deep), and dilutions ranged from 1:100,000 to 1:1,000 dilbit:water. These values were chosen to represent a regression of real-world spills equivalent to the 50th to 99th centiles in North America over the last two decades. Two additional limnocorrals untreated with dilbit and fish caught from the open lake serve as references. Adult male and female finescale dace (*Phoxinus neogaeus*) were released in the limnocorrals 21 days after oil addition, while incidental juvenile fathead minnows (*Pimephales promelas*) were present in the enclosure from oil addition onwards. Here we report the effects of chronic exposure (>21 days) on reproductive health of adult fish, development of juveniles, and physiological and molecular responses in both stages. Assessed metrics of reproductive health and metabolism include calculation of male and female gonadal somatic indices,

egg diameter, histological development of gonads in both sexes, and condition factor. Gills were scored for deformities, and hepatocyte volume indexes were examined as well as mRNA upregulation of Cyp1a. Results indicate that in the lower exposures (<1:10 000) there are no significant effects on the health of adult or juvenile small-bodied fish, but in the higher exposures (>3:10 000) there is a significant increase in mortality.

Polycyclic aromatic hydrocarbons in Canada's Athabasca Oil Sands Riverine Region as measured using passive samplers (PO)

Lucie Levesque[1], Julie Roy[1], Nancy Glozier[1], Kerry Pippy [1], Leah Dirk[1], Cari-Lyn Epp[1]

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Investigations into the use of passive water quality (WQ) monitoring approaches were initiated in the oil sands region of Alberta, Canada in 2012 as a part of the Joint Oil Sands Monitoring (JOSM) Program, a collaboration between Alberta Environment and Parks (AEP) and Environment and Climate Change Canada (ECCC). Semi-permeable membrane devices (SPMDs) were deployed in the Athabasca, Slave and Peace rivers upstream, within, and downstream of the oil sands minable area (OSMA) to determine their effectiveness in long-term monitoring of dissolved concentrations of polycyclic aromatic compounds (PACs) within the region. Seventy-five potentially toxic, bioavailable compounds (PACs) were measured in both SPMD samples and conventional water quality (WQ) grab samples. On average, 75% of the analytes from WQ grab samples were below analytical limits of detection, limiting the information available to identify changes in concentrations between sampling locations and over time. SPMDs analysed for the same PACs from the same sites had significantly higher proportions of detectable analytes, illustrating the occurrence of strong spatial and temporal trends in PAC concentrations that were not evident from grab samples. These findings contributed to the development and release of several standard operating procedures and the incorporation of SPMD sampling into the long-term operational water quality monitoring program of the AEP-ECCC OSM Program in 2017. This work illustrates that routine use of SPMDs in long-term water quality monitoring programs reinforces the ability of water managers to make informed decisions that may not be possible with water quality grab samples alone.

Embryotoxicity of fathead minnow exposed to Cold Lake Blend diluted bitumen spilled at the IISD-Experimental Lakes Area (PO)

Linda Lara-Jacobo[1], Lauren Timlick[2], Lucie Baillon[1], Jose Luis Rodriguez-Gil[3], Jules Blais[3], Mark Hanson[2], Bruce Hollebone[4], Diane Orihel[5], Vince Palace[2,6], Valérie Langlois[1]

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The biological effects of oil in marine environments have been well studied, but there is a critical lack of information regarding the impacts of oil spills undergoing natural weathering in freshwater lakes. The specific aim of this project is to study the potential effects in wild living fish embryos of Cold

Lake Blend diluted bitumen (CLB dilbit) in a contained model spill at a Northwestern Ontario boreal shield lake. For this, three subsequent experiments were conducted in one of the IISD-ELA lakes (Lake 260) in July 2018. Fertilized embryos of wild fathead minnow (FHM; *Pimephales promelas*) were exposed to water obtained from nine large limnocorrals (10m diameter) in which oil was naturally weathered. The treatments included seven CLB concentrations of dilbit in a gradient design and two untreated reference enclosures. Each FHM exposure lasted 7 days. Mortality rate, malformation occurrence, and/or hatching time were recorded after 7, 14 and 21 days. Preliminary data suggest that at the tested concentrations the spilled dilbit does not alter fish embryo survivorship but is likely inducing malformations. Future work includes 1) a recovery exposure to be performed in summer 2019 to assess fish embryonic recovery, and 2) a throughput transcriptional investigation of all time-points, including targeted real-time RT-PCR analysis of a series of genes related to xenobiotic detoxification and oxidative stress (e.g., *cyp1a* and *gst*). This research is part of a large research effort, the results of which will help in understanding the direct and indirect repercussions of a dilbit spill in freshwater ecosystems.

Simulating diluted bitumen spills: Environmental weathering and submergence in model freshwater systems (PO)

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Over the past few years, a number of high-level governmental scientific reports highlighted the numerous knowledge gaps that currently exist in relation to the weathering, fate, behaviour, and environmental effects of a potential spill of diluted bitumen, (dilbit) into aquatic systems. These reports emphasized the need for field research to address these gaps, especially in freshwater systems. The BOREAL project (Boreal lake Oil Release Experiment by Additions to Limnocorrals) was established to address these research needs with a large-scale field simulation of a dilbit spill in a boreal freshwater system. Here we report results related to the environmental weathering and submergence of dilbit from a pilot study, as well a large in-lake study conducted at the IISD-Experimental Lakes Area. Following dilbit application to water, rapid decreases of volatile hydrocarbons in the dilbit slick were observed within the first 24 hours of the experiment, and were attributed mainly to evaporation. These changes in chemical composition of residual dilbit coincided with increasing density, viscosity, and water uptake. Relatively rapid increases in hydrocarbon concentrations were detected in the water column following the spill, mainly alkylated polycyclic aromatic compounds, which constitute a large proportion of the aromatic fraction of dilbit. Our study provides new insights into the environmental fate, behaviour, and risks of dilbit in a freshwater environment. In particular, we are the first to demonstrate the propensity for dilbit to sink under ambient environmental conditions in fresh waters typical of many boreal lakes across the Canadian Shield.

Multi-generational Ecotoxicology and Epigenetics

The antidepressant fluoxetine is a transgenerational neuroendocrine disruptor of stress and behaviour (PL)

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Antidepressants such as fluoxetine (FLX), the active chemical in Prozac, are widely prescribed to treat affective disorders, especially in pregnant and breast-feeding mothers, a particularly vulnerable population. FLX crosses the placenta and it is also excreted in breast milk, so the developing baby may be exposed in utero and/or as a child. Although risks to babies and potentially to their descendants are beginning to be assessed in humans, such transgenerational studies will require many decades of analysis. Additionally, numerous antidepressants are widely detected in aquatic ecosystems, and environmental levels have been found to affect the behavior, food intake and reproduction of some fish species. Small bodied, with a short generation time, the zebrafish *(Danio rerio)* is an excellent model system for transgenerational toxicology. We have found that a short (6 day), early-life exposure to FLX disrupts the stress axis by reducing cortisol synthesis and associated transcriptional signatures in the kidney-adrenal complex across multiple generations in the adult male zebrafish. Current data implicate an epigenetically inherited hypocortisol phenotype linked to significantly reduced exploratory behaviour (novel tank test). Time- and sex-dependent effects are also evident. Thus far, we have established that maternal transcripts involved in endocrine stress axis development and regulation, epigenetic (*de novo* DNA methyltransferases) and post-transcriptional (miRNA pathway components and specific miRNAs) regulation of gene expression are differentially deposited in unfertilized eggs of control versus FLX-exposed females. These results have both human and ecosystem-related health implications.

Do epigenetic marks contribute to sensitivity to polycyclic aromatic hydrocarbons (PAHs) in birds? (PL)

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Lipophilic environmental contaminants such as dioxin-like compounds (DLCs) and polycyclic aromatic hydrocarbons (PAHs) can be found in high concentrations in the eggs of wild birds. We are interested in how this early-life exposure to contaminants relates to individual differences in sensitivity to re-exposure later in life, and whether epigenetic mechanisms are involved. In the current study, the DLC tetrachlorodibenzo-*p*-dioxin (TCDD) or the PAH benzo[*k*]fluoranthene (BkF) was injected into fertilized chicken eggs prior to incubation. At embryonic day 19, livers were harvested and slices of the tissue were grown in culture. These liver slices were then re-exposed to graded concentrations of each of the test chemicals. Both BkF and TCDD were associated with dose-dependent induction of several

genes associated with the aryl hydrocarbon receptor (AHR) response pathway including *cyp1a4, cyp1a5,* and *ahrr* (but not *ahr* or *arnt*). A large degree of variability was observed in the responsiveness of liver slices cultured from different individuals, with a larger proportion of highly responsive individuals present in the BkF pre-treated group. This sensitivity to induction was associated with small but significant increases in methylation of the *cyp1a4/5* shared promoter. Ongoing work is examining the role of histone acetylation in the response to re-exposure to AHR ligands at this locus. We are also investigating whether embryo mortality associated with a low degree of *cyp1a* inducibility is responsible for the effects we have observed. Understanding the molecular basis for individual variability in sensitivity to DLCs is important for improving risk assessment for these ubiquitous environmental chemicals.

The fungicide tebuconazole causes persistent changes to the DNA methylome of zebrafish (*Danio rerio*) (PL)

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Exposure to some chemical stressors during early stages of development can impair physiology of adults via induction of mitotically stable alterations to the DNA methylome. In the current study, zebrafish (*Danio rerio*) embryos were exposed from 1-hour post-fertilization until 24-hours post-hatch to 0, 10, or 1000 $\mu g \cdot L^{-1}$ of tebuconazole, a fungicide used globally to control pathogenic fungi found on a variety of crops. After termination of the exposure, embryos were transferred into clean water and reared until sexual maturity. Exposure of embryos to 1000 $\mu g \cdot L^{-1}$ of tebuconazole caused significant changes in expression of the brain isoform of aromatase (*cyp19b*) in male and female fish and decreased concentrations of estradiol (E2) in female fish, relative to controls. However, there were no significant effects of the early life stage exposure on reproductive performance. Exposure to tebuconazole also resulted in significant alterations to the DNA methylome and expression of enzymes that regulate the methylome. Specifically, mRNA abundances of ten-eleven-translocases (*tet*) and DNA methyltransferases (*dnmt*) were significantly different from controls in gonads and brain from male and female fish exposed as embryos to tebuconazole. Using reduced representation bisulfite sequencing (RRBS), 16 and 227 differentially methylated regions were identified in gonads from females exposed as embryos to 10 and 1000 $\mu g \cdot L^{-1}$ of tebuconazole, respectively. Implications of effects on DNA methylation are being explored.

Micro and Nanoplastics in the Environment

Effect of polystyrene micro- and nanoplastics on *Drosophila melanogaster* development, motility and reproduction (PL)

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The degradation of plastic waste into microplastics (MPs; 100nm <size <5 mm) and nanoplastics (NPs; <100nm) is a growing environmental concern. Despite being the largest sink for plastic pollution, research on the interactions of MPs and NPs in terrestrial models is scarce. Here, we use the fruit fly (*Drosophila melanogaster)* to study the toxicity of MPs and NPs. Commercial 1 µm red fluorescent polystyrene (PS) and 20 nm green-yellow PS spheres were dialyzed in 2L de-ionized water for seven days with frequent water changes to remove potentially toxic preservatives and surfactants. They were then mixed into food at concentrations of 0, 0.01, 1, 10, 50, and 100 ppm. First instar larvae were exposed for 13 days with endpoints such as development and mortality being recorded daily, and locomotion being tested during the 3rd instar larvae stage (day 3) and the adult stage (day 13). Three mating pairs of emerged adults from each treatment were then switched into vials with clean food, and the number of eggs produced was counted. There was no significant difference in mortality, rate of development, or fertility at various concentrations of either size of PS. Significant differences in locomotion were observed between controls and exposed groups of adults, with treatment groups demonstrating lower motility. These measurements will contribute to determining the mechanism of toxicity of MPs and NPs in the model organism, *Drosophila*, and provide insight into their potential impact on human health.

Nanoplastics toxicity of mangroves vs Canadian oysters (PL)

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Plastics accumulation in aquatic ecosystems observed in recent decades has led the scientific community to study the ubiquitous contamination of microplastics (MPs) in the oceans. More recently, the existence of nanoplastics (NPs) which result from industrial synthesis residues or (mostly) from environmental degradation of MPs has triggered questions concerning the toxicity of these small-sized molecules. As NPs less than 100 nm can cross biological barriers and accumulate in cells and tissues, they represent an ecotoxicological risk to aquatic organisms. In addition, NPs are known to adsorb contaminants, and in particular, metals. Therefore, NPs can participate in increasing the bioavailability of metallic contaminants to aquatic fauna, especially for bivalves. The high filtration rate of bivalves for respiratory and nutritional purposes have made them tolerant to high concentrations of metals, rending them good bioindicators of pollution. In the light of this ecotoxicological risk, the study of NPs by trophic transfer to bivalves, an under-documented entry route for the bioavailability of NPs and metals,

should be investigated. This project focuses on the impacts of several NPs on the growth of phytoplankton species. NP-contaminated phytoplankton was used to feed Guadeloupean mangrove oysters (*Isognomon alatus*), which were contaminated with or without arsenic. Similar experimental design will be performed on Canadian oysters native to Eastern Canada (*Crassostrea virginica*) and species responses will be compared. This project will shed light on the effects of NPs in oysters and will provide data on the differential responses between sub-tropical versus sub-Arctic oysters species.

Effects of nanoplastics on zebrafish embryo at the early developmental stage (PO)

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The quantity of plastics released into the environment has been rising rapidly since the mass production of plastic in the early 1940s. The annual global production of plastics was estimated to be over 340 million metric tons in 2017. Approximately 10% of global annual plastic waste ended up in the ocean, making plastic one of the most significant marine pollutants for aquatic organisms. In the past decade, most research has focused on the effects of microplastics, while the impact of nanoplastics on aquatic organisms has only recently attracted attention. Micro-sized plastics can be broken down to nano-sized plastics which may have different effects on aquatic systems due to their smaller size and higher specific surface area. This study investigated the hatching success of zebrafish embryo in terms of malformation (including pericardial edema, yolk sac edema and spinal curvature), oxidative stress, and locomotor activity of hatched larvae after exposed to nanoplastics for 96 hours. The results will improve understanding of the environmental impact of nanoplastics on aquatic species.

A fluorescence assay for the detection of polystyrene nanoplastics in aquatic organisms (PO)

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A simple fluorescence-based methodology for the detection of polystyrene nanoplastics (NP) in biological tissues is proposed, using hydrophobic-sensitive fluorescent probe Nile Red. NPs were found to display autofluorescence at 430 and 650 nm during excitation at 400 nm and emission at 650 nm. Although NPs alone did not influence Nile red fluorescence, a characteristic hypsochromic shift in the emission spectra was found when the dye and NP were incubated with subcellular tissue fraction. To explain this, the probe and NPs (50 and 100 nm) were prepared in the presence of increasing concentrations of two detergents (Tween®20, Triton™ X100). The data revealed that both NP sizes readily increased fluorescence values when the detergents were added, and Tween®20 blue shifted the emission spectra from 648 to 627 and 633 nm for 50 and 100 nm NPs respectively. The addition of NPs in tissue extracts blue shifted further the emission spectra to 623 nm from the normal Nile Red-"lipid droplet" peak at 660 nm. The fluorescence intensity was proportional to the NP concentration. A methodology is thus proposed for the detection of NPs in laboratory-exposed organisms based on the

solvatochromic properties of Nile Red. The methodology was used to detect the presence of NP and changes in lipid contents in *Hydra attenuata* and revealed that NPs were detected and increased lipid droplets in Hydra.

Microplastics in the diet of nestling double-crested cormorants (*Phalacrocorax auritus*), an obligate piscivore in a freshwater ecosystem (PO)

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Anthropogenic debris, namely plastic, is a concern across aquatic ecosystems worldwide, with freshwater systems being understudied relative to marine systems. In this study, we quantified and characterized debris in the diet of double-crested cormorant chicks (*Phalacrocorax auritus*) from three sites in two of the Laurentian Great Lakes to (1) determine whether or not the diet of double-crested cormorants in the Laurentian Great Lakes includes anthropogenic debris, (2) characterize the size, shape, and type of debris incorporated, and (3) examine relationships between the amount of debris ingested and their proximity to industrial–urban centres. Overall, >86% of cormorants in our study had anthropogenic debris (mostly fibers) in their digestive tracts with no correlation between site and the amount of debris ingested. The ingested debris includes microplastics, natural fibres from textiles, and other anthropogenic materials (e.g., glass). To the best of our knowledge, this is one of the first studies to examine anthropogenic debris in a diving bird in the Laurentian Great Lakes and one of few studies investigating this in freshwater birds.

Toxic Effects in Aquatic Organisms: Integrating Biochemical, Physiological, and Ecological Responses

Energy modulation of P-glycoprotein neuroprotection: A behavioural assessment (PL)

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The presence and role of P-glycoprotein (P-gp) in the blood brain barrier (BBB) of fish is relatively unknown. Normal fish behaviour is related to central nervous system (CNS) health, which may rely on the BBB for protection against endogenous and exogenous neurotoxic compounds. This study assessed the neuroprotective role of P-gp through behavioural endpoints. Zebrafish were injected intraperitoneally with a CNS neurotoxicant ivermectin (IVM) and/or the known P-gp inhibitor Cyclosporin A (CysA) under two different diet conditions. Fish were regularly fed (FED) or fasted for seven days (FAST). Treatment groups were: saline, DMSO (0.01%), IVM (2.24 μmoles·kg^{-1}), CysA (5 μmoles·kg^{-1}), IVM (2.24 μmoles·kg^{-1}) + CysA (1 μmoles·kg^{-1}), IVM (2.24 μmoles·kg^{-1}) + CysA (3 μmoles ·kg^{-1}) and IVM (2.24 μmoles·kg^{-1}) + CysA (5 μmoles·kg^{-1}). Following injections, fish were placed into a two chambered trough that allows free movement. In a food attraction test, mean speed, maximum swimming speed, number of passages, circling swimming, 90° turns, survivorship, position in the water column, posture and fish activity were measured. IVM affected most of these endpoints, which was intensified in the presence of CysA. No difference were observed between fed and fast fishes. These results suggest that P-gp plays an role protecting teleost brain and that this protection is prioritized even during periods of fasting.

The effects of 17α-ethinylestradiol (EE2) and hydroxypropyl-β-cyclodextrin (HPβCD) on the heart rate of embryonic Japanese medaka (*Oryzias latipes*) (PL)

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Estrogen toxicity has been an area of priority in aquatic toxicology over the last 20 years. Recent evidence has indicated that a rapid, non-genomic, non-classical estrogen signaling pathway exists via the G protein coupled estrogen receptor (GPER). GPER is expressed in many biological systems, with roles in the cardiovascular system and metabolic pathways. The primary objective of this research was to investigate the effect of 17α-ethinylestradiol (EE2) on the heart rate of embryonic Japanese medaka (*Oryzias latipes*). A 5–20 % decrease in embryonic heart rate was observed in 120 and 144 hour post fertilization medaka embryos exposed to 0.1 ng·L^{-1}, 1 ng·L^{-1}, 10 ng·L^{-1}, 100 ng·L^{-1}, and 1000 ng·L^{-1} EE2 ($P \leq 0.05$). This effect was attributed to the activation of GPER through the use of GPER and ERα and ERβ agonists and antagonist, highlighting a novel mode of action for EE2 toxicity. The secondary objective was to determine if the presence of the odour suppressant, hydroxypropyl-β-cyclodextrin (HPβCD), could alter the toxicity of EE2. HPβCD is an amphiphilic toroidal compound that can bind non-polar

compounds such as fragrances and pharmaceuticals within a central cavity. The combination of EE2 and HPβCD in a 1:4 molar ratio (EE2:HPβCD) resulted in an embryonic heart rate that was significantly greater than EE2 alone at 120 and 144 hours post fertilization ($P \leq 0.05$). EE2:HPβCD was also not significantly different from the control at 144 hours post fertilization, indicating that HPβCD reduced the effect of EE2 on embryonic heart rate ($P > 0.05$). This research suggests that EE2 can cause a decrease in the heart rate of embryonic Japanese medaka through GPER activation, non-classical estrogen signaling, and this effect can be reduced by the presence of HPβCD.

Assessing the effects of environmentally-relevant concentrations of metformin to fathead minnows exposed over a full life cycle (PL)

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Metformin is a commonly used glucose-lowering drug that has been detected in municipal wastewater effluents at $\mu g \cdot L^{-1}$ concentrations. We exposed fathead minnow (*Pimephales promelas*) over a full lifecycle to three concentrations of metformin: 3, 30, and 300 $\mu g \cdot L^{-1}$. The low concentration is similar to metformin concentrations in rivers, and the mid concentration similar to metformin concentrations in municipal wastewater effluents (MWWEs). Mean measured concentrations of metformin were 3.0, 31, and 322 $\mu g \cdot L^{-1}$. During the life-cycle exposure, no significant changes were observed in survival of fathead minnows. Growth and maturation of the fathead minnows was unaffected by metformin exposure. There were no significant differences in condition factor, gonadosomatic index, or liver-somatic index in the metformin-exposed fish. Mean time to first breeding was significantly delayed by 10 days in 31 $\mu g \cdot L^{-1}$ metformin treatment (and was delayed, but not significantly, in the 322 $\mu g \cdot L^{-1}$ treatment). Overall, the metformin-exposed fathead minnows produced similar numbers of eggs as control fish. Egg quality was very good and was unaffected by metformin, with % fertilization 92-96 %, and 70-77 % hatching success in F1 fry. Eggs hatched in five days, severe deformities in fry were low (2-4 %), and there were no effects on survival or growth of F1 larvae at nine and 16 days post-hatch from any metformin treatment compared to controls. Exposure to the metformin at environmentally-relevant concentrations (i.e. 3 and 31 $\mu g \cdot L^{-1}$ metformin) over a full lifecycle caused no adverse effects in fathead minnows.

Characterizing fluoxetine-induced molecular toxicity pathways in embryonic and juvenile white sturgeons (*Acipenser transmontanus*) (PL)

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Fluoxetine (FLX), an emerging contaminant of the pharmaceutical group “selective serotonin reuptake inhibitors” (SSRIs), is commonly found at sub-therapeutic levels in surface waters. FLX is of significant concern in the aquatic environment due to its ability to induce inadvertent sub-lethal effects

in aquatic organisms exposed at low concentrations. However, the molecular mechanisms of FLX toxicity in aquatic organisms are not well understood, particularly in fish species native to North America. The objective of this study was to characterize molecular pathways associated with FLX toxicity in different life stages of white sturgeon (WS) using RNA-Seq technology. Freshly hatched embryos and 1.5 year-old juvenile WS were exposed for four days to FLX and transcriptomic analysis was performed in whole body embryos and excised livers from juveniles. Raw reads were analyzed following the quasi-mapping approach and the significance of differentially expressed transcripts was assessed using the quasi-likelihood pipeline with an FDR (false discovery rate) <0.05 cut-off. Although results showed differences in specific transcript-level responses between embryonic and juvenile stages, molecular pathways in both life stages were predominantly associated with disruptions related to brain-function plasticity. Additionally, sterol biosynthesis and catabolism pathways were affected in juvenile WS, whereas embryonic responses included pathways associated with development, such as collagen formation and phenotypic impairment. This study showed that major toxicologically relevant molecular pathways were conserved across life stages which are in alignment with the read-across hypothesis; however, there were distinct life stage-specific responses of WS to FLX exposure.

Differential selenium uptake by periphyton in boreal lake ecosystems (PL)

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Selenium (Se), an essential trace element with a narrow margin between essentiality and toxicity, is released into aquatic environments by certain industrial practices. Excess Se is rapidly and efficiently assimilated into food webs in a site-specific manner by primary producers and transferred to higher trophic levels through dietary pathways. More research is needed regarding the effects of increased Se loading in cold freshwater systems and how certain site-specific factors influence the incorporation of Se into food webs by periphyton. The objective of this study was to quantify Se uptake at the base of food webs in representative boreal lake ecosystems by investigating if concentration-dependent differences exist in periphyton enrichment functions (EFs) of Se, and if water chemistry variables play a role in Se uptake. Periphyton was naturally grown in five lakes with variable water chemistry and exposed to environmentally-relevant concentrations of Se in a static renewal system for eight days. Aqueous and periphyton total Se concentrations, and periphyton community composition were quantified. Significant concentration-dependent differences in EFs were found among the five study lakes, with the highest EFs in four of the five lakes occurring in the lowest Se treatment. EFs generally decreased with increasing Se concentration, and significant differences in EFs were also found among lakes at the two highest Se treatments. The results of this research provide insight into the biodynamics of Se assimilation at the base of boreal lake food webs, which can potentially inform ecological risk assessments in cold freshwater ecosystems in North America.

Development of a standardized toxicity test method using a native amphibian (PO)

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Although there is growing evidence of the sensitivity of amphibians to contaminants and thus a growing demand for their use in regulatory frameworks, amphibian toxicity data are currently under-represented in risk assessments. Few standardized methods are available, which in part contributes to this under-representation. In addition, none of the available aquatic methods pair whole-organism chronic endpoints with species that are relevant to Canadian environments. To address this gap, Environment and Climate Change Canada has invested in developing a standardized test method for assessing contaminants with a native amphibian species (*Lithobates pipiens;* Northern leopard frog). We will discuss some lessons learned from laboratory research and data from recent inter-laboratory testing rounds to validate the new test method. For the inter-lab project we used sodium chloride thyroxine and perchlorate as model compounds in this multi-laboratory experiment. Environment and Climate Change Canada's amphibian test method will continue along the usual path for standardization of a toxicity test method, including development of quality control criteria, improvement of methodology, text and peer review. The result will be the first Canadian standardized toxicity test method using a native amphibian species.

Proposed experimentation to characterize the toxicity to fathead minnows of groundwater from a legacy contaminated industrial site (PO)

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Legacy contaminated industrial sites represent a specific scenario of contamination where persistent parent molecules, metabolites, and by-products form complex mixtures that may affect humans and ecosystems. Consequently, it is essential to define the principal mode of toxic action of these mixtures and to identify what the key active elements leading to toxicity are. In this project, we propose to address this issue by applying a toxicity pathway framework using an *in vivo* fish model, fathead minnow (*Pimephales promelas*), in combination with targeted *in vitro* assays to characterize the potential hazards posed by groundwater from a legacy contaminated pesticide manufacturing site. In order to establish a relationship between the exposure to the complex mixture and potential hazards to aquatic organisms, this study will link molecular toxicity patterns with physiological and behavioral changes and associated adverse outcomes. After acute and sub-chronic exposure of fathead minnows to incremental dilutions of groundwater samples from the legacy contaminated site, test organisms will be subjected to an integrated biomarkers analysis to (1) observe effects on survival, reproduction and development, (2) assess impact on different organs using histopathology, (3) evaluate cytotoxic and genotoxic effects in blood, and (4) characterize specific mechanisms and molecular pathways of toxicity. Finally, we will also conduct mesocosm experimentation to (5) validate toxicological responses under environmentally realistic conditions. The results obtained from the aforementioned experimentation

will help to link the biological adverse outcomes to the exposure with contaminants of concern detected in the mixture in a parallel study. Preliminary results will be presented.

Toxicological properties of municipal effluent and rainfall overflow discharge sites in caged freshwater mussels (*Elliptio complanata*) (PO)

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In the context of climate change, increased frequency and intensity of rainfall events will contribute to discharges of wastewaters/sewage overflows where the toxic impacts to resident fauna are not well understood. The purpose of this study was to compare the toxicity of two sewage overflow sites with a primary-treated wastewater dispersion plume and an upstream site in the St. Lawrence River using caged mussels for three months. At the end of the exposure period, mussels were collected and analyzed for tissue-contaminants burden, metallothioneins (MT), glutathione S-transferase (GST), lipid peroxidation (LPO), inflammation (arachidonate cyclooxygenase aCOX), and DNA damage. The data revealed that mussels collected downstream of the treated wastewater plume had increased fecal coliform loadings, GST, gonad DNA strand breaks and MT with lower digestive gland DNA strand breaks and aCOX. With respect to mussels exposed to rainfall/sewage overflows, increased levels of digestive gland MT and GST were observed. Mussels readily accumulated venlafaxine and acebutolol at the wastewater site, while mussels collected at the overflow sites contained more atrazine and its metabolite. The mussels placed at the sewer overflow sites were more closely related to the upstream site than the wastewater site but showed some signs of stress, based on MT levels and GST activity, which suggests the input of heavy metals and organic contamination. In conclusion, the data suggest that mussels near two major sewage overflows are less impacted than those exposed to treated municipal effluents at some 8 km downstream of the dispersion plume in the St. Lawrence River. Further research is underway to examine more closely the influence of rainfalls over the years.

Effects of Nanomaterials in the Environment

Characterizing the effects of engineered nanoparticles released from painted surfaces due to weathering, in zebrafish (*Danio rerio*) (PL)

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Titanium dioxide ($nTiO_2$) and silver (nAg) are among the most common engineered nanoparticles (ENPs) used in paint for their whitening and anti-microbial properties. Weathering processes such as rainfall and exposure to sunlight can cause the release of such ENPs from outdoor painted surfaces into aquatic systems, though little is known about the toxicity of the released particles. The objective of this study is to use two aquatic models, a zebrafish (ZF) embryotoxicity test (OECD TG-236) and a ZF liver cell line (ATCC CRL-2643), to characterize the effects of $nTiO_2$ and nAg released from painted panels subjected to simulated weathering treatments: sonication and sunlight exposure. Zebrafish liver cells were exposed to 10 concentrations of sonicated pristine $nTiO_2$ or nAg (0–200 $\mu g \cdot ml^{-1}$), base paint (without ENPs), unpainted panel, or painted panels containing $nTiO_2$ (0–10 $\mu g \cdot ml^{-1}$) or nAg (0–2 $\mu g \cdot L^{-1}$). Pristine nAg decreased cell viability to 87±3.8% and 70±3.5% at 100 and 200 $\mu g \cdot ml^{-1}$, respectively. Pristine $nTiO_2$, unpainted panel, base paint, and $nTiO_2$ and nAg released from panels failed to decrease viability at any concentration. While no cytotoxic effect of paint-ENPs was observed, analyses to examine effects related to oxidative stress (gene expression, enzyme activity), and ZF embryo experiments are ongoing. This work will help us understand the potential risks to aquatic organisms of ENPs released from outdoor painted surfaces. Additionally, in line with growing interest in alternative testing strategies, the use of cells and embryos can contribute information to deepen our understanding of these methods in ecotoxicology.

Molecular mechanism of cadmium – titanium dioxide nanoparticle mixtures when co-exposed to the nematode *Caenorhabditis elegans* (PL)

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Nanoscale titanium dioxide (nTiO2) is probably among the most relevant engineered nanomaterials with a projected accumulation rate in European river sediments of 2 $mg \cdot kg^{-1} \cdot yr^{-1}$. To evaluate the environmental risk of nTiO2, the impact of co-exposure with other aquatic contaminants like cadmium (Cd) should be considered. While Angelstorf (2014) had shown that the toxicity of nTiO2 (P25) to the nematode *Caenorhabditis elegans* increases under simulated solar radiation (SSR), Samet (2017) found synergistic inhibitory effects by 80 % on the nematode when co-exposed to nTiO2 and Cd. The effect again was only observed when worms were irradiated during exposure. We present here experimental data which may explain this synergistic effect: Cd induces intracellular Ca-signalling as part of protective cell processes. Thus, the effect of Cd/ nTiO2-mixtures on calcium (Ca) ion channels in the

intestinal membrane of *C. elegans* was investigated with three different calcium ion channel blockers (NS8593 for TRPM7-, Lanthanum for TRPM3- and Heparin for IP3-channels). In order to understand whether SSR has any impacts on the chemical structure of nTiO2-Cd-agglomerates, not-irradiated and irradiated samples were characterized by different analytical methods at pH 4 and 7 to test the influence of pH changes during gut passage of agglomerates. Further experiments are currently being undertaken to look into the impact of nTiO2-Cd-agglomerates on membrane integrity under SSR by applying propidium iodide and hexokinase to check for non-specific effects of the Cd/ nTiO2-mixture on the cells under SSR.

Fate of nanoparticles released from Canadian municipal wastewaters (PL)

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[1]Environment and Climate Change Canada

Natural transformation processes such as aggregation or degradation control the environmental fate and behavior of nanoparticles (NPs) released into the environment. Water chemical properties such as organic carbon have significant impacts on the fate and changes in forms. This study investigated the fate of silver (Ag) and cerium oxide (CeO_2) nanoparticles in municipal wastewaters and NP transformation products in different types of natural water. Wastewater samples were analyzed by the technique of single particle/ICP-MS (SP-ICP-MS) to identify and characterize their content in nanoparticles. Nano-sized Ag would account for less than 5% of the total Ag released from municipal effluents. AgNPs formed aggregates in water with relatively high values of organic carbon concentrations, while degraded forms were also observed. In contrast, no Ce was found in the truly dissolved fraction, indicating no evident NP degradation for CeO_2. More than 90% of NPs' CeO_2 was found as large colloids. High levels of natural organic matter in water can reduce the global toxicity of the NPs Ag and CeO_2. Future research on nanotoxicity should consider potential transformation for an improved risk assessment of released nanoparticles.

Field exposure assessment of nanocarriers and pesticides in agricultural soils (PO)

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Over the past few years, the use of nanoparticles for agricultural purposes has been increasing, along with concern about their ecological risks. In order to assess the effects of polymeric nanocarriers and nanopesticides on agricultural soils, strawberries were cultivated under field conditions (n= 5) and exposed to several treatments (no treatment; nanocarrier; Bifenthrin; Azoxystrobin; nanocarrier containing Bifenthrin; nanocarrier containing Azoxystrobin). After 3 months of cultivation, soil enzyme activities were measured after extraction of 0.5 g of dried and sieved (2 mm) soil using 25 mL of buffer (pH= 6.9) during 30 minutes under constant stirring, followed by centrifugation (5 minutes at 1882 x g) and incubation in multi-well plates (24 hours, 30°C). Fluorescence intensities of 4-MUB-β-D-glucopyranoside, 4-MUB-sulfate, 4-MUB-phosphate and L-leucine-AMC were measured using excitation

wavelengths of 330 nm (MUB) and 360 nm (AMC), with fluorescent emission measurements at 460 nm (Infinite M200, Tecan). The results showed no differences between the soil enzyme activities of the different treatments when compared to the controls after the 3-month exposure. The pesticide content in the soil was also evaluated in the middle and at the end of the experiment by extracting 2 g of dried and sieved soil using 4 mL of acetonitrile, followed by liquid chromatography quadrupole time-of-flight mass spectrometry (recoveries of 95%- 104%). In general, the pesticide concentrations (nano and non-nano) decreased over time. Further experiments, currently underway, are measuring the release rate of the pesticides from the nanocarriers and the bioavailability of the nanopesticides to the strawberries.

Emerging Organics

Pharmaceuticals and estrogenic compounds in Manitoba rivers and wastewater treatment plant influent/effluent (PL)

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Pharmaceuticals and estrogenic compounds (estrogens) in aquatic environments are contaminants of emerging concern, and their potential to cause adverse effects to aquatic life, wildlife and human health has received international attention. The primary source of pharmaceuticals and estrogens in surface waters is industrial and municipal wastewater treatment facilities, which discharge effluent directly to receiving waters. In Canada, the majority of wastewater treatment facilities cannot fully eliminate pharmaceuticals and estrogens during the treatment process. The current state of knowledge on pharmaceuticals and estrogens suggests that a variety of factors may govern their concentration, bioaccumulative nature, toxicity, and environmental fate, such as the physical and chemical properties of the compounds and characteristics of the receiving water body. In recognition of the potential widespread contamination of pharmaceuticals and estrogens in surface waters, governments have established water quality monitoring programs to assist in the development of management actions to minimize environmental impacts. In 2012, Manitoba Sustainable Development initiated a water quality monitoring program to determine background concentrations and to understand the spatial and temporal variability of pharmaceuticals (n=40) and estrogens (n=19) in the Red, Assiniboine and Winnipeg rivers. In addition, influent (raw untreated) and effluent (treated) samples were analyzed for pharmaceuticals and estrogens to investigate upstream versus downstream effects of large wastewater treatment facilities on river concentrations. The results of the study are presented.

Loadings of active pharmaceutical ingredients from manufacturing facilities in Ontario (PL)

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Recent evidence has revealed that cities with pharmaceutical manufacturers have elevated concentrations of active pharmaceutical ingredients (APIs) in their receiving water bodies. The purpose of this study was to gather information on direct sewer discharges of APIs during their manufacturing and processing from five pharmaceutical manufacturing facilities in Ontario, Canada. Drug classes and maximum reported concentrations ($ng \cdot L^{-1}$) for which APIs were directly discharged included: antidepressants (paroxetine - 3380 and sertraline - 5100); mood stabilizer (carbamazepine - 575,000); antibiotics (penicillin - 14,300); analgesics (acetaminophen- 461,000; codeine - 49,200; ibuprofen - 344,000; naproxen - 253,000 and oxycodone 21,000); cardiovascular drugs (atorvastatin - 893 and metoprolol - 7,333,600) and drugs used for blood pressure control (amlodipine - 22,900; diltiazem - 1,160,000; furosemide - 1,200,000 and verapamil - 7340). Based on flow and water usage data from the individual facilities, the maximum concentrations for acetaminophen, ibuprofen, carbamazepine,

diltiazem and metoprolol correlate to approximately 200, 220, 390, 420 and 14,200 g, respectively, of lost product being directly discharged to the sewers daily during active manufacturing. This survey demonstrates that direct point source discharges from pharmaceutical manufacturers represent a key source of pharmaceutical pollution to receiving sewersheds. Onsite recovery of product or treatment at pharmaceutical manufacturing or processing facilities in order to reduce the sewage loadings to receiving treatment plants, product loss, and potential environmental loadings is strongly recommended.

Trophodynamics of substituted diphenylamine antioxidants and benzotriazole UV stabilizers in aquatic food webs from Hamilton Harbour and Lake Joseph (PL)

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Substituted diphenylamine antioxidants (SDPAs) and benzotriazole UV stabilizers (BZT-UVs) are anthropogenic additives widely used in industrial and commercial products such as plastics, rubbers, fuels, lubricants, and personal care products. They are persistent in the aquatic environment, and chronic exposure to these contaminants may lead to adverse effects in organisms. Despite an increasing number of reports on the occurrence and bioaccumulation of SDPAs and BZT-UVs, the knowledge of their trophodynamics remains extremely limited. Thus, the present study collected organisms such as plankton, insect, mussel and fish to investigate the trophodynamics of SDPAs and BZT-UVs in food webs from Hamilton Harbour (HH) and Lake Joseph (LJ), Ontario, Canada. Organisms from HH showed higher concentrations of SDPAs and BZT-UVs compared with samples from LJ. This is the first time to report the levels of SDPAs and BZT-UVs in insects. SDPAs such as 4,4'-bis(α,α-dimethylbenzyl)-diphenylamine (diAMS) and dinonyl-diphenylamine (C9C9), as well as BZT-UVs 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol (UV234) and 2-(2H-benzotriazol-2-yl)-4,6-di-tert-pentylphenol (UV328) were frequently detected in insects (e.g., dragon fly, *Anisoptera*) with concentration up to 22 $ng \cdot g^{-1}$ for UV328 (wet weight). Biodilution of SDPAs was found in both food webs. The levels of SDPAs were positively correlated with $\delta^{13}C$, indicating benthic species had higher exposure to SDPAs compared to pelagic species. In contrast, UV234 showed biomagnification in the HH food web, suggesting greater exposure risks of higher trophic level organisms to this contaminant. Such variations of trophodynamics indicate the differences in accumulation and elimination pathways of SDPAs and BZT-UVs and require further elucidation of underlying mechanisms.

PollutionTracker: Emerging contaminants in the marine environment (PL)

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PollutionTracker is the first long-term marine pollution monitoring program in Canada (pollutiontracker.org). Established in 2015, the program currently operates coast-wide in British Columbia (BC) with over 60 sampling sites established to date. Collaboration with coastal First Nations,

government agencies, port authorities, industry, and community groups enabled the completion of Phase 1 in 2017 and the implementation of Phase 2 in 2018. Mussels and nearshore subtidal sediment are being used to monitor spatial and temporal trends of both legacy and emerging contaminants of concern. Over 450 individual analytes from 14 contaminant classes are being measured using high-resolution analysis, including emerging contaminants, such as alkylphenols, perfluorinated compounds, flame retardants, and pharmaceuticals and personal care products. Phase 1 results indicate that all emerging contaminant classes investigated were detected in sediment, and all but tetrabromobisphenol A (TBBPA) were detected in mussels. Alkylphenols were the most frequently detected emerging contaminant class (73% and 100% of sediment and mussel samples analyzed, respectively), while TBBPA was the least frequently detected (3% and 0% for sediment and mussels, respectively). The highest levels were measured in Burrard Inlet and Victoria Harbour, consistent with proximity to urban areas. These results provide important baseline information for emerging contaminants of concern and inform on environmental fate and potential exposures of marine organisms. With a growing network of collaborators, *PollutionTracker* is well-positioned to enable long-term monitoring of both legacy and emerging contaminants on the BC coast, as well as expansion to Canada's other coastlines.

Assessing potential impacts of pharmaceutical and personal care products (PPCPs) in aquatic ecosystems (PO)

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Pharmaceuticals are defined as prescription or over-the-counter drugs used to prevent or treat human and animal diseases, whereas personal care products are used mainly to improve quality of daily life. Considerable quantities of PPCPs enter the freshwater ecosystems due to inefficient removal by wastewater and effluent treatments. PPCPs and their metabolites are biologically active and can impact the non-target aquatic organisms. A systematic literature review was conducted on toxicological effects of PPCPs on aquatic organisms. PPCPs which were known to cause toxicity in aquatic organisms were given priority in the literature review. Additionally, analysis of secondary data was performed for 145 PPCPs sampled from 35 aquatic sites in British Columbia, Canada. The detectable values of the compounds were compared with their lethal threshold values from the literature, to determine their level of toxicity. For those PPCPs where lethal values were not available, their toxicity was determined by examining literature about behavioural alterations they produce in aquatic organisms. Seventy-four compounds out of 145 in the secondary dataset were detected in at least one location. The detected values were well below the published lethal values. However, the systematic literature review suggests that PPCPs bioaccumulate in aquatic organisms across different trophic levels, even when present at low concentrations. This adversely effects the organisms' development, growth, and reproduction and causes toxicity in them. Priority PPCPs that cause toxicity in aquatic organisms as identified from the literature and measured at detectable levels in Canada remain a cause of ecological concern.

Automated methodological development for the analysis of personal care products and drug residues in water (PO)

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Technological advances (computer and electronic) have contributed to the development of an automated methodological system for solid phase extraction by high performance liquid chromatography coupled with tandem mass spectrometry. In addition, the lowering of instrumental detection limits has allowed very low detection limits for several families of compounds to be reached without requiring any laboratory preconcentration steps. The methodology consists in automatically evaluating the best conditions for extraction on different cartridges at different pHs for washing and rinsing for the mobile phases as well as the chromatographic column offering the best performance. The detection and quantification of compounds of interest are performed using a tandem mass spectrometer. The methodology allows evaluation of 72 combinations of extraction, elution, purification, and chromatography conditions. The results show that counter-intuitive methods produce unpredictable, unexpected, and very satisfactory results. Performing these tests manually requires several days of laboratory work, whereas this methodology evaluates and quantifies the results in less than 48 hours. The methodology was applied to the measurement of personal care products, drug residues, and antineoplastic drugs. The development of these methods allows analytical results to be obtained at detection limits similar to traditional extraction methods used in laboratories, using small volumes of aqueous samples.

Shedding light on the toxicity of benzotriazole UV stabilizers and a benzothiazole to aquatic invertebrates (PO)

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Benzotriazoles (BZTs) and benzothiazoles (BZThs) are high production volume chemicals that are environmentally persistent, have the potential to bioaccumulate, and have been detected in environmental samples; however, the available toxicity information for aquatic invertebrates is scarce. Thus, we conducted spiked-sediment exposures to assess the toxicity of three BZTs and one BZTh to *Hyalella azteca*, *Hexagenia* spp., *Tubifex tubifex*, and *Daphnia magna*. Test compounds were UV234 (phenol, 2-(2H-benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)-), UV326 (phenol, 2-(5-chloro-2H-benzotriazol-2-yl)-6-(1,1-dimethylethyl)-4-methyl-), UV329 (phenol, 2-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)-), and MBTS (2-mercaptobenzothiazole disulfide). Maximum nominal sediment concentrations tested were 100 and 1000 $mg \cdot kg^{-1}$ (dry weight) for BZTs (UV234, UV326, and UV329) and BZTh (MBTS), respectively. Tests were 21-42 days in duration, and survival, growth, and reproduction were examined. Results are based on nominal sediment concentrations, as chemical analysis is ongoing. Survival of *Hyalella*, *Hexagenia*, *Tubifex*, and *Daphnia* was not affected, nor was

growth of *Hyalella* and *Daphnia*. However, growth of *Hexagenia* was significantly reduced at 1-100 $mg \cdot kg^{-1}$ UV234 and 1000 $mg \cdot kg^{-1}$ MBTS. Cocoon production of *Tubifex* was unaffected by BZTs, as was cocoon hatch success with the exception of 1 $mg \cdot kg^{-1}$ UV326, which was significantly lower. Juvenile production of *Tubifex* was significantly reduced at 10 and 100 $mg \cdot kg^{-1}$ UV329. Cocoon production, cocoon hatch success, and juvenile production were all significantly lower at 1000 $mg \cdot kg^{-1}$ MBTS. *Daphnia* reproduction was unaffected by BZTs, but increased significantly at 1000 $mg \cdot kg^{-1}$ MBTS. The results of this study will be compared to environmental concentrations, and will support risk assessments to determine the impacts of these compounds on aquatic organisms.

General Ecotoxicology

Characterization of the "Festival of Sacrifice"-period wastewaters and its effect on the inlet polluting roads in Istanbul municipal wastewater treatment plants (PL)

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Pollution is caused during the annual Festival of Sacrifice (also called "Sacrifice Feast," *Kurban Bayrami*, or *Eid al-Adha*), a five-day public holiday period in Turkey when slaughtering of certain types of animals is carried out as an Abrahamic tradition for the purposes of thanksgiving. In order to deal with this additional potential pollution, Istanbul Water and Sewage Administration pays special attention to detect possible negative impacts on the wastewater treatment plants (WWTPs) and to minimize such pollution. For this purpose, a parallel monitoring program was carried out in Istanbul's seven municipal WWTPs, in which 24-hour composite samples were taken for five consecutive days of the festival each year and once every month over a three-year period. The physicochemical parameters and heavy metal (mercury, nickel, lead, zinc, arsenic, selenium, cadmium, copper, cobalt, and zinc) concentrations were measured. In addition, acute toxicity tests were performed with DAPHTOXKIT FTM (*Daphnia magna*) and BioTox luminometric (*Vibrio fischeri*) toxicity tests on the busiest day of the Festival. The analysis results showed that five WWTPs' influent loads and toxicity levels were lower than the annual averages, with the exception of two WWTPs which were located right next to the slaughterhouses. These same WWTPs' acute toxicity tests with *Daphnia magna* had the highest percentage mortality rate and low levels of luminescence rate for the *Vibrio fischeri*. Although the slaughtering of animals affects the toxicity levels of inlet polluting roads, it is more significant if the WWTPs are near the slaughterhouses. Levels were not evident in other WWTPs due to dilution effects in sanitary sewers and reduced industrial activity, that is due to insignificant levels of industrial discharging during the five-day holiday.

In vitro-in vivo extrapolation of uptake and biotransformation of benzo[*a*]pyrene in the fathead minnow (PL)

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An ever-increasing number of chemicals are used by society and eventually released into the environment. Current regulations to assess the toxicological risks associated with these chemicals rely on extensive live-animal testing. Because of the huge costs and ethical concerns with regard to animal testing, the 3R principle (i.e., reduction, replacement, refinement) demands that animal experiments be substituted with alternative test methods whenever possible. Computational models have been proposed as powerful *in silico* alternatives. Toxicokinetic (TK) models have received particular

attention. These models can be used to predict the time course of a toxicant's concentration at the target site, and thus help in interpreting and extrapolating toxicological effect data. Currently, the TK models designed for fish are appropriate for neutral organic chemicals, but lack specificity for chemicals that are actively biotransformed. To bridge this gap, fathead minnows were aqueously exposed to the model chemical benzo[*a*]pyrene (BaP) at concentrations of 0.3 $\mu g \cdot L^{-1}$, 0.8 $\mu g \cdot L^{-1}$ and 1.3 $\mu g \cdot L^{-1}$ with the objective of characterizing *in vitro* transformation kinetics of BaP, develop TK models for *in vitro-in vivo* extrapolation of biotransformation, and validate model predictions using data from *in vivo* flow-through exposures to graded concentrations of water-borne BaP. Analysis of BaP metabolites showed a significant dose-dependent increase in BaP metabolites. However, no difference in biotransformation rates were observed when ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GST) activity was analyzed. Therefore, a first-order *in vitro-in vivo* extrapolated biotransformation rate was applied to a physiologically-based TK model for fathead minnows. Based on data acquired to date, we conclude that TK models in combination with *in vitro* assays are a powerful alternative to *in vivo* experiments.

A tail of two birds: contrasting contaminant trends in herring gulls and double crested cormorants (PL)

Shane de Solla[1], Kimberly Hughes[2], Glenn Barrett[1], Craig Hebert[1]

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Diet is an important factor for determining exposure of bioaccumulative persistent organic pollutants to high trophic level vertebrates such as colonial waterbirds. The Great Lakes Herring Gull Monitoring Program has proved valuable in contributing to our understanding of food web dynamics and the importance of understanding diet in interpreting contaminant and related data. We assessed relationships between diet, as assessed using stable isotopes ($\delta^{15}N$, $\delta^{13}C$) and fatty acids, and contaminant burdens in eggs of herring gulls (HERGs) from 2015 to 2018 from 15 colonies in the Great Lakes. Eggs of double-crested cormorants (DCCO) from some of the same colonies were also collected and analyzed for the same suite of contaminants, providing a unique opportunity to compare similarities and differences in contaminant burdens between a generalist omnivore (HERG) and an obligate piscivore (DCCO), as well as the extent to which diet can explain interspecific differences in body burdens. Although good relationships were observed in burdens between DCCOs and HERGS for at least some contaminants (e.g. PCBs r^2 =0.72), other compounds had marked differences; relationships between HERGs and DCCOs were particularly poor for contaminants that had putatively substantial terrestrial sources. The flame retardant Dechlorane Plus was ubiquitously detected in HERGs but was mostly undetected in DCCOs. Polychlorinated biphenyl (PCB) levels in eggs of HERGs were proportional to the degree that they fed in aquatic foodwebs. Intercolony variability in diet had more profound effects on egg burdens in HERGs than DCCOs; both $\delta^{15}N$ and $\omega 3$:$\omega 6$ fatty acid ratios influence contaminant burdens in eggs of HERG more than DCCOs. DCCOs clearly had a greater aquatic diet than HERGs. Despite their similarities and differences, cormorants and gulls provide complementary information about food webs and contaminant exposure in the Great Lakes.

Contrasting sources and temporal trajectories of legacy and current use flame retardants in Herring Gull (*Larus argentatus*) eggs in the Laurentian Great Lakes (PL)

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Following the discovery of widespread adverse reproductive and developmental impairment in fish-eating colonial waterbirds nesting in the Canadian Great Lakes, Environment and Climate Change Canada started monitoring contaminants in herring gull (*Larus argentatus*) eggs in 1974. Here we contrast the spatial and temporal trends of both legacy contaminants, some of which are flame retardant chemicals, such as polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs), with flame retardants currently in production, such as organophosphate esters (OPEs) and Dechlorane Plus (DDC-CO) isomers. In general, the temporal trajectories of legacy compounds in gull eggs were consistent with first order exponential declines. An exception was PCNs in gull eggs from colonies immediately downstream of the Detroit River, in which concentrations peaked again in the early 2000s. PCN concentrations appeared to have a temporary resurgence following dredging of sediment for remediation of contamination within the Detroit River, the effect of which was detectable at least as far downstream as eastern Lake Ontario. Compared to legacy contaminants, concentrations of DDC-CO have increased over time, whereas temporal trends of OPEs were inconsistent. Spatial trends of all persistent contaminants (e.g., PCBs, PCNs and DDC-COs) appear to be associated primarily with industrial activity or nearby centers of human population. Conversely, OPE temporal trends in gull eggs were only weakly related to the trends seen in sediment, and body burdens appeared to be driven not by municipal density but instead are heavily influenced by metabolism in the maternal birds or by embryos.

Comparing the embryo-larval toxicity of microinjected selenomethionine in the fathead minnow and white sturgeon (PO)

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Selenomethionine (SeMet) is a bioaccumulative and toxic organic form of the essential element "selenium" (Se). Where anthropogenic activities have increased Se loading to aquatic environments, resultant toxicity has been attributed to the biotransformation of aqueous Se oxyanions to SeMet, which subsequently bioaccumulates due to SeMet's dose-dependent substitution for the essential amino acid "methionine," and ultimately produces redox cycling metabolites. SeMet poses particular risks to oviparous animals dependent upon aquatic environments as it is maternally transferred to their developing eggs, which exposes their progeny to teratogenic and/or lethally toxic doses of the chemical during embryogenesis. This study compares the embryo-larval toxicity of SeMet in the fathead minnow and white sturgeon using microinjection as an analogue for maternal SeMet transfer. Experimental conditions included negative control, sham injection, and environmentally-relevant low (8.9 μg $Se \cdot g^{-1}$),

medium (13.3 µg $Se \cdot g^{-1}$), and high (20 µg $Se \cdot g^{-1}$) dose treatments, with all exposures confirmed to be within $\pm$ 15% of their nominal concentrations. SeMet exposure induced significant deformity and mortality across species at similar concentrations and induced non-significant increases in measures of oxidative stress in whole-body sturgeon at swim-up, though survivorship bias likely obscured this trend in the high-dose treatment. These results corroborate literature assessments of the embryo-larval toxicity of SeMet in fish from contaminated systems and emphasize the importance of continued assessments of populations at risk of SeMet exposure, such as the white sturgeon of the San Francisco Bay Delta. Transcriptomic analyses are currently underway to determine sensitive predictive indicators of SeMet toxicity in these species.

Levels of trace metals, PAHs and TBT in surface sediment samples from a broad marine area of Isla 25 de Mayo (King George Island), South Shetland Islands, Antarctica (PO)

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Western Antarctic Peninsula (WAP) has the highest human presence in "the white continent" and represents the area most affected by global warming worldwide. Human presence constitutes a potential risk of pollution by both persistent organic pollutants (POP) and metals, and global warming could modify dynamics and transport of such pollutants with increasing summer water runoff, ice melting and iceberg scouring. To reach a deep understanding of the presence and dynamics of pollutants, we performed a regional-scale monitoring of POPs, polycyclic aromatic hydrocarbons (PAHs), organotin compounds (OTCs; biocides in hull paints) and trace elements (Cr, Co, Ni, Cu, Zn, As, Pb, Cd and Hg), as well as Fe and Mn, in surface sediment from 64 sites comprising six different areas in Maxwell Bay, Isla 25 de Mayo (King George Island). Based on the values observed for the reference areas, baseline levels for the studied elements were defined for the studied region. A regional enrichment in Cu, related to the widespread mineralization of volcanic rocks, was observed. The most anthropized area (South Fildes) showed sediment class 3 (moderately polluted) for Pb, Cd and Hg, with some samples revealing highly contaminated hot spots. Moderated evidence of pollution with PAHs and OTC were detected in the same area, with some samples showing total PAHs as high as 100 $ng \cdot g^{-1}$ dry weight. Tributyltin (TBT) was detected only in five samples. This work represented the first regional-scale attempt to define the anthropogenic impacts in this region of WAP and provided the first data about Hg concentration in surface sediment of the study area.

Tree swallows as a sentinel species for assessing pre-remediation conditions at Randle Reef, Hamilton Harbour (PO)

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As part of an assessment of pre-remediation environmental conditions at Randle Reef, several parameters relating to reproduction, health, and contaminants were studied in tree swallows

(*Tachycineta bicolor*) nesting in Hamilton Harbour from 2013–2018. This species feeds on emergent aquatic insects in close proximity to its nesting site and has been used widely as an indicator of local contaminant conditions in the Great Lakes. Nest boxes were installed at two locations in Hamilton Harbour, Randle Reef (adjacent to the remediation site) and Bayfront Park (3 km west of Randle Reef), and at Long Point on Lake Erie (reference site). Measures of reproductive success were generally high at all study sites. While sum-concentrations of polychlorinated biphenyls (PCB) were significantly higher in eggs from the two Hamilton Harbour locations compared to those from the reference site, these were well below those associated with effects on reproduction. Exposure to polycyclic aromatic hydrocarbons (PAHs) was greatest at Randle Reef relative to other sites, based on PAH concentrations in stomach contents of nestlings (reflective of chick diet) and in air using polyurethane foam passive air samplers that were deployed near nest boxes. Increased ethoxyresorufin-O-deethylase (EROD) activity (enzyme induced by PAH exposure and responsible for PAH metabolism) was found in nestlings (liver) in one of three study years. Biochemical health effects typically associated with contaminant exposure were found, including those associated with thyroid function (plasma) and oxidative stress (lungs) in nestlings. Remediation of Randle Reef is currently ongoing, with post-remediation monitoring activities of tree swallows expected to commence in 2020.

Automated water quality monitoring under the Oil Sands Monitoring Program in the Peace-Athabasca Delta (PO)

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The water quality monitoring program outlined by the Joint Canada-Alberta Implementation Plan (JOSM) began in 2012. The Plan was designed to address recommendations for the development of an integrated monitoring program which would be able to identify changes in environmental condition over time in Canada's oil sands region. Through the program, several monitoring approaches have been evaluated, including automated water quality monitoring deployments. Automated monitoring technologies present an opportunity to establish baseline characteristics and potentially provide an early warning monitoring system based on a subset of physical-chemical parameters. This is particularly applicable in remote locations such as the Peace-Athabasca Delta (PAD), where logistical and financial constraints make frequent site visits challenging. Since 2013, the program has been conducting deployments of water quality monitoring instruments (typically referred to as a sonde) in the PAD at remote sites on the Slave (M11A) and Athabasca (M9) rivers. Sondes (YSI 6600 series) were deployed for a month at a time during the open water season and were programmed to log water temperature, pH, specific conductivity, turbidity, and dissolved oxygen (DO) at hourly intervals, 24 hours per day. As the program has developed, new automonitoring sites have been added on the Peace River (M12) in 2015 and Rivière des Rochers (M10) in 2018. Additionally, YSI 6600 sondes were updated to the next generation YSI EXO sondes in 2018. Standard operating procedures have been published and made available online and automated water quality data is now uploaded annually to Environment and Climate Change Canada's open data website.

Assessment of contaminants in the American lobster, *Homarus americanus*: A baseline survey for Boat Harbour remediation (PO)

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There is little research on contaminant levels in the American lobster, *Homarus americanus*, which is economically and ecologically important in Nova Scotia. Moreover, the remediation of Boat Harbour, previously a treatment facility for pulp mill effluent, is scheduled to occur, which may release contaminants into an active fishing region in the Northumberland Strait. This survey was conducted to establish a baseline of contaminants in *H.americanus* as part of the monitoring throughout Boat Harbour remediation. In the summer of 2018, we identified inorganic and organic contaminants and their amounts in the tissues of three lobster age classes (adults, sub-adults, and juveniles) from three sites along the coastal region of the Northumberland Strait, Nova Scotia. Three additional objectives were to 1) determine if contaminants in tissues exceeded any guideline values; 2) record the differences in contamination between sites; and 3) test for any bioaccumulation effect in lobsters. Overall, there were a few exceedances of guideline values, notably arsenic levels, but metal concentrations were generally within the range of metal concentrations as reported in other studies. There was no clear trend in contaminant levels based on site; rather, it varied depending on the contaminant. Finally, a bioaccumulation effect was evident with most contaminants apart from a few exceptions, most notably aluminum and iron levels in juveniles. These results will be valuable as a baseline comparison in future studies to effectively monitor the effects of Boat Harbour remediation on the lobster population in the Northumberland Strait.

Soil Ecotoxicology and Soil Ecological Risk Assessment in Canada

Bioaccumulation and phytotoxicity of microcystins in agricultural plants: Meta-analysis and risk assessment (PL)

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Microcystins (MCs) are cyanotoxins produced by many species of cyanobacteria. As specific inhibitors of serine/threonine protein phosphatases and potent tumor promoters, they are hepatotoxic to humans and poisonous to animals. In addition, there is evidence of MCs toxicity to many plant species in aquatic and terrestrial environments. Terrestrial plants are susceptible to MCs toxicity because these amphiphilic molecules tend to form micelles and ion-pairs, which prevents them from bonding with charged surfaces of soil minerals and organo-minerals, so they remain in soil solution where they are bioavailable to plants. The objective of this work was to determine how MCs exposure affects the physiology and growth of agricultural plants, estimate bioaccumulation rates, and consider the human health risk of plants containing MCs. We conducted a formal meta-analysis to evaluate the bioaccumulation and phytotoxicity of MCs in agricultural plants and answered the broad question, "which species of agricultural crops are more vulnerable as exposed to MCs and accumulate more MCs?" Most agricultural plants are exposed to MCs present in water sources used for irrigation. Organic fertilizer made from cyanobacteria are another source of MCs exposure for agricultural crops. The mechanisms governing MCs uptake in plants and the consequences of MCs toxicity at the cellular and organismal level is discussed. Finally, the human health risk of MCs in agricultural plants was evaluated with a risk assessment model.

Quantification of nano zero-valent iron in soil for toxicity testing of soil invertebrates (PL)

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Nano zero-valent iron (nZVI) has been used for several years in the remediation of contaminated groundwater and has more recently been proposed as a method for soil remediation. It can reduce the toxicity of contaminants such as polycyclic aromatic hydrocarbons, pesticides, and heavy metals that are resistant to microbial degradation and weathering. Before nZVI can be widely used in soil remediation efforts, toxicity testing must be done to compare the toxicity of the existing contaminants to potential toxicity of the nZVI. Some literature exists on the toxicity of nZVI to soil biota, but these studies report nominal concentrations or reduction in bioactivity of a contaminant rather than measured concentration of nZVI, which undermines confidence in the data. The purpose of this research is to adapt a method of nZVI quantification from groundwater research that uses indigo disulfonate as a specific chemical redox probe. A standard curve was developed by reacting indigo disulfonate with known concentrations of nZVI in water to produce a colour change measured by ultraviolet-visible spectroscopy. This curve was then used to quantify nZVI in soil pore water extracted

from nZVI amended soils. Toxicity to the soil invertebrates *Folsomia candida* and *Eisenia andrei* was then assessed using the endpoints of avoidance, lethality, and reproduction. These endpoints were combined with the method of measuring nZVI to express toxicity thresholds in terms of the realized dose in the soil. This allows for better extrapolation to field exposure scenarios and development of guidelines for the use of nZVI in soil remediation.

Characterizing a novel springtail for soil ecotoxicology testing (PL)

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Springtails (Subclass: Collembola) are one of the most commonly studied groups of test organisms in soil ecotoxicology thanks to their ease of use in laboratory culture, significant ecological role, and consistent response to environmental contaminants. *Folsomia candida* (Family: Isotomidae) is a globe-spanning parthenogenetic collembolan found in agricultural soils, riparian systems, caves, and forests. It has been identified as relatively insensitive to exposure to some contaminants compared to other Collembolan species. However, due to standardized test methods, it is the subject of most soil ecotoxicology arthropod studies. *Arrhopalites caecus* (Family: Arrhopalitidae) is a novel (to soil ecotoxicology) globular springtail species. Like *F. candida*, it has a worldwide distribution and can be found in a wide range of habitats, including cave systems and forest leaf litter. A. caecus also reproduces through parthenogenesis. Unlike *F. candida*, the sensitivity of *A. caecus* to environmental contaminants is largely unknown. This research aims to characterize the life cycle of the springtail *Arrhopalites caecus,* as well as the optimal culture and test conditions and its sensitivity to environmental contaminants. The sensitivity of *A. caecus* and *F. candida* exposed to insecticides in soil matrix was compared, representing the most common springtail exposure scenario. The results suggest that *A. caecus* is more sensitive to acute insecticide exposure. In order to further characterize differences in sensitivity, these species were exposed to insecticides through a feeding assay that was developed for use in this investigation.

Contaminated sites management in Quebec: A perspective on sustainable development? (PL)

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In the most recent version of its *Guide d'intervention* (Beaulieu 2019), Quebec's *Ministère de l'Environnement et de la Lutte contre les changements climatiques* (MELCC) ranked risk assessment as one of the least sustainable contaminated site-remediation strategies. Nonetheless, elsewhere in Canada and internationally, risk assessment has become a tool of choice for the management of contaminated sites. Through a case study that considers risks pertaining to emissions generated by

excavation work and transport and disposal of soils, this presentation will discuss the sustainability of risk-based site remediation compared with traditional approaches.

The potential of pre-industrial Boat Harbour sediment to support growth and survival of estuarine primary producers *Zostera marina* and *Spartina alterniflora* (PL)

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Boat Harbour, a tidal estuary located in Nova Scotia, Canada, has been receiving effluent from a pulp and paper mill since 1967 and will cease to be the receiving site upon remediation efforts. Contaminants are strictly associated with the top layer of sediment, which will be removed in the initial steps of the cleanup process. A critical step in the remediation is to test the suitability of the underlying layer of pre-industrial sediment to support primary producers. Large diameter cores were extracted from Boat Harbour and nearby Pomquet Harbour, NS, and were inserted into Pomquet Harbour, a productive estuary, mimicking desired post-remediation conditions in Boat Harbour. Estuarine plants *Zostera marina* and *Spartina alterniflora* were transplanted into the cores and were monitored for growth and survival in summer 2018. Results indicated no significant difference in growth and survival of either plant with varying sediment type and upon addition of fertilizer to *Zostera* cores and addition of *Geukina demissa* to *Spartina* cores. The findings of this study indicate that Boat Harbour's pre-industrial sediment will support the summer growth and survival of two important estuarine plants, *Zostera* and *Spartina*. The implication for remediation is that if the upper layer of contaminated sediment can be effectively removed from Boat Harbour, it seems that these two ecosystem engineers can be re-established in the system, once communication with the ocean is restored. This bodes well for the eventual goal of the planned remediation, which is establishment of a self-sustaining estuarine ecosystem in Boat Harbour.

Data analysis

Lessons from a multi-biomarker study in French rivers: Insight into challenges for biomonitoring (PO)

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Biomarkers are used as part of the regulatory monitoring of marine environment (Marine Strategy Framework Directive). In French marine ecosystems, the *2012-17-12 Decree on the definition of good ecological status of marine waters* requires the measurement of five biomarkers on bivalves and/or fishes. In contrast, the Water Framework Directive does not currently require biomarkers measurement in freshwater, and only refers to them as a method that can be used to complement an environmental monitoring strategy. This study aims to assess the spatiotemporal variability of biomarkers response when applied to freshwater streams. To achieve this, seven biomarkers were measured on European chub (*Squalius cephalus*) in September 2016 during the environmental monitoring of the fish compartment in French rivers. Fish were caught on streams where EDF (Électricité de France) electricity production sites are located. Genotoxicity, neurotoxicity, and immunotoxicity, as well as endocrine disruption, biotransformation and development indices were accounted for. Biomarkers were selected in accordance with the *European Technical Report on Aquatic Effect-Based Monitoring Tools* (2014). This work provides insight into challenges for biomonitoring, and especially into the determination of reference values and on the natural biological variation that can make the interpretation challenging. It focuses on the interpretation of biomarker responses by using an integrative index, which processes a set of responses into one single score: the Integrated Biomarker Response (IBR). The pros and cons of two IBR versions will be weighed in order to offer a critical review of this methodology when it comes to ecotoxicological assessment.

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