Proceedings of the 44th Annual Canadian Ecotoxicity Workshop: October 1–4, 2017, Guelph, Ontario

Comptes rendus du 44e Atelier annuel canadien en écotoxicologie : du 1er au 4 octobre 2017, Guelph, Ontario

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Canadian Technical Report of Fisheries and Aquatic Sciences 3309

Rapport technique canadien des sciences halieutiques et aquatiques 3309

Canadian Technical Report of Fisheries and Aquatic Sciences

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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.

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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.

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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.

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PROCEEDINGS OF THE 44TH ANNUAL CANADIAN ECOTOXICITY WORKSHOP: OCTOBER 1-4, 2017, GUELPH, ONTARIO

Editors:

P.K. Sibley[1], R.S. Prosser[1], C. Dubetz[2] and R. Allen Jarvis[3]

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Preface

For 41 years, the annual Aquatic Toxicity Workshop (ATW) has been 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 44th annual CEW was held at the Delta Hotel in Guelph, Ontario from October 1 to 4, 2017. The workshop included 163 platform presentations and 56 poster presentations. Total attendance was 354.

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.

Préface

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 44ième Atelier canadien annuel sur l'écotoxicité a eu lieu à l'Hôtel Delta à Guelph (Ontario), du 1er au 4 octobre 2017. L'atelier a donné lieu aux 163 présentations orales et 56 présentations par affiche. Trois-cents cinquante-quatre 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 totally 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 Jill Watson dans la préparation de ces comptes rendus.

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Toxicokinetics and bioaccumulation of PACs in wood frog tadpoles exposed to Athabasca oil sands sediment

Julie Bilodeau[1], Linda Kimpe[1], Juan Gutierrez- Villagomez[1], Vance Trudeau[1], Jules Blais[1]

[1]*University of Ottawa*

Many polycyclic aromatic compounds (PACs) are toxic, carcinogenic, and mutagenic. Research on PACs in aquatic biota often overlooks the role of amphibians, alkylated PACs, and sediment. In order to study the accumulation and toxicokinetics of PACs following sediment and aqueous exposure, and to compare the bioaccumulation potentials of parent and alkyl PACs, two accumulation-elimination experiments using wood frog tadpoles (*Lithobates sylvaticus*) of Gosner stage 28-32 were conducted (one evaluating exposure to contaminated sediment and water, and the other to contaminated water alone). A complementary field study was then conducted near Fort McMurray, Alberta, to assess PAC body burdens in field-collected amphibian larvae and to determine whether PAC body burdens are related to exposure to sediment and/or water in the field. Our results show that PAC concentrations and uptake rates in wood frog tadpoles were highest when they were exposed to PAC-contaminated sediment. Consequently, we determined that the dominant route of exposure of wood frog tadpoles to PACs is sediment ingestion rather than water. In both the laboratory and field study, alkyl PAC concentrations exceeded those of parent PACs in wood frog tadpoles, characteristic of petrogenic PACs. *L. sylvaticus* tadpoles seemed to be efficient at eliminating and metabolizing both parent and alkyl PACs. However, the elimination of some compounds, such as C4-naphthalenes, was not as efficient. Furthermore, C3-fluorenes and C2-dibenzothiophenes were isolated as potential markers of amphibian larvae exposure to PAC-contaminated sediment due to their positive correlation with the wetland sediment concentrations. The results of this study are the largest, most comprehensive set of toxicokinetic and bioaccumulation information of PACs (52 analytes) in the amphibian larvae *L. sylvaticus* obtained to date.

Toxicity of vanadium to freshwater organisms representative of the Athabasca Oil Sands Region

Stephanie Schiffer[1], Karsten Liber[1]

[1]*University of Saskatchewan*

Vanadium (V) is an elevated trace metal in oil sands coke, which is produced and stored on-site of major Athabasca oil sands companies in northern Alberta, Canada. Recently, coke has been evaluated as a treatment option for the removal of organic acids in oil sands process-affected water (OSPW) through adsorption processes. However, past studies have found coke to leach toxicologically relevant concentrations of V (>1 $mg \cdot L^{-1}$) into solution upon contact with water, including OSPW. Therefore, given limited knowledge of V toxicity, coke leachates or coke-treated OSPW containing V could pose risks to aquatic organisms in

nearby freshwater systems. To address this data gap for V, this research aimed to generate the needed toxicity data to derive sound V water quality (WQG) benchmarks for use in this region. To do this, several acute and chronic toxicity tests were conducted on a diverse suite of aquatic organisms of different trophic levels, using both comparable laboratory standard test-organisms and organisms more regionally relevant to northern Alberta. Data generated here was then combined with data from the peer-reviewed literature into separate acute (23 species) and chronic (21 species) species sensitivity distributions (SSDs). From these SSDs, the acute and chronic hazardous concentrations to 5% of species tested (HC5) were estimated as 0.64 and 0.05 mg $V \cdot L^{-1}$, respectively. These new data for V toxicity to aquatic organisms ensure that there are now adequate data available for regulatory agencies to develop appropriate water quality guidelines (WQGs) for use in the Athabasca Oil Sands Region and elsewhere.

Assessment and Management of Contaminated Soils: Ecotoxicological Assessment and Remediation

Derivation of soil standards for the beneficial reuse of excess soils in Ontario

Chi Hoang[1], Adam Leus[1]

[1]*Ontario Ministry of the Environment and Climate Change*

A significant amount of excess soil is generated annually during excavation from construction sites across Ontario. As soil is a valuable resource, it is essential that excess soil is managed in a manner that promotes sustainable reuse while being protective of human and ecological health. As part of the provincial excess soil management policy framework, the Ontario Ministry of the Environment and Climate Change is developing effects-based numeric standards to support the beneficial reuse of excess soils in Ontario. These standards are derived through the consideration of a number of component values that are developed to provide sensitive human or ecological receptors with acceptable levels of protection from contaminants via different exposure pathways. This presentation will provide an overview of the standard derivation process and highlight some key fate and transport processes of contaminants in soils and exposure pathways, which include (1) movement of contaminants from soil to groundwater (2) inhalation of vapours from soil to indoor air, and (3) direct soil contact and ingestion by human and ecological receptors.

Are we too stringent in our assessment of ecotoxicological risks when managing contaminated soils?

Agnès Renoux[1]

[1]*SANEXEN Services Environnementaux*

After many years of experience using the ecotoxicological risk assessment (ERA), it is becoming obvious that this approach is less and less appealing for the management of contaminated soils. Site managers are complaining about the fact that, whatever the site or jurisdiction (federal or provincial, at least in Quebec), the conclusion of an ERA (using exposure modelling and toxicological benchmarks) is always the existence of risk with respect to the components of the ecosystem. On top of that, toxicity testing or field studies are hardly considered in the ERA process, because they are either expensive, too long, or uncertain. This leads almost systematically to the excavation and disposal of the soils, based on generic criteria, with all the ensuing environmental costs associated with this type of site management and thus avoids the time and money-consuming steps of the ERA. Using some examples, the objective of this presentation is to provide a different perspective regarding the failures of the ERA and the challenges we face as scientists from government, industry, and academia.

Bridging the gap between research and risk assessment in ecotoxicology

Rick Scroggins[1], Juliska Princz[1], Ellyn Ritchie[1], Chris Fraser[1], Heather Lemieux[1], Dina Schwertfeger[1], Patrick Boyd[1], Lee Beaudette[1], Stephanie Kvas[1], Alden Crossman[1]

[1]*Environment and Climate Change Canada*

Canada has committed to the assessment of environmental and human health risks for priority chemical substances as part of Canada's Chemicals Management Plan (CMP). The assessments are usually based on available experimental data and predictive modeling tools, with focused research conducted to fill data gaps where feasible. Gaps exist for the soil compartment, particularly for chemical substances known to partition to soil, for which the persistence, bioaccumulation and inherent eco-toxicity potential are uncertain. As a result, research efforts have focused on filling some of these information gaps for various substances (e.g., inorganic and organic-containing metals). A few case studies will be presented to demonstrate how standard soil test methodologies and a suite of soil microbial health tests are used to provide effect estimates and confirm model predictions for the risk assessment of chemical substances in the environment. The studies will focus on metal-containing inorganic and organic substances, where in some instances it is uncertain whether the metal ion, the parent molecule or the organic moiety has the greatest toxicological significance. As a result, the evaluation of a group of priority substances sharing a common metal moiety (e.g., zinc and copper) will be discussed. The results of these studies demonstrate the importance of research for the development and confirmation of tools applicable to better understanding and predicting the impacts of chemicals in the environment.

Communicating herbicide toxicity to northern Saskatchewan communities to facilitate informed, collaborative decision-making for vegetation management on boreal rights-of-way

Jenna Zee[1], Chelsea Voinorosky[1], Tegan Brock[1], Maureen Reed[1], Katherine Stewart[1]

[1]*University of Saskatchewan*

Limiting the growth of tall trees under power lines is essential for preventing power outages and wildfires caused by contact with the lines. Managing vegetation along power line rights-of-way (ROW) is particularly challenging in remote boreal forests across northern Canada. Integrated vegetation management (IVM) has the potential to increase the efficiency and lower the cost of ROW maintenance, reduce associated environmental impacts, and meet other ecological and socioeconomic objectives. However, the use of herbicides—an integral aspect of many IVM plans—is raising concerns from local stakeholders and Indigenous communities, especially where traditional land use activities may occur along power lines. These concerns are resulting in (1) a need to better determine the efficacy, persistence and toxicity of herbicides used on ROWs in northern boreal ecosystems and (2) improved community engagement for environmental decision-making that can support co-management of ROW vegetation. We are examining the impact, translocation and dissipation of triclopyr under boreal conditions. Translocation of

triclopyr applied via basal bark (Garlon RTU) and dissipation of triclopyr in foliage when applied by both basal bark and low volume foliar (Garlon XRT) will be determined under field conditions. From previous work in the boreal forest of the Yukon Territory, the DT50 (dissipation time until 50% of the initial concentration remains) for triclopyr in soils was as low as 1 day, and dissipation in *Salix glauca* foliage was estimated at 11.5 days. Impacts of triclopyr on litter decomposition and soil-dwelling invertebrates (*Collembola*) will be determined using northern soil and plant materials under laboratory conditions. Soil invertebrates (*Enchtreaus crypticus, Folsomia candida* and *Oppia nitens*) in northern boreal soils appear to be relatively insensitive to triclopyr; however, little is known regarding the sensitivity of these invertebrates to herbicide-contaminated leaf litter. The information from these studies will be used to directly support decision-making processes regarding herbicide use on the I3P line (Island Falls to Key Lake, Saskatchewan). Given a lack of locally relevant scientific information, community concerns about herbicide use, and challenges associated with early engagement, there are strained relationships between industry, government, and Indigenous communities. Working in partnership with SaskPower and the Lac La Ronge Indian Band Lands and Resources Management Board, we are examining barriers to, and the principles of, good-practice engagement. In addition, using both local Indigenous and scientific knowledge, we have implemented an education program in four local schools to promote questioning and learning through observation of nature and to teach the concepts and practice of IVM. By simultaneously working to provide relevant toxicological data and to improve engagement practices, our project aims to provide both the information and good-practice framework to support a collaborative decision-making process.

Toxic effects of rare earth elements to radish and durum wheat

Amanda Pellegrino[1], Bianca Pereira[1], Beverley Hale[1]

[1]*University of Guelph*

Rare earth elements (REEs), or rare earth metals, are 17 elements including the lanthanide series, yttrium and scandium, that occur naturally together in mineral deposits and have similar properties. Use of REEs has increased in recent years, and as such, mining of them has increased as well. Establishment of REE mining in Canada is being considered, which would lead to increased prevalence of REEs in the Canadian environment. While there is little phytotoxicity information currently available for these elements, it has been proposed that plant response to them via soil exposure would follow a hormetic dose response, whereby at low concentrations REEs would be stimulatory and at high concentrations toxic. In OECD soil, EC_{25} values for cerium (Ce) and neodymium (Nd) were 310 and 540 $mg \cdot kg^{-1}$ respectively. In order to determine the effects of several REEs to plants, 14 day toxicity tests were completed. Radish was grown in Ce-, europium (Eu)- and Nd-amended soils at a range of concentrations including those expected at very contaminated sites, and based on values from previous studies$\cdot kg^{-1}$ respectively. At the end of the study, plant root and shoot lengths, biomass, and REE concentrations in soils and plant tissues were determined. Clear dose responses were seen for each of the REEs tested, with potential for hormesis to be identified. A similar study using both radish and durum

wheat will be undertaken in order to test more concentrations of each metal, and also to determine the effect of REEs in mixtures. Research is currently ongoing and further results will be presented.

Bioavailability and effects of bismuth-contaminated soil on the earthworm

Pierre Yves Robidoux[1], Zohra Omouri[2], Michel Fournier[2]

[1]AGAT Laboratories, [2]Institut National de la Recherche Scientifique (INRS)-Institut Armand Frappier

Bismuth (Bi) is used increasingly to replace lead in several industrial applications including the production of alloys and munitions formulations. However, little information is available on the environmental fate and ecological effects of Bi. This paper summarizes the acute toxicity (LC_{50}=416 mg Bi·kg^{-1} dry soil) and bioaccessibility of Bi, and describes bioavailability and chronic effects of bismuth on the earthworm *Eisenia andrei*. The bioaccessible fraction of Bi in soil was determined using KNO3 soil extraction. In reproduction tests, adult earthworms were exposed to natural sandy soil spiked with Bi citrate. The tested soil concentrations were based on the previous acute toxicity study and the measured concentration of Bi ranged from average 75 to 289 mg·kg^{-1}. Results indicate that Bi significantly decreased reproduction parameters at concentrations ≥ 116 mg·kg^{-1}. The number of hatched cocoons and juveniles were sensitive endpoints. Bismuth did not affect *E. andrei* growth and survival at the tested concentration, and had little effect on phagocytic efficiency of coelomocytes. The low immunotoxicity effect might be explained by other mechanisms, i.e., Bi sequestered by metal-binding compounds as metallothioneins. Indeed, after 28 days of exposure, Bi concentrations in earthworm tissue increased with increasing Bi concentrations in soil, reaching a stationary state at 21.37 mg Bi/kg dry tissue for 243 mg Bi·kg^{-1} dry soil. Data also indicate that after 56 days of exposure, the average fractions of Bi available in soil without earthworms varies from 0.0051 to 0.0229 mg·kg^{-1}, while in presence of earthworms Bi concentration ranged between 0.310 to 1.347 mg·kg^{-1} dry soil. This increase in metal bioaccesssibility could be explained by the mucus and chelating agents produced by earthworms and the microorganisms in the soil or/and earthworm gut, as well as the dermal and ingestion routes of uptake.

Toxicity of metal mixtures in soil and the effect of non-metal factors

Pooja Arora[1], Beverley Hale[1]

[1]University of Guelph

Metals co-occur in the environment at toxic concentrations as a result of mining and industrial activities. Risk assessment of metal mixtures in soils relative to these impacts is of high priority for Canada's mining industry and federal agencies as they develop regulatory frameworks for metal-elevated sites. Current risk assessment tools are based on dose-response studies of single metals. There are limited data available on the phytotoxicity of mixtures, particularly in field application. The proposed research will evaluate the concentration addition (CA) model in predicting the phytotoxicity of a ternary

metal mixture—As, Cu, and Z—on higher plant species. Barley (*Hordeum vulgare L.*) and tomato (*Lycopersicon esculentum*) have been assessed for growth parameters (seed emergence, root and shoot length, and dry weight) and metal uptake. These growth studies utilize naturally aged and leached field-contaminated soils from a decommissioned gold mine site. Field soil conditions are often depauperate in nutrients required for successful plant germination and establishment. The high variability of soil characteristics within a large site can lead to a complex matrix of factors that influence plant growth. Toxic units (TUs) are widely used in the comparison of single compounds when assessing mixture toxicity to extrapolate laboratory data to more field-applicable results. This study will use current regulatory NOECs as single metal TUs to predict toxicity of metal mixtures, to test deviation from the CA model. Outcomes of this research will serve as a predictive risk-assessment tool for mixtures and their interactions in soil at precious-metal mine sites across Canada and worldwide. This study will reduce the need for site-specific toxicological testing by providing a robust method for determining estimates of metal mixture toxicity to fulfill land reclamation objectives.

Using Diffusive Gradient Thin Films (DGT) and Ion Exchange Technique (IET) to relate bioaccessibility and phytotoxicity of rare earth elements

Bianca Pereira[1], Amanda Pellegrino[1], Beverley Hale[1]

[1]*University of Guelph*

The extractive mining and use of rare earth elements (REEs) has increased in recent decades, thus increasing deposition of emissions and waste associated with the mining and manufacturing processes to surrounding environments. There are insufficient data describing the ecotoxicity of this group of metallic elements to establish regulatory soil quality guidelines. Unlike data-rich metals such as Cu and Ni, toxicity associated with REEs has not yet been well demonstrated to relate to the soil REEs that are readily available for uptake from the soil (also known as bioaccessibility). Bioaccessibility of REEs will be measured using Diffusive Gradient Thin Films (DGT) (Figure 1) and Ion Exchange Technique (IET) to determine which is more predictive of the true exposure of plants, thus better correlated with toxicity. It will also be compared to the theoretical values predicted by the modeling program WHAM 7, with the expectation that the values determined by IET and WHAM will be more similar than DGT and WHAM. This work will fill in data gaps that will contribute to the development of federal soil-quality guidelines as well as site-specific risk assessment protocols. Results of ongoing research will be presented.

Nuclear magnetic resonance studies of interactions between organic contaminants and soil organic matter

James Longstaffe[1]

[1]*University of Guelph*

Soil organic matter (SOM) plays a key role in the retention, transport, and availability of many organic contaminants in the environment. For hydrophobic compounds, the role of

SOM is rationalized as that of a hydrophobic partitioning domain. While this approach is useful for developing easily applied metrics for use in risk assessment models, there is a general consensus that the underlying mechanisms responsible for these interactions are more complex than simple partitioning. This is especially true for hydrophilic contaminants. Nuclear Magnetic Resonance (NMR) spectroscopy is emerging as a powerful tool to help elucidate the molecular-level nature of the interactions that occur between organic contaminants and SOM. By focusing on the use of organofluorine compounds as model hydrophilic compounds, multinuclear 1H/19F NMR experiments are useful for probing many attributes of the intermolecular associations between these hydrophilic compounds and both colloidal humic materials and whole organic soils, including: the presence of preferred binding sites, the orientation of the hydrophilic compounds during their interaction, and the dynamics of these interactions. This presentation will discuss the use of these NMR experiments to explore the role that the physical and chemical structure of soil organic matter may play in interactions with these small hydrophilic compounds, which may in turn play a role in governing the overall fate and behaviour of these compounds in the environment.

Quantitative polymerase chain reaction (PCR) pipelines: Linking soil management to microbial ecosystem processes

Lori Phillips[1]

[1]*Agriculture and Agri-Food Canada*

Soil biologists now routinely extract and analyse the DNA of the entire soil microbiome and use different molecular tools to explore the previously hidden diversity of the below-ground ecosystem. One of these tools, quantitative PCR (qPCR), is a rapid, cost-effective, and sensitive method for determining the capacity of the soil microbiome to perform a particular function. In agricultural research, qPCR is typically used to investigate how different management practices impact microbe-mediated biogeochemical cycling processes. Most studies quantify specific genes relevant to the process under investigation; for example, a study assessing how tillage impacts greenhouse gas production might analyse one or more genes associated with denitrification. Microbes, however, don't exist as uni-functional communities and no single process occurs in isolation. This talk will provide specific examples of how a qPCR pipeline that targets multiple inter-related processes can be used to shed light on microbial functioning in the soil environment. This integrated approach may help predict how soil management strategies will impact the beneficial ecosystem processes that are provided by soil microbiomes.

A functional assessment of the mycorrhizal community at Steeves Lake shoreline, Colomac Mine, Northwest Territories

Rebekah Hamp[1], Kevin Stevens[1]

[1]*Wilfrid Laurier University*

The mining industry has played a large role in Canada's economy since the beginning of the twentieth century and is expected to play a larger role in the future. As the industry grows, so do the impacts: economic, social, and environmental. This study will focus on some of the environmental impacts of mining and potential techniques to mitigate them. Bioremediation, the use of living organisms to remediate pollutants, has been gaining attention as it is a less invasive method of remediation and has greater public acceptance than other methods. The living organisms that will be used in this study are northern plants and mycorrhizae collected from Colomac Mine, a study site in the Northwest Territories. The objectives of this study will be to (1) evaluate the mycorrhizal and plant communities at the study site to assess whether there have been negative effects on them due to historic contamination, (2) quantify the effects of petroleum hydrocarbon contamination on northern plants and associated mycorrhiza, and (3) assess the capacity of northern plants and associated mycorrhiza to remediate petroleum hydrocarbon- impacted soils. The results of the field study combined with our controlled studies will increase our understanding of how these communities are affected by petroleum hydrocarbon contamination and provide new insight in the area of bioremediation.

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

Metabolic and respiratory consequences of wastewater exposure in bluegill sunfish *(Lepomis macrochirus)*

Sherry Du[1], Graham Scott[1]

[1]*McMaster University*

Municipal wastewater effluent is a major worldwide source of aquatic pollution. We sought to determine the metabolic consequences of exposure to wastewater effluent on fish and whether physiological adjustments help fish cope in effluent-dominated water. We caged bluegill sunfish *(Lepomis macrochirus)* at two sites downstream of discharge from a tertiary wastewater treatment plant (WWTP) and at an uncontaminated reference site for three weeks. Survival was reduced in fish caged near the outfall of the WWTP (< 50 m away) and a short distance (830 m) downstream, when compared to the negligible mortality in fish from the reference site. Fish at both wastewater-contaminated sites were exposed to elevated levels of pharmaceuticals and personal care products, as reflected by measurements of a suite of target compounds in the water and by the accumulation of synthetic musks (e.g., Galaxolide® and Tonalide®) in the gills and livers of fish by the end of the exposure period. Resting rates of oxygen consumption increased by 30-36% in fish at contaminated sites, reflecting a metabolic cost of wastewater exposure. Exposed fish expanded the gill surface area available for gas exchange by reducing the interlamellar cell mass and thus exposing more lamellar surface to the water. Blood-O_2 affinity also decreased in exposed fish, facilitating O_2 unloading at respiring tissues. Exposure also improved the quality of isolated liver mitochondria by increasing respiratory capacities for oxidative phosphorylation (assessed using single and multiple inputs to the electron transport system) and succinate dehydrogenase (but not citrate synthase) activity, while decreasing the emission of reactive oxygen species (ROS). We conclude that exposure to wastewater effluent invokes a metabolic cost that leads to compensatory respiratory improvements in O_2 uptake, delivery, and utilization.

The cardiorespiratory effects of acute naphthalene and pyrene exposure in adult zebrafish (*Danio rerio*)

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Naphthalene (NAP) and pyrene (PYR), important petrogenic polycyclic aromatic hydrocarbons (PAHs), are not as well studied as benzo[*a*]pyrene (BaP). We hypothesized acute exposure (48h) to NAP and PYR will cause sublethal cardiorespiratory impairment similar to acute BaP exposure in adult zebrafish (*Danio rerio*). To investigate this hypothesis, adult zebrafish were aqueously exposed to PAHs (NAP, 37, 370, and 3700μg·L^{-1}/L; PYR, 0.25, 2.5 and 25μg·L^{-1}) using static renewal (24 hours) and compared to

dimethysulfoxide controls. At 48 hours, fish were subjected to high-frequency cardiac ultrasound (n=16 fish/group) or swim-tunnel analysis (n=12 fish/group). Zebrafish from the 370µg NAP·L^{-1} group increased stroke volume (SV) without alterations in end-systolic volume (ESV), heart rate or cardiac output (CO). The paradoxical lack of response at 3700µg NAP·L^{-1} may be related to the whole-body edema observed upon dissection. PYR changes in cardiac function were only observed at 25µg PYR·L^{-1}; decreased ESV, increased ejection fraction, and decreased heart rate without alterations in SV or CO. NAP showed a U-shape response to increased standard metabolic rate (SMR), but not PYR. With increasing PYR, active metabolic rate (AMR) decreased, resulting in a similarly decreasing factorial aerobic scope (FAS). This was associated with minimal changes in swimming endurance with either NAP or PYR. In conclusion, acute aqueous NAP- and PYR-exposure cardiac effects do not resemble those of BaP, while effects on respiration, metabolism and swimming do resemble those of BaP. High concentrations of NAP have additional cardiorespiratory toxic effects, making it a greater concern for acute sublethal toxicity in adult fish.

The effects of diluted bitumen on the growth, burst swimming performance, and hepatic detoxification status of juvenile pink salmon (*Oncorhynchus gorbuscha*)

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Recent expansions in the transportation of diluted bitumen (dilbit) products via pipeline, railway, and marine terminals in coastal regions of British Columbia potentially increase the risks of exposure to populations of Pacific salmon at various life stages. Juvenile pink salmon (*Oncorhynchus gorbuscha*) inhabit estuarine/marine environments and are constantly challenged by a variety of natural stressors that including dynamic temperature and salinity regimes. The combined effects of a dilbit exposure under varying environmental conditions have the potential to alter the success of these fish as measured by alterations in survival, physiology (e.g., growth/swim performance), biochemistry, and gene expression. The objective of this study was to investigate the potential toxicity of dilbit under different salinity and temperature conditions in early life-stage pink salmon. Juvenile pink salmon (5 months) were subchronically exposed to dilutions (100, 50, 25, 0%) of water-accommodated fractions (WAFs) of the Cold Lake blend (CLB) dilbit in seawater at three salinity levels (7.5, 15, and 30 parts per thousand (ppt) at a temperature 12.5°C) or temperatures (8.5, 12.5, and 16.5°C at a salinity of 30 ppt) for three months. Fish were monitored for survival, growth, and burst swimming ability (Uburst). Several biochemical measurements associated with swimming performance were also measured: white muscle [protein], [glucose], [lactate], and [glycogen]. Gill Na^+/K^+-ATPase activity was also measured to evaluate potential effects on osmoregulatory ability. The expression of genes associated with phase I and II xenobiotic biotransformation, oxidative stress, energy metabolism, mitochondrial activity, temperature stress, salinity regulation, and inflammation were measured in liver. Fish exposed to WAFs of dilbit in every treatment group were found to have increased liver somatic ratios compared to controls. Exposed

individuals exhibited reduced tolerance to the two temperature extremes (8.5 and 16.5°C) at the highest salinity (30 ppt). The survival and growth of fish exposed to the highest WAFs at these two temperature and salinity conditions were significantly lower than in controls. At 30 ppt, Uburst was decreased in fish exposed to the highest WAFs (100%) at all three temperatures (8.5, 12.5, and 16.5°C). Level of genes associated with phase I, energy metabolism, mitochondrial activity and inflammation showed significant increases with dilbit exposure and temperature stress. These results suggest that the combined effects of dilbit exposure and environmental stressors can cause adverse alterations in the growth and swimming performance of juvenile pink salmon, and alterations in hepatic biochemistry and gene expression.

The influence of the gill microenvironment on the toxicity of lampricides

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The lampricide TFM (3-trifluoromethyl-4-nitrophenol) is a pesticide that is used to control invasive sea lamprey populations (*Petromyzon marinus*) by specifically targeting their larval stage in the Great Lakes tributaries. Although TFM has minimal non-target effects, its toxicity is highly influenced by water chemistry, such as pH and alkalinity. Decreases in water pH and alkalinity lead to a greater proportion of the lampricide being in its un-ionized (phenol) form, which increases its uptake rates, therefore making it more toxic to aquatic organisms. Although water chemistry is carefully monitored and the rates of TFM release are adjusted accordingly, TFM applications in the field may affect non-target organisms due to sudden changes in pH or due to alterations in the gill microenvironment. Therefore, this study will focus on how changes in the pH of the gill microenvironment affect TFM speciation and toxicity in two non-target fishes: the TFM-sensitive juvenile lake sturgeon (*Acipenser fulvescens*) and the TFM-tolerant rainbow trout (*Oncorhynchus mykiss*). We hypothesize that discrepancies in pH between the gill and bulk water may explain how TFM is still being taken up even in alkaline waters, where almost no TFM is present in its un-ionized form. To test this, fish were fitted with a latex mask and placed into a divided chamber, with a surgically implanted opercular catheter (PE50) to measure pH of the water at the gill, under different bulk water chemistries: pH 6.5, 7.8 and 9.0, with alkalinity of 50, 150, and 250 $mg{\cdot}L^{-1}$ $CaCO_3$. Radiolabeled TFM (14C-TFM) was used to track rates of TFM uptake and clearance, which were then modeled to reflect the proportions of TFM in its un-ionized form in the waters of varying chemistries. These experiments will be repeated in sturgeon with the mouth occluded, to mimic their natural feeding behavior as a bottom-dwelling fish. We hypothesize that when the mouth is occluded, the fish change their gill ventilation strategy from tidal to quasi-tidal, making them less effective at removing CO_2, which leads to a decrease in water pH at the gill. Lower pH affects the speciation of TFM where a higher proportion of it is in its un-ionized form, therefore increasing its uptake rates and its toxicity. We predict that differences between ventilation strategies, which alter the buffering capacity of the gills, can explain the higher sensitivity of lake sturgeon to TFM.

Microplastic particle morphology: Implications for impacts and managing sources

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Microplastic particles (MPPs)—plastic particles less than 5 mm in size—present in Great Lakes waters became a widely publicized issue in 2012 and contributed to the movement to remove plastic microbeads from personal care products. However, sampling in the Great Lakes has shown that there are numerous sources contributing microplastics to the lakes. Most studies classify MPPs in broad categories such as fragments, film, foam, fibers, and pellets/beads. Such classification can be subjective, and the defined categories may not be helpful for directing management actions and measuring the success of such actions. This presentation will highlight some of the morphological characteristics of microplastics that can be used to expand the categorization of MPPs to support management. For example, rigid plastic particles resembling shavings, cuttings, and trimmings clearly generated by mechanical means, normally categorized as "fragments", are indicative of commercial and building activities. Such material could comprise an additional category such as "commercial fragments", and management of such a source type would be very different from MPPs due to fragmentation of post-consumer plastic debris. Our sampling for microplastics in nearshore Great Lakes waters, wastewater effluents, streams and urban runoff indicate that in some areas the contributions due to commercial fragments can dominate over other types of particles. For example, in 2015 manta trawl surface-water samples from Humber Bay off Toronto, an area which has been found to have the greatest abundance of microplastics measured in sediments and waters of the Great Lakes to date, the commercial fragments category contributed >50% to the total of all MPPs found. Given the MPP morphologies observed in lake water, laboratory tests were undertaken with three species of freshwater fish—fathead minnow (*Pimephales promelas*), rainbow trout (*Oncorhynchus mykiss*), and white sucker (*Catostomus commersonii*)—to examine whether MPPs of a particular morphology were more likely to accumulate in fish when ingested. Ingested particles tended to pass through the digestive tracts of the fish and no definitive differences between particle types and shape were observed on tendency to accumulate. Our sampling demonstrates that manageable sources beyond microbeads from personal care products are present in and entering Great Lakes waters, indicating that multiple management strategies will be needed to reduce the occurrence of MPPs in the Great Lakes.

Microplastics in a eutrophic prairie system

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Recent studies have shown that wherever we look, microplastics are ubiquitous. Microplastics can be defined as any plastic that has a diameter of 5mm or less, and the contamination of environments, as well as ingestion by freshwater organisms, is a growing concern. Problems associated with these plastics, such as contamination of both marine and freshwater environments and ingestion by aquatic organisms, may be problematic. Our

study confirms the presence of microplastics in a prairie creek and lake downstream of Regina, Saskatchewan. Water samples and five species of fish were collected from sample sites upstream and downstream of a waste water treatment plant (WWTP) discharging into the creek in the summers of 2015 and 2016. Sediment samples were also collected from Pasqua Lake, a eutrophic lake downstream from the urban areas of Regina and Moose Jaw, which receives wastewater from those centres. Material in water was sampled using fine mesh net set in the creek for 5 minutes. Fish were collected using gill and seine nets. A gravity corer was used to obtain a 60 cm long core from the deepest point in the lake that was sectioned at 0.5 cm intervals. Lead-210 dating confirmed the ages of the sediment intervals. Water and sediment samples were digested in a $Fe(II)/H_2O_2$ solution, whereas a NaClO solution was used to digest the fish stomachs. Digested samples were dried and observed under a microscope where plastics present were enumerated by colour and type. At least one microplastic was detected in 74% of fish gastrointestinal tracts, 96% of water samples, and in a number of sections of the core. The vast majority of plastics observed were fibers. There was no evidence of differences upstream and downstream of the WWTP and our study provides baseline conditions on the presence of plastics in the creek prior to a major upgrade of the WWTP completed in late 2016.

Canadian Oil and Gas Industries: Advancing Towards a Sustainable Future

Investigation of oil sands groundwater quality and a case for its release to surface water-receiving environment

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The chemistry, and acute and chronic toxicity of groundwaters associated with Shell Canada's Albian Sands operations in northern Alberta were characterized as part of an effort to develop a burden of evidence that the release of groundwaters would pose a negligible and understandable risk to a receiving aquatic environment. Concentrations of conventional chemicals such as nutrients and metals were generally below concentrations considered to pose a negligible risk of harm in long-term exposures. Concentrations of naphthenic acids were variable, due in part to the analytical method employed, but routinely in excess of 1 $mg \cdot L^{-1}$. Groundwaters rarely caused lethality to fish and invertebrates in acute toxicity tests. The oil sands groundwaters caused similar or less sublethal toxicity than has historically or recently been produced by effluents regulated by the *Pulp and Paper Effluent Regulations* or the *Metal Mining Effluent Regulations*. These groundwaters have somewhat high oxygen demand associated with nutrients and organic substances, and therefore have the potential to enrich receiving surface-water environments if untreated, depending on ratios of mixing with a receiving environment.

Temporal and spatial variation in snowpack deposition of metals in the Athabasca Oil Sands area

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Atmospheric deposition of metals in snowpacks and their release into freshwaters during spring snowmelt has been a concern in the Athabasca Oil Sands Region of Alberta. This study was designed to evaluate the loadings and distribution of metals in springtime snowpack and how they vary over time. We examine consecutive years of metal loadings data (2011-2016) collected through a rigorous federal monitoring program, the Joint Oil Sands Monitoring (JOSM). Snowpack samples were collected in late winters (1st week of March) from 2011–2016 at varying distances from the main developments. For vanadium (V) and aluminum (Al), we also included 1978 (January sampling) and 1981 (February sampling) data from a study published by the Alberta government. To be comparable, the calculated loadings for all the years were corrected for the number of snow days prior to sampling by standardizing to the same number of snow days. This was determined to be

120 days based on the average number of snow days for JOSM from 2012-2016. Temporal trends were divided into sites less than 8 km from a major industrial site (AR6), 8-50 km from AR6, and >50 km from AR6. Results indicate that deposition of V decreased over time, as did Al, but to a smaller degree.

The effect of water chemistry representative of Alberta oil sands on the acute and chronic aqueous toxicity of vanadium to model aquatic organisms

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In recent years, Alberta's oil sands coke generation has surpassed 10 million tonnes. This coke has been proposed as a sorbent to reduce concentrations of organic chemicals in oil sands process water (OSPW). However, coke contains up to 5% vanadium (V) by weight and during the treatment process V is released from the coke, reaching levels up to 7mg·L^{-1} in "treated" OSPW. Little information is available on how common water-quality variables influence the toxicity of V to aquatic organisms. Here those relationships are quantified to better understand how site-specific surface water characteristics representative of the Alberta oil sands region affect the adverse effect of V to *Daphnia pulex* and *Oncorhynchus mykiss*. Results to date indicate that when *D. pulex* was exposed for 48 hours to an increase in pH, a threshold relationship was found where toxicity increased between pH 6 and 7, and then leveled off. When alkalinity (70 to 600 mg·L^{-1} CaCO3) increased, the toxicity of V decreased. Also, when sulphate increased from 30 to 380 mg·L^{-1}, the LC_{50} to *D. pulex* slightly increased from 0.95 to 1.31 mg·L^{-1} of V. When the exposures were extended to 21 days, only sulphate resulted in a slight increase in chronic V toxicity to *D. pulex*, an opposite trend to that seen in the acute study. In addition, testing of two environmentally representative mixtures of sodium and sulphate for 48 hours, and alkalinity and sulphate, on V toxicity to *D. pulex* showed that only increasing alkalinity and sulphate decreased V toxicity to *D. pulex*. Finally, when *O. mykiss* fry were exposed to similar ranges of alkalinity and sulphate as tested with *D. pulex*, similar relationships were observed between alkalinity and V, and sulphate and V, to those found for *D. pulex*.

Naphthenic acid extracts decrease larval and metamorphic survival in the wood frog, *Lithobates sylvaticus*

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Due to their dual aquatic and terrestrial life, amphibians are amenable models for understanding the potential aquatic toxicity of process waters produced by bitumen extraction. Naphthenic acids (NAs) are known to be toxic constituents of oil sands process-affected water (OSPW) stored in tailing ponds. Our lab studies have revealed effects of NAs from Merichem (a commercial source) and those extracted from raw OSPW, on wood frog

(*Lithobates sylvaticus*) development and metamorphosis. Toxicity estimates (LC_{50}s based on nominal concentrations) for early larval exposures (Gosner Stage 8-10) were 9.9 mg L^{-1} for Merichem NAs. This contrasts a lower toxicity of 81 mg L^{-1} for Merichem and 52 mg L^{-1} for OSPW NAs when tadpoles were exposed at the hatchling stage (Gosner Stage 20-21) of development. Analysis of developmental abnormalities and growth parameters indicates that embryonic wood frogs exposed to 8-12 mg L^{-1} (EC_{50}) of Merichem NAs were significantly smaller in size and showed compromised structural integrity of the gut. Hatchlings exposed to the same NA concentrations exhibited few alterations until higher NA concentrations (48 mg L^{-1}), thus suggesting a critical window of exposure for this species. To determine if the final stage of tadpole development is also sensitive to NAs, a novel metamorphic emergence assay was developed. At metamorphic climax, (Gosner Stage 42) tadpoles were exposed to Merichem NAs (0.3-3 mg L^{-1}) and allowed to complete tail resorption and emerge onto land. While the rate of tail resorption was only marginally decreased at 3mg L^{-1}, 50% of animals in the 1 and 3 mg L^{-1} treatment died before completing metamorphosis. A field-based experiment was also conducted in which eggs of wild wood frogs were first exposed to a gradient of NA concentrations (1-50 mg L^{-1} OSPW NAs), and then transferred to floating cages in a natural wetland in Ontario. Exposure to NAs resulted in a significant decrease in body size of hatchlings and larvae, but not metamorphs. Survival of tadpoles in the wetland cages decreased in relation to the NA concentration when they were exposed to as eggs. Altogether, our data indicate that multiple amphibian life-history stages are sensitive to NAs. Efforts are underway to find the causative agent(s) in the very complex mixture of NAs and to understand whether or not effects observed in the laboratory would occur with exposures in the wild. It will be essential to incorporate data from amphibians into a species sensitivity distribution so that appropriate treatment targets can be developed. Acknowledgements: NSERC Strategic Program (VLT, JB), Banting and Liber Ero Fellowships (DO), and University of Ottawa Research Chair (VLT). The help and encouragement of Bruce MacLean and the Mikisew Cree First Nation (Fort Chipewyan, AB) is appreciated.

In vitro assessment of endocrine-disrupting potential of organic fractions extracted from hydraulic fracturing flowback and produced water

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The potential environmental impacts of horizontal drilling with high-volume hydraulic fracturing (HF) have drawn significant public concern, especially on the HF flowback and produced water (HF-FPW), which is a complex mixture of wastewater containing large amounts of salts, metals, natural organics, fracturing fluid additives, and potential secondary byproducts from downhole reaction. In 2015 alone, there were more than 113 spills of HF-FPW fluids into the environment documented in Alberta, Canada. The consequent salts, metals, and organics pollutants detected in HF-FPW often greatly exceeded the maximum contamination levels for water quality guidelines. However, knowledge about the potential hazards of this wastewater on the health of aquatic ecosystem is still very limited. Previous studies indicated HF-FPW can significantly disrupt

biotransformation and endocrine gene expression. Our most recent study focuses on the disruptive effects of HF-FPW organic extracts on receptor-mediated signaling using reporter gene assays. Generally, 12 organic fractions were extracted from HF-FPW samples collected from 2 different sites with 6 timepoints. PAH contents and total organic profiles were analyzed by GC-MS and HPLC-Orbitrap. The agonism/antagonism activities of different extracts on aryl hydrocarbon receptor (AhR), androgen receptor (AR) and estrogen receptor (ER) were also screened using H4IIE-luc, MDA-kb2, and MVLN-luc cell lines, respectively. The results demonstrated a complex profile of receptor-signaling mediated effects, especially on AhR activation, based on the extracts from different locations and timepoints. This study also provided novel information on the endocrine-disruptive potentials of various HF-FPW samples on different temporal and spatial scales, and suggested that remediation of HF-FPW spills may need different strategies based on the various properties of their geological and physiochemical information.

Bioremediation approaches and tools for benzene remediation under anaerobic conditions

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Benzene, toluene, ethyl benzene, xylene (BTEX) and other aromatic hydrocarbons typically degrade faster under aerobic conditions than under anaerobic conditions. When hydrocarbon contaminated aquifers become anaerobic, aerobic bioremediation is not always feasible and anaerobic bioremediation approaches become favorable. To address this need, anaerobic cultures capable of complete degradation of BTEX have been developed at the University of Toronto (UofT). The cultures have been characterized and key organisms have been identified. SiREM, UofT and Federated Cooperatives Limited are currently engaged in a three-year project to advance anaerobic benzene degradation from the lab to the field, funded in part by Genome Canada and the Province of Ontario. The objectives of the project include scale-up of an anaerobic benzene culture to field volumes, demonstrating its effectiveness for bioaugmentation in treatability studies and in field tests. The culture is currently being assessed using microcosms constructed with materials from hydrocarbon-contaminated sites. Information generated will include inoculum density requirements, degradation rates and the range of geochemical conditions required for optimal performance of the culture, and will be used to design field trials. Molecular genetic tools to quantify and track key microbes and functional genes involved in benzene degradation are also being developed. These tools will allow assessment and monitoring of enhanced bioremediation applications.

Sublethal effects of hydrocarbons on blue mussel (*Mytilus edulis*) exposed to conventional and non-conventional crude oils spilled

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Canada possesses large reserves of non-conventional oil associated with bituminous sands. Despite the lack of knowledge of its toxicity on marine wildlife, Canada hopes to increase its export before 2030. Nevertheless, its transport in the form of diluted bitumen (dilbit) involves significant risks of spillage in the marine environment. In this case, Transport Canada could allow the use of chemical dispersant as an intervention measure, with the aim of quickly decreasing the concentration of hydrocarbons in an impacted environment. The low toxicity of the dispersing agents was demonstrated for numerous aquatic species, but the combination of dispersants and crude oil may represent a greater toxicity. Our tests on the blue mussel (*Mytilus edulis*) show strong mortality in summer conditions after 48 hours of exposure, destabilization of the lysosomal membrane, and critical effects on their DNA. Comparing chemical and physical dispersal with conventional and non-conventional oils, our results show that the dilbit scattered chemically is more toxic than conventional oil that is scattered chemically or physically.

Physiological and transgenerational effects of an acute exposure to one conventional and two unconventional crude oils under winter conditions with ice cover on blue mussel (*Mytilus edulis*)

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With the potential development of TransCanada's Energy East Pipeline project, the St. Lawrence River is foreseen as an optimal exit gate for unconventional Canadian oil in its diluted form (dilbit) to reach international markets. However, the St. Lawrence system undergoes harsh environmental conditions with very low temperatures and an important ice cover during winter. The St. Lawrence Estuary is also known as a highly diverse ecosystem providing great incomes for commercial fisheries and aquaculture. No research has yet been conducted on possible disastrous environmental consequences of a dilbit spill in the marine environment with a winter ice cover, making our capacity for tackling such an event highly deficient. To our knowledge, a long-term ecotoxicological monitoring of blue mussels (*Mytilus edulis*) and their offspring following acute exposure to unconventional oil has not yet been conducted in the field or in the laboratory. To evaluate the effects of a winter spillage on blue mussels, a small-scale oil spill was simulated in an outdoor 3,500-litre mesocosm filled with seawater from the St. Lawrence Estuary. Mussels (n=288) were exposed to one conventional crude oil sample (Heidrun from Norway) and two dilbits (Cold Lake Blend and Access Western Blend from Alberta, Canada) for seven

days under a persistent layer of ice and then kept alive for several months until the spawning season. During the post-exposure period in winter, oxygen ingestion and assimilation rates were monitored in concert with the measurement of cellular stress and gametogenesis development. At the end of winter, adults were maintained in a controlled environment reproducing spring conditions (gradual increase of temperature and appearance of an algal bloom) one month before spawning stimulation. The growth and survival of mussel larvae were monitored for nine days, corresponding to their period of dependence on energy reserves accumulated in the egg (mother effect). Bioaccumulation of dissolved hydrocarbons in adult mussels was detected after only three days of exposure, and after seven days, cellular and physiological responses were monitored. Despite an obvious depuration period of adults, significant negative effects were noted on the survival and development of larvae produced by genitors exposed to crude oil and dilbits a few months earlier. Both conventional and unconventional oil samples triggered a slower larval development when compared with the control treatment. At the end of the 9-day sampling period, the larvae produced by the exposed parents expressed an important delayed growth (5 days below control). A significantly higher larval mortality of mussels exposed to dilbit was observed at every sampling event. Overall, both immediate physiological effects and a delayed transgenerational toxicity were observed. Results also showed higher toxicity of the two diluted bitumen when compared to the third, conventional crude oil.

Water use and quality at Syncrude's mineable oil sands operation in northern Alberta, Canada

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The oil sands industry, located in northern Alberta, Canada, makes a significant contribution to Canada's economy. The deposits contain an estimated 1.7 trillion barrels of oil, of which 300 billion barrels are recoverable using current technology. Depending on the depth of the deposit, raw bitumen is produced by either in-situ well technology or mining. After mining, bitumen is recovered from the oil sand using a warm water extraction process, generating a tailings material composed of oil sands process-affected water (OSPW), sand, silt, clay and some unrecovered bitumen. The oil sands industry is very efficient at recycling water, which minimizes the amount of freshwater drawn from the Athabasca River. OSPW is stored on site for reuse in large settling basins, also known as tailings ponds. Since commercial bitumen production from the oil sands began in the 1960s, there has been no approved release of OSPW to the environment from the mineable oil sands region. Efficient water reuse must be balanced with water quality. With perpetual recycling, dissolved constituents present in the oil sands ore (including sodium, chloride, and sulfate, and organic compounds such as naphthenic acids) become concentrated in the OSPW. This presentation will review how Syncrude Canada Ltd. uses the available water resources, and will present OSPW quality relative to regulatory guidelines for the protection of human health and aquatic life. Two technologies to further improve OSPW quality to ensure safe release in compliance with government regulations will be outlined. The oil sands industry is required by law to return all affected lands and waters to state of

equivalent capability, including the safe and responsible return of water to the environment.

Water quality status of the first pit lake in the Athabasca Oil Sands Region

Carla Wytrykush[1], Tara Penner[1], Dallas Heisler[1], Warren Zubot[1]

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An oil sands pit lake is an area where overburden and oil sand has been removed for mining and, subsequently, filled with tailings materials to allow development into an acceptable closure feature within the reclamation landscape. These lakes may contain any combination of oil sands process-affected water (OSPW; from the bitumen extraction process), freshwater, fine fluid tailings, treated fluid tailings and other solids (for example, coarse tailings sand or overburden). The Base Mine Lake (BML) is the first commercial-scale demonstration of a pit lake in the mineable oil sands industry. It is located in the former original mine at Syncrude's Mildred Lake site. Following mining, the pit was filled with FFT (silt, clay, process-affected water and residual bitumen). Today, the FFT underlies a water layer composed of a mixture of OSPW and fresh water. The configuration of FFT overlain by a water cap in BML is defined as water capped tailings technology. Based on twenty-five years of previous research and modelling, the prediction is that with time, the water quality will improve and the FFT will remain sequestered, and ultimately, the lake will be a component of the closure landscape. Since lake commissioning in 2013, water quality in BML has improved, as demonstrated by the decreased concentrations of a number of key variables (e.g., major ions, nutrients, total suspended solids (TSS)/turbidity, polycyclic aromatic hydrocarbons and toxicity). As well, early in the lake's development, most water quality parameters are already present at concentrations less than provincial and federal guidelines for the protection of freshwater aquatic life. Based on standard acute bioassays, evidence to date indicates that the lake's surface water is no longer acutely toxic. The BML Surface Water Quality program is one component in a comprehensive research and monitoring program designed to further our understanding of trends in lake performance and the processes and mechanisms responsible for those trends. Knowledge gained from this program is valuable for the design, operation, and eventual certification of oil sands pit lakes across the region.

Genomic Approaches in Environmental Assessment and Monitoring: From Individual Species to Whole Ecosystems

Best approaches for DNA-based ecological assessment

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DNA-based tools can potentially enrich traditional environmental assessment and monitoring programs by adding another layer of information that can be used to detect target species, bioindicator assemblages, and whole communities. As technological and bioinformatics methods have improved, the capacity (number of field samples that can be processed), throughput (amount of sequence data that can be produced from these samples), and resolution (ability to detect individuals through to whole community assemblages) have also improved. Here we review some of the most commonly used DNA-based tools used for biodiversity analysis, their typical resolution, and how they can be used to answer ecological questions. After a review of the literature, we focus on amplicon sequencing, also called DNA metabarcoding, as a tool that can be readily incorporated into traditional environmental assessment and monitoring programs. We talk about the DNA markers that can be used to target particular taxonomic groups and review some of the most popular analysis tools and databases. We also present the results of our leave-one-out testing of a new CO1 training set that can be used with the naïve Bayesian classifier available from the Ribosomal Database Project to make rapid and accurate taxonomic assignments of arthropods and chordates while also providing a measure of confidence for assignments at each rank. We show how technological improvements are driving advances in bioinformatics and how studies can be scaled-up by adopting standardized methods, using automation, and multiplexing. We also specifically demonstrate the advantage of using a purpose-built taxonomic assignment tool over using the more general, but still widely used, top BLAST hit method to facilitate high throughput taxonomic assignments in a reasonable time frame and to reduce rates of false positive assignments.

The SHAPE of the future: Using high-throughput microscopy to create unialgal isolates for streamlined genomic library/phylogeny construction

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Environmental risk assessment of phytoplankton has often been limited to time-consuming destructive methodologies. The advent of DNA sequencing, such as DNA barcoding, increases the potential accuracy and speed with which community composition can be screened, but is likewise destructive and relies on the limited existing phytoplankton sequences/isolates to accurately anchor and resolve reconstructions of this multi-phyletic group. We have developed a reliable and novel Single-cell High-throughput Assessment of Phytoplankton from the Environment (SHAPE) microscopy technique that allows for the simultaneous analysis of phytoplankton community structure, individual and community growth rate, as well as the isolation of large numbers of unialgal isolates for further study. The creation of unialgal isolates identified by microscopy will greatly accelerate the generation of finer resolution genetic maps for use with genomics-based reconstructions, thus increasing the sensitivity of these techniques to changes in local populations, while at the same time creating a unialgal library for further biotechnological and research analysis.

High-Throughput Sequencing can provide core data for freshwater biomonitoring in Canada

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Despite a strong bias toward the use of chemistry-based approaches, pollution is essentially a biological phenomenon, and it is has been standard practice for many years to combine chemical assessment with observation of biological components in freshwater monitoring. However, processing constraints mean the number of biological samples is typically constrained, as well as limited to subsets of the overall community, usually macroinvertebrates, at a variably coarse taxonomic resolution. The advent of high-throughput sequencing approaches such as DNA metabarcoding provides an opportunity to overcome such bottlenecks, and Environment and Climate Change Canada, in collaboration with other federal departments, has initiated targeted studies across Canada to accelerate the incorporation of DNA metabarcoding methods for ecosystem monitoring. These include a comparison of metabarcoding with existing CABIN surveys in Atlantic Canada, comparison with standard monitoring in a southern Ontario watershed to evaluate potential disturbance from agricultural practices, and a tool to study oil sands impacts on wetlands in northern Alberta. We demonstrate how such monitoring systems can be optimized to (1) identify the magnitude of impairment at a location, and (2) inform users about the most likely causes. Nonetheless, implementing a monitoring program based on DNA metabarcoding still faces several challenges, and we discuss how these may be overcome. Finally, with the capacity to evaluate diverse biological communities on large spatial scales, we can bridge the historic divide between small-scale experimental biology

and large-scale ecosystem observation. We describe how inferences based on observations of compositional change in biological communities could be compared and validated with ecotoxicological experiments to support the development of diagnostic biomonitoring tools for use in ecological risk assessment.

DNA metabarcoding and morphological macroinvertebrate metrics reveal the same changes in boreal watersheds across an environmental gradient

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Cost-effective, ecologically relevant, sensitive, and standardized indicators are requisites of biomonitoring. DNA metabarcoding of macroinvertebrate communities is a potentially transformative biomonitoring technique that can reduce cost and time constraints while providing information-rich, high-resolution taxonomic data for the assessment of watershed condition. Here we assess the utility of DNA metabarcoding as a means of providing aquatic indicator data for evaluation of forested watershed condition across Canadian eastern boreal watersheds, subject to natural variation and low-intensity harvest management. We do this by comparing the similarity of DNA metabarcoding and morphologically derived macroinvertebrate metrics (i.e., richness, ratio of Ephemeroptera, Plecoptera and Trichoptera to Chironomid) and the ability of DNA metabarcoding and morphological metrics to detect key gradients in stream condition linked to forested watershed features. Our results show consistency between methods, where common DNA metabarcoding and morphological macroinvertebrate metrics are positively correlated and indicate the same key gradients in stream condition (i.e., dissolved oxygen, and dissolved organic carbon, total nitrogen and conductivity) linked to watershed size and shifts in forest composition across watersheds. Our study demonstrates the usefulness of macroinvertebrate DNA metabarcoding for future application in broad-scale biomonitoring of watershed condition across environmental gradients.

eDNA as an early detection tool for the potential spread of zebra mussels (*Dreissena polymorpha*) to Saskatchewan lakes

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Invasive zebra mussels (*Dreissena polymorpha*), native to Europe and introduced to the North American Great Lakes, have been fundamentally altering Canadian aquatic ecosystems. Zebra mussels cost Ontario $75-$91 million per year to clear from infrastructure such as docks and submerged pipes and to run inspection stations and invasive species education programs. These mussels drastically alter ecosystems through high filtration rates and mat-like colonies that outcompete native species and increase water clarity, allowing light to reach greater depths and thereby increasing vegetative growth. Following introduction in Ontario they have been steadily moving west, mainly via

pleasure boats and other watercraft transferred from lake to lake. As of 2013 they have become established in Lake Winnipeg, and most recently veligers have been found in Cedar Lake, MB, and in the state of Montana near the Saskatchewan and Alberta borders. Current detection methods include plankton netting for veligers or physical sighting of adult mussels. It is likely that colonies will be established before they are physically encountered, especially in large lakes, thus hindering eradication efforts. Environmental DNA (eDNA) techniques may prove to be a more sensitive detection method than plankton netting or physical sighting. Since DNA floats freely throughout a water body, it is possible to detect the presence of zebra mussels with a water sample from shore. This technique could drastically decrease monitoring costs and increase their effectiveness. Using sites of known infestation in Lake Winnipeg, we are validating the use of eDNA as a monitoring tool by coupling quantitative polymerase chain reaction (qPCR), gel electrophoresis and sequencing. This has proven the reproducibility and repeatability of the technique, but seasonal timing of sampling may affect levels of ambient eDNA because of differences in animal life-stage and activity. At 9 sites in Lake Winnipeg, mean total eDNA extracted was 30.3 ng/uL (range 12.8-77.8) in summer samples (August 2016), while in the fall (October 2016) average eDNA extracted at the same sites was 8.3 ng/uL (range 2.1-24.6), which declined further in the- winter (January 2017) to 6.1 ng/uL. Despite these seasonal differences in eDNA concentration, qPCR for summer and fall sampling produced similar results, with positive detections at 8 of 9 sites in the summer and 7 of 9 sites in the fall. The eDNA approach is being incorporated into Saskatchewan's Aquatic Invasive Species Strategy.

A point-of-need environmental DNA (eDNA) platform to facilitate the real-time monitoring of aquatic ecosystems

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The polymerase chain reaction (PCR)-based detection of free and intracellular genetic material in the aqueous environment, with no obvious biological source, has greatly increased our capacity to detect, monitor and manage vulnerable aquatic taxa and ecosystems. By processing water samples alone, practitioners are able to infer presence and occupancy of specific taxa, metacommunity structure, and increase the spatial and temporal scope of ecosystem studies with increased cost-effectiveness and minimal disturbance to sensitive habitats and species of interest. However, the current workflow paradigm to conduct eDNA surveys is susceptible to various sources of systematic error due to the spatial and temporal separation of individual pipeline stages, decreasing the accuracy of acquired data and lessening confidence in their interpretation. We present a point-of-need platform that unifies all previously separate stages of contemporaneous eDNA surveys, mitigating their sources of error, with the added benefits of 1) reducing data-acquisition time from potentially weeks to just a couple of hours; and 2) possessing the functionality to disseminate data, via the cloud, to geographically distant personnel for immediate analysis and enactment. We propose that our system will greatly benefit those who wish to expedite environmental protection rapid-response plans to protect species-at-

risk, keystone ecological health indicator species, and fragile aquatic ecosystems themselves.

Evaluating environmental DNA metabarcoding for assessment of benthic impacts of salmon farms in British Columbia

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Aquaculture production of marine finfish has been increasing rapidly in past decades and plays an important role in the Canadian economy and human food supply. The sustainable development of finfish aquaculture requires effective monitoring methods to assess potential impacts on the benthic environment. To date, traditional benthic monitoring methods typically include biochemical (pore-water sulfide, dissolved oxygen, and organic content) and biological attributes (macrofaunal diversity). Traditional measurement of benthic species diversity using morpho-taxonomic methods requires highly specialized expertise and is cost- and time-intensive, hence is not practicable for routine industry monitoring. Environmental DNA (eDNA) metabarcoding is a novel, cost-effective, and rapid method of assessing biodiversity in environmental samples, and thus has high potential to assist in the assessment of benthic impacts of fish farms. Here we present results from 84 sediment samples collected along organic enrichment gradients (based on porewater sulfide and organic content) in the dominant current direction at two fish farms in British Columbia, Canada. We employed an eDNA metabarcoding approach using three genetic markers targeting foraminifera, meiofauna, and eukaryotes, respectively, to identify eDNA-based bioindicators of benthic organic enrichment. To do this, we characterized benthic biodiversity using traditional morpho-taxonomy of benthic macrofauna and measured a variety of physico-chemical parameters of sediments for comparison with eDNA data. Results presented here are findings to date within a larger study that will analyze 252 sediment samples at six farms with different sediment types and organic contents in British Columbia to firmly establish relationships in this geographic region between eDNA data and more traditional morpho-taxonomic and physico-chemical methods. The goal is to evaluate the performance of eDNA metabarcoding for routine benthic monitoring at soft-bottomed finfish aquaculture sites. Findings will be used to inform the national standard for regulatory monitoring of benthic impacts in Canada.

Development and application of environmental DNA methods for survey and monitoring of freshwater species: Case study with white sturgeon (*Acipenser transmontanus*)

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Rapid and accurate assessment of biota present in aquatic habitats is a core activity of Stantec's Environmental Services. Traditional survey methods are labour intensive, can be expensive, can take a long time for results to be obtained, and are subject to uncertainty especially when species are rare, their distribution is poorly understood or unknown, or they have cryptic life stages. Environmental DNA (eDNA) can help us to confirm the presence of species and their identity in the habitat where they live, using water or sediment samples, without having to collect the target species. The purpose of the white sturgeon (*Acipenser transmontanus*) eDNA research project is to develop and refine freshwater eDNA field sampling and laboratory methods using white sturgeon as the test species. The methods developed will be used to collect information on the distribution of white sturgeon in BC (populations of which are listed as threatened and endangered). The project is addressing research questions around applying eDNA to commercial projects such as the sensitivity of the lab protocols for concentration and amplification of eDNA and the persistence of eDNA in water through time. The results of this work will be presented.

Plasma proteomics provides a retrospective glimpse into the recovery of fish health in Jackfish Bay, Lake Superior

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Jackfish Bay is located on the northern shore of Lake Superior, Ontario, Canada. Jackfish Bay was identified as an Area of Concern (AOC) by the International Joint Commission in 1987 under the Great Lakes Water Quality Agreement between Canada and the United States. Pulp and paper effluents from the pulp and paper mill in nearby Terrace Bay were identified as one of the major contributors to poor water quality, contamination of sediment, and fish and fish habitat destruction, along with impairment of populations of sediment-dwelling organisms in Jackfish Bay on Lake Superior. In May 2011, all remediation projects in Jackfish Bay were complete, and the bay was re-designated as an Area in Recovery. Since that time, long-term monitoring programs have been implemented to determine if remediation efforts were successful in restoring the pre-existing beneficial use impairments. To provide proof-of-concept, a retrospective study was undertaken to determine if plasma proteome analyses could inform and assist in the remediation and monitoring efforts at other AOCs in the Great Lakes. Plasma samples collected from 20 male and 20 female white sucker (*Catostomus commersonii*) on seven separate occasions between 2000-2013 from Jackfish Bay and a reference site (Mountain Bay) were located from storage at –80°C. Non-targeted proteome analysis was conducted on all samples using reverse-phase liquid chromatography coupled to a Quadrupole-Time-of-Flight mass

spectrometer. In total, over 6500 proteins were identified in both sexes from each sample site across all of the years. We then performed functional biological analysis using Qiagen's Ingenuity Pathway Analysis software on all detected proteins. Based upon the functional categorizations, it was apparent that both male and females at Jackfish Bay had decreased stress responses over time, which was supported by protein expression related to acute phase response, xenobiotic metabolism, and arylhydrocarbon receptor signaling. The results demonstrate that use of plasma proteomic profiling in environmental effects monitoring could benefit in the identification of areas in recovery and in de-listing areas of concern within the Great Lakes.

Chronic Testing in Aquatic Toxicology: Assessing Cumulative Sub-lethal Effects to Biota

Lifecycle exposures of fathead minnows (*Pimephales promelas*): Linking effects in lab fish to the real environment

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Fathead minnow lifecycle exposures have been conducted in our lab for several pharmaceutical compounds (ethinylestradiol, propranolol, venlafaxine) and their mixtures. We have also assessed complex effluents from pulp mills, municipal wastewaters, and oil sands sediments containing mixed polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs. The lifecycle exposures encompass all "sensitive windows" of a fish lifecycle, from hatching and larval growth to sexual development and maturation, and breeding and reproduction. They also can detect delayed effects, which are especially important for endocrine-disrupting substances (EDS). These delayed effects occur when the EDS affects one life stage (e.g., gonadal differentiation in larval-juvenile fish) but the effects are manifest later on (as an altered sex ratio, or as decreased breeding in mature adults). Although these lifecycle tests are difficult and costly, they provide a complete picture of long-term effects, especially if exposures include concentrations that are environmentally relevant.

Determination of acute and sub-chronic toxicity of emerging contaminants in early life stages of three Canadian fish species

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In recent years, emerging contaminants have gained notoriety due to their ubiquity in the aquatic environment as well as the lack of data available regarding their toxicity to wildlife and humans. Emerging chemicals (ECs) of concern such as flame retardants, pharmaceuticals, and nanoparticles primarily enter the aquatic environment as mixtures through municipal wastewater effluent (MWWE). MWWE is a complex mixture of industrial and domestic wastes that may be released into receiving waters with minimal treatment, which is not uncommon in rural Canada. Most data to date has been garnered using standard laboratory species, which may not be particularly relevant to northern species, considering the potential role of life history, trophic level, physiology, and climate on the species-specific toxicity of chemicals. Consequently, inaccurate extrapolation from standard laboratory species to species native to northern ecosystems is a cause for concern and represents a significant uncertainty factor in ecological risk assessment. In this study, *Oncorhynchus mykiss*, *Salvelinus namaycush*, and *Esox lucius* gametes were exposed to six waterborne concentrations of 17α-ethinylestradiol (EE2), fluoxetine (FLX), silver

nanoparticles (AgNP) and two concentrations of MWWE. Lowest doses were selected based on environmental relevance and were increased incrementally thereafter. Exposures were continuous flow-through and subsamples were collected at critical developmental stages to assess acute and sub-chronic toxicity of all test chemicals. Initial findings suggest that these three species vary significantly in their sensitivities towards the aforementioned ECs. Ongoing work aims to fully elucidate biochemical and histological anomalies associated with exposures to the six ECs and focuses on characterizing the effects of these ECs on native fish species in comparison to one another, as well as to standard laboratory fish models. Overall, this work will aid in the development of more appropriate environmental risk assessment strategies for native fishes to EC of concern.

Method improvement through the examination of growth and survival differences between fed and unfed nymphs of *Hexagenia* spp. in various sediments

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Recently, Watson-Leung *et al* (2015) published a letter to the editor extolling the virtues and value of using *Hexagenia* spp., an ecologically important burrowing mayfly, in sediment toxicity assessments. *Hexagenia* spp. have been routinely incorporated into Ontario Ministry of the Environment and Climate Change (MOECC) sediment quality assessments in the province of Ontario, Canada, for almost 25 years. In addition, *Hexagenia* spp. is incorporated into Environment Canada's (EC) BEAST (BEnthic Assessment of SedimenT) protocol and has been in use for over 20 years. There is a significant difference between these two methodologies however. The EC test protocol requires that the organisms be fed over the duration of the test while the MOECC method does not. In general, *Hexagenia* spp. has been shown to be intermediate in sensitivity, between the common sediment toxicity test species *Chironomus dilutus* (least sensitive) and *Hyalella azteca* (most sensitive). However, of 36 field-collected contaminated sediments in which all three species were tested in our laboratory, where significant impairment was seen in at least one species, *Hexagenia* spp. was ranked as the most sensitive species in 28% of the sediments. While studies have shown that *Hexagenia* spp. nymphs have to increase their feeding rate effort in low organic carbon (OC) sediments to meet their dietary needs, in our studies with field-collected sediments there was high variability in the relationship between OC in control and reference sediments and *Hexagenia* spp. wet weight at test termination (R^2=0.14, n=43). However, the relationship was significant (paired t-test p=0.0005). To assess the impact of feeding on the survival and growth of *Hexagenia* spp. in the 21-day sediment toxicity test, *Hexagenia* spp. were exposed to four sediments with varying OC levels. Half of the test replicates were fed weekly, and half were unfed. Feeding the *Hexagenia* spp. significantly affected the growth of the organisms, while survival rates were less impacted. The OC content of the sediments also influenced the survival and growth rates of the organisms, but not to the same extent as feeding. The implications of this methodology change will be discussed.

The Sins of the Father: Multi-generational effects of copper on the freshwater snail *Planorbella pilsbryi*

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Traditional toxicological testing can be limited to assessing the risk posed to an organism at only one life stage or portion of its life cycle, and tend to only assess the immediate, observable effects caused during exposure. This can be due to the time and resources required to maintain an exposure through a full life cycle, especially for larger or long-lived organisms. In order to better understand whether these partial-life span tests are representative of long term and population-scale effects of chronic contaminant exposure, we used the freshwater snail *Planorbella pilsbryi* to conduct a two-generation study on the effects of copper in a pulse exposure scenario. The test involved an initial 7-day exposure of the parent generation to sub-lethal Cu concentrations. The exposed parents were transferred to clean water to lay eggs and the eggs were allowed to hatch and develop. The resulting juvenile snails were then exposed to Cu for 72 hours and LC_{50}s were determined for each parent treatment group. By comparing the LC_{50}s, we determined that the level of parental Cu exposure influenced the Cu sensitivity of naïve first-generation juveniles. The Cu LC_{50} of naïve juveniles laid by non-exposed parents, 29.25 µg Cu·L^{-1} (95% CI 22.17-36.32 µg·L^{-1}), was significantly higher than the LC_{50} of naïve juveniles born to parents who were exposed to copper, 11.57 µg Cu·L^{-1} (95% CI 3.71-19.43 µg·L^{-1}). In addition to copper sensitivity, several other sub-lethal endpoints were monitored (such as egg-mass production, egg viability, and time to hatching) but none were significantly influenced by parental exposure to copper. These results suggest that despite minimal difference in parent reproduction and first-generation development, parental exposure caused increased sensitivity of the first naïve generation. This finding supports the continued investigation of multi-generational toxicity studies with snails for use in risk assessment.

Validating the use of chronic *Hyalella azteca* and *Chironomus dilutus* methods with six common sediment contaminants of concern

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In Ontario, contaminated sediments present a major impediment to the restoration of degraded aquatic conditions. Conventional short term sediment toxicity exposures can help to identify high levels of contamination, but may not be as effective at assessing the long-term impacts of more marginally contaminated sediments. Chronic and life-cycle standard laboratory assays are thus useful in estimation of potential chronic toxicity not always captured in conventional acute exposures. In this study, we assess the performance of the U.S. Environmental Protection Agency *Chironomus dilutus* life-cycle test and 42-day test for survival, growth and reproduction in *Hyalella azteca*, as well as the draft 42-day *H. azteca* method being developed by Environment and Climate Change Canada for six different contaminants of concern. Each method will be performed on field-collected sediments

contaminated with polychlorinated biphenyls (PCBs), perfluorooctane sulfonate (PFOS), arsenic, metals, polycyclic aromatic hydrocarbons (PAHs) or pesticides, and the uncertainty and resource requirements of each method will be compared. There is a need within the province of Ontario for standardized laboratory assays enabling estimation of sublethal endpoints, and these experiments will inform decisions about which methods meet the needs of our laboratory.

Systems Biology for Ecotoxicology: From Gene to Ecosystem

Whole embryo transcriptomic profiling of contaminant exposure in amphibians: Benefits and challenges for interpretation

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Embryonic and larval development of amphibians to tadpole through the various metamorphic stages fascinates all who have encountered frogs in the wild. For endocrinologists, amphibian embryos represent exquisitely sensitive systems in which to determine mechanisms of development. Embryonic models allow toxicologists to understand how development can be disrupted by environmental contaminants. Assessment of the full expression profile (the transcriptome) of early embryos gives insight into organogenesis, the role of hormones and liganded transcription factors, and subsequent developmental and metabolic processes. Nevertheless, application of such data to ecotoxicology remains challenging. Two cases and approaches will be presented. One example will illustrate the complexity of responses in an early embryo up to the first four days of development, revealing both the elegance and limitations of the approach. These data will include the transcriptomic response of *Silurana tropicalis* embryos to a naphthenic acid extract. The second example will be a targeted gene expression analysis by qPCR with an a priori hypothesis that naphthenic acids disrupt thyroid hormone-dependent gene expression (receptors, deiodinases) in *S. tropicalis*. While easier to interpret on first analysis because there are less data, the dominant (true) response may go undetected using targeted qPCR for a limited number of genes. Moreover, critically missing with whole-embryo analysis is the site of expression within the developing organism. As ecotoxicologists, we need to follow the lead of developmental biologists and take steps toward more precise expression analysis at the cell and tissue levels (i.e., *in situ* hybridization, laser microdissection coupled to expression analysis). This will increase the interpretive value of gene expression within the adverse outcome framework, and more directly contribute to risk assessments. Supported by NSERC and the North American Society for Comparative Endocrinology.

Genomics resources for true frogs and their application to ecotoxicology

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Ranids are the largest family of frogs in the world. Common examples include the wood frog (*Rana sylvaticus*), Northern leopard frog (*Lithobates pipiens*), and the North American bullfrog (*Lithobates catesbeiana*). Amphibians are regarded as sensitive sentinels of the environment. However there is a paucity of genomics resources available for application in

determining bioindicators linked to adverse outcome pathways. We have developed several new genomics tools including the first true frog genome from the North American bullfrog, *de novo* assembly transcriptome pipelines, and RNA-seq analysis pipelines that we have applied to identify potential thyroid hormone (TH) endocrine disruption in bullfrog tadpoles. We examine the responsiveness of multiple tissues to the thyroid hormones thyroxine and 3,3',5-triiodothyronine, and relate these responses to disruptions in development, behavior, and chemosensation. Novel bioindicators were then applied to evaluate the efficacy of removing TH-disrupting activity in municipal wastewater treated with secondary treatment. These studies demonstrate the utility of systems-level analysis for the linkage of molecular bioindicators to adverse outcomes. Acknowledgements: Supported by: NSERC Canada, Compute Canada, and Genome British Columbia.

Transgenerational effects of prenatal A1221 exposure on the reproductive axis in the rat

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Polychlorinated bisphenyls (PCBs) are organic compounds and known endocrine-disrupting chemicals. The PCB mixture A1221 disrupts neuroendocrine pathways involved in reproduction and behavior, which are linked at least partially to an estrogenic mode of action at the gene-expression level. The current study investigates the hypothesis that prenatal A1221 exposure not only disrupts hypothalamic gene expression profiles within an individual's lifespan, but results in alterations of these parameters in subsequent generations (F1-F3), which have been exposed to A1221 directly as fetus (F1), indirectly via exposure of F1 germ cell development (F2) or never exposed (F3). Given the estrogenic mode of action of A1221, we employed a study design containing exposure of pregnant female founder rats (F0) to A1221, a positive estrogenic control (EB), and a negative control (DMSO). The expression of targeted (n=48) genes involved in the neuroendocrine control of reproduction were quantified using TLDA cards in two hypothalamic nuclei, the arcuate nucleus (ARC) and the anteroventral periventricular nucleus (AVPV), known to respond to organizing effects of estrogen and A1221 in SD rats. Given the sexually dimorphic roles of both nuclei in the neuroendocrine regulation of the reproductive axis, gene expression profiles of the selected genes were analyzed in both sexes and within maternal and paternal lineages. Under the experimental design used, we found prenatal A1221 treatment in F0 females to affect gene expression in the AVPV more profoundly than in the ARC, with generally larger number of differentially expressed genes in F1 and F2 generations, respectively. Specifically, prenatal A1221 exposure decreased hormone (Avp, Gal) and hormone receptor transcripts (Ar, Esr1, Esr2, Mcr3) and increased expression of the arylhydorcarbon receptor (Ahr) in the AVPV of F1 males, an effect largely mimicked by EB treatment. Conversely, prenatal A1221 exposure resulted in increases in the expression several transcripts (Dnmt3a, Arntl, Esr1, Gria2, Tac2) in F2 males within the paternal lineage, an effect not mimicked by EB treatment. Prenatal exposure to A1221 and EB increased expression of Tgfa in F3 generation males within the maternal linage. In the ARC, prenatal A1221 treatment resulted in gene expression changes in the F3 generation only:

Maternal lineage females and males exhibited increased expression of Crhr1 and decreased expression of Oxtr, respectively, both mimicked by EB treatment. Our study also identified estrogen-dependent changes in gene expressions across generations: In the arcuate nucleus, EB treatment significantly decreased expression of genes involved in stress and growth axis in F1 males (Avp, Pomc, Ghrh, Igf1) and increased expression of several genes coding for hormones and hormone receptors (Avp, Oxt, Pdyn, Ar), neuropeptides (Crh2), glutamate neurotransmission-related transcripts (Grin1, Grin2d) and circadian rhythm (Clock, Per1, Dbp) in paternal lineage F3 generation males. In the AVPV of F3 males within the maternal lineage, EB treatment increased expression of transcripts (Dnmt3a, Srd5a1, Tgfb, Thra, Thrb) and decreased expression of glutamate receptor component Grin2d. Together, the results show that prenatal A1221 exposure differentially regulates the expression of several neuroendocrine genes across generations via both estrogen-dependent and independent mechanisms, and that male offspring appear to be more vulnerable to both A1221 and estrogenic effects on neuroendocrine gene expression across generations and lineages.

21st-century environmental risk assessment and 20th-century science: The regulatory challenge

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Formulating regulatory exposures limits for environmental media on a chemical-by-chemical basis, although unsophisticated, has been successful for decades. Changes in regulatory policy emphasis—including reducing test animal usage, addressing huge numbers of chemicals, and considering multiple media and species—requires an efficient regulatory paradigm and much more testing data. *In vitro* testing, plus linking changes in biochemical processes through levels of biological organization (LBO) to whole-organism adverse-apical effects (adverse outcome pathways) is a major thrust of 21st-century new policy initiatives. However, such advanced approaches must address a myriad of issues, both new ones related to the novel 21st-century approaches, and old ones remaining from 20th-century policy paradigms and associated scientific methods. Aspects of the science and policy challenges are compared and contrasted in four areas: (1) Dose-response. Although acknowledged as important, the influences of many toxicity-modifying factors are poorly understood; e.g., bioavailability. It is an important confounding influence, affecting the dose surrogate chain from external exposure through whole organism then organism subcompartments to the subcellular site(s) of toxic action. Additionally, solvents used in most *in vitro* testing protocols have a substantial, unquantified bioavailability-based toxicity modifying influence. (2) Mode/mechanism of toxic action. There are many schemes but no single, phylogenically-relevant (plants and bacteria through fish to birds and mammals), broadly applicable, widely accepted scheme for identification, classification, and relative potency estimation for either modes/mechanisms of toxic action or adverse outcome pathways. (3) Levels of Biological Organization (LBO). In this concept, newly emergent properties that emerge from translevel integration of lower-level properties are not necessarily predictable. Thus, it is difficult to establish single, direct, deterministic

causal links through multiple LBO levels. Effects observable at upper LBO levels may be induced by multiple unrelated initiating events occurring at lower LBO. Also, *in vivo* toxicology is a "middle-out" approach with an inordinate focus on tests whose outcomes are for narrowly defined statistical populations poorly linked to higher LBO. (4) Data quality/validity/relevance. The largely neglected issue of primary validity and task-specific relevance for both *in vivo* and *in vitro* toxicity testing data. Use of accepted testing methods is not a guarantee of validity or relevance and weight-of-evidence schemes rarely address either adequately.

Unifying theories about toxicological and generalized stress responses across different types of biological communities

Doug Bright[1]

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Marine benthic or pelagic ecologists (freshwater ecologists with a varied focus on wetland, lotic or lentic systems), vegetation ecologists, and microbial ecologists have each sought to understand the normal or "healthy" state or function as well abnormal characteristics of biological communities within their respective domains. An environmental toxicologist with a marine benthic focus will likely understand and make interpretations based on an implicit or explicit understanding of K-dominance curves, trophic guilds, or Pearson and Rosenberg's model describing peak of opportunists. A scientist with a longstanding interest in contaminant- or stressor-responses of stream invertebrates will have a good understanding the scientific knowledge that supports metrics such as the Ephemeroptera Plecoptera Trichoptera (EPT) Index, or the Karr *et al* Index of Biological Integrity (IBI). A forest ecologist will be familiar with the pros and cons of forest health interpretations based on the Normalized Difference Vegetation Index (NVDI) derived from satellite imagery. The recent published literature in ecology or environmental toxicology, however, contains remarkably few examples of crossover theories and observations that seek to understand community-level effects from a broader and unified theory. This presentation provides a quick (and very incomplete) survey of some of the interpretative models for community responses to contaminants and stressors that have become popularized among environmental toxicologists in the different ecological domains. The overarching similarities in contemporary ecotoxicological theories are then explored, with some parallels drawn to Selye's model of stress and health impairment in humans. Some of the overarching conceptual frameworks discussed include species sensitivity distributions (SSDs), bioenergetics, and system cybernetics.

Environmental Drivers of Contaminant Variability in Wildlife

Amphibian breeding phenology in relation to pesticide exposure in Ontario

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Amphibians often are exposed to pesticides while breeding in agricultural landscapes, and the timing of breeding may affect their exposure. The phenology of amphibian lifestage varies among frog species, as do their calling and breeding behaviours. Our objective was to identify how breeding phenology alters exposure to pesticides of breeding adults or their egg or tadpole lifestages post-breeding. Using frog calling intensities from intensive acoustic surveys for eight frog species across Ontario, we estimated the behavioural phenologies and onset of breeding for each species. Data from Environment and Climate Change Canada's surveys of pesticides in surface water throughout Ontario were used to quantify temporal changes in concentrations of pesticides in surface waters. Seasonal changes in pesticides were characterized by both individual pesticides as well as mixtures. For earlier breeders, such as leopard frogs and spring peepers but particularly wood frogs and chorus frogs, the pesticide concentrations in surface waters generally were higher post breeding. Consequently, although estimated only semi-quantitatively, exposure would generally be highest during egg or tadpole lifestages for earlier breeders. Conversely, pesticide exposure in late breeders was similar for adults compared to eggs or tadpoles. Data relating pesticide exposure with amphibian lifestage and breeding behaviour is important for assessing risk, and for designing appropriate exposure regimes for toxicological studies.

Contaminants in Arctic Caribou and Reindeer

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Caribou and reindeer (*Rangifer tarandus* spp.) are an important food resource around the Arctic and are an integral part of traditional culture for many northern peoples. However, there is growing concern for Arctic food security. As part of Canada's Northern Contaminants Program, we analyzed kidney, liver and muscle of animals from 27 caribou herds (9 Barren-ground, 1 Peary, 15 Northern Mountain Woodland, the George River and the Dolphin and Union) from northern and Arctic Canada for a range of elements. These data were compared with data from two Barren-ground caribou herds in Greenland, two reindeer herds in Sweden and one reindeer herd in the Russian Federation. We used principal components analysis and cluster analysis to investigate covariation among elements and multivariate element patterns across the herds. These assessments revealed no consistent patterns; therefore we applied a more targeted modeling approach to

evaluate the relative influence of different predictors (herd, year, season, age, sex) of variability for each element within an information-theoretic framework. Preliminary results show strong annual and inter-herd variation in all elements, with specific patterns varying across elements. Environmental (season) and biological (age, sex) drivers also vary in the extent to which they influence element concentrations. These results highlight the complex patterns in contaminant profiles for a key Arctic species and represent a critical first step in understanding the implications of changing environmental conditions for individuals and populations of this species and other northern wildlife.

Changes in spring phenology and its influence on mercury levels in Arctic-breeding shorebirds

Katelyn Luff[1], Kirsty Gurney[2]

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Mercury is disproportionately deposited in the Arctic ecosystem through long-distance atmospheric transport, and can accumulate in wetlands during spring thaw. In addition, Arctic ecosystems are rapidly changing and exhibiting earlier spring onset in response to climatic variability. Some studies have indicated Arctic shorebirds may be responding to earlier spring conditions by shifting their nesting phenology, but whether earlier nest initiation will influence contaminant levels in these species is unknown. The purpose of this research is to determine how climate and life-history strategies interact to influence contaminant levels found in select Arctic shorebird species. Sample collection will occur at three sites in the central Canadian Arctic, utilizing the most locally abundant nesting shorebird species at each site. We will test predictions related to the hypothesis that early nesters are exposed to higher contaminant levels than later nesting cohorts, due to temporal overlap during nest initiation and mercury-deposition events in early spring. Preliminary results collected during the 2016 field season will be discussed. This research will contribute to baseline contaminant monitoring in Arctic biota and to an improved understanding of interactions between life-history strategies and environmental variables in Arctic-breeding species.

Using a tiered experimental approach to assess the effects of diquat, an aquatic herbicide, on non-target biota

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Invasive aquatic plants are an increasing threat to the health of aquatic ecosystems. As Canadian aquatic ecosystems face the stressors of invasive aquatic plants, there is likely to be an increased demand for chemical control options to manage aquatic invasive plants. Currently, data on the effects of chronic, ecologically relevant concentrations of current-use aquatic herbicides on non-target biota are limited. The objective of our research was to

examine the impacts of direct application of herbicides to water for control of aquatic invasive plants. The research focused on the active ingredient diquat, the only currently registered herbicide in Canada approved for control of submerged plant species. We used a commercial formulation according to label directions to represent an ecologically realistic application of the herbicide. We integrated different experimental designs that represent increasing environmental relevance: greenhouse and laboratory exposures and outdoor mesocosm exposures. This tiered approach provided toxicity information for single-species exposures under controlled environmental conditions, and the mesocosm exposures provided more realistic ecosystem-related toxicity information but with lower variability and increased replication compared to field studies. We focused on biota that are representative of and important to Canadian aquatic ecosystems. Several species were added to the mesocosms, including invasive and native plants, an amphipod species, an amphibian species and algal communities obtained from nearby waterbodies. The mesocosms were maintained for six weeks to measure chronic, lethal and non-lethal endpoints (e.g., life-history traits and physiological biomarkers (i.e., oxidative stress)). Our single-species controlled exposures were conducted separately on the aquatic plant and amphibian species assessed in the mesocosms. The combined use of controlled greenhouse/laboratory and semi-natural mesocosm measurements allowed us to compare results between study systems to better understand variability in exposure and effects of diquat to non-target biota. Overall, we had good agreement in our results from the different study systems and we used subsequent greenhouse exposures to establish the minimal effective dose for some plant species (i.e., ≤ 0.4% of the label rate (4.7 $\mu g \cdot L^{-1}$)). Results from our combined approach suggest that a lower label rate, at least in waterbodies with low turbidity, would provide control of target species while reducing effects on non-target biota.

Role of δ34S in the variation of mercury levels in thick-billed murre (*Uria lomvia*) prey

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Mercury (Hg) biotransformation and biomagnification are processes that affect both the environment and humans in the Arctic. Seabirds are often used to monitor levels of Hg in the ocean because they integrate exposure/signals over large areas and bring that signal back to a central location (colony) where it can be easily sampled. To interpret variation in Hg in seabirds, it is important to understand how Hg biomagnifies through the food web. Stable isotope ratios have been widely used to explain Hg accumulation in organisms. Due to the central role of sulfate-reducing bacteria in the production of methylmercury (MeHg), the most toxic form of Hg, δ34S has been used to understand variation in Hg concentrations among otherwise similar organisms. In this study, we evaluate the use of δ34S, along with that of two commonly used stable isotope signatures (δ15N and δ13C), for the determination of possible sources of Hg in various Arctic organisms. Hg and the three isotope ratios were measured for 55 individuals of 15 species of fish and invertebrates that are common prey for thick-billed murres (*Uria lomvia*), an Arctic seabird widely studied as a monitor species for contaminants. Multiple regressions showed that Hg is correlated with

δ34S when data were grouped by species (R^2=0.72) and with a combination of δ15N and δ34S when data were not grouped (R^2=0.82; mixed model, with species as a random effect). Our results demonstrate the usefulness of δ34S to account for variation in Hg among marine animals and to study the possible underlying effects that MeHg production may have on Hg pathways in Arctic ecosystems.

Assessing variation in egg contaminant levels in bird species from a low Arctic site

Kirsty Gurney[1], Ray Alisauskas[1], Dana Kellett[1], Katelyn Luff[2]

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Environmental contaminants, including trace elements and organic compounds that are transported to Canada's Arctic across long distances, pose a risk to the northern environment, and some migratory birds that breed in the Arctic have contaminant burdens that exceed established thresholds for biological effects. Contaminant profiles, however, vary across species for reasons that are not entirely clear. Further, because avian fauna are highly mobile and use multiple habitats throughout the year, contaminants identified in Arctic-breeding birds may be a consequence of exposure outside of the Arctic, but sources of contaminant exposure for these birds are not well understood. We aim to evaluate factors—such as dietary preference, migration strategy, and wintering ground provenance—that might influence contaminant concentration in Arctic avifauna, by measuring contaminants in the eggs of migratory birds with a range of annual distributions and life-history strategies. Preliminary results suggest that contaminants measured in eggs are linked to local food webs, and our ongoing studies in a low-Arctic site will provide new insights about variation in contaminant exposure for Arctic wildlife and will help inform policies and conservation initiatives to protect wildlife and ecosystem health in Canada's North.

Behavioural Toxicology

How oil sands process-affected water affects feeding in *Daphnia magna*

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Oil sands process-affected water (OSPW), a byproduct of the extraction of bitumen in the surface-mining oil sands industry, is currently stored in on-site tailings ponds. This study explored the effects of OSPW on feeding behaviour of *Daphnia magna*. To determine the mechanisms of effect on feeding rate, the present study also investigated the role of OSPW's principal components, dissolved component (DC) and suspended particulate matter (SPM). Feeding rates of one-week-old *D. magna* were assessed following 24 hours of exposure to each OSPW at 20, 10, 5, 2.5, and 1.25% concentration. Based on the regression inhibitory curve, IC50 and IC75 of OSPW on feeding rate of *D. magna* was calculated at 5.3 and 10.1% OSPW. To establish mechanisms of effect, three groups of *D. magna* were exposed to DC, SPM and total components of 10% OSPW for 24 hours. Digestive enzyme activity, beating of thoracic limbs, mandible rolling or post-abdominal rejections, peristaltic activity and hind gut content of exposed daphnids was investigated. There was no change in digestive enzymes trypsin or amylase except for the daphnids exposed to total OSPW, which showed reduced trypsin activity. Mandible rolling or post-abdominal rejections, which are indicators of feeding rate and food palatability, were not affected by any treatment. Beating of thoracic limbs, which provides water flow toward the feeding groove, was reduced in SPM and total OSPW treatments. Peristaltic activity was reduced in DC treatment, resulting in reduced digestion time. All treatments showed an increase in the number of intact algae cells in the hindgut and excreted material of *D. magna*. These results suggest that both DC and SPM inhibit feeding behaviour of *D. magna* by impairing the actions of the digestive system but not by reducing the ingestion rate.

The impacts of municipal wastewater on fish behaviour, physiology and community structure

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When the treated effluent from a wastewater treatment plant is returned to the environment it often alters water quality and is a source of anthropogenic pollutants. To better understand the impacts of wastewater effluent on aquatic organisms, we caged two different fish species—native bluegill sunfish (*Lepomis macrochirus*) and invasive round goby (*Neogobius melanostomus*)—for three weeks at different distances from wastewater effluent outflow. We also characterized the fish community composition along this wastewater effluent exposure gradient. Fish (of both species) caged close to the outfall had lower survival compared to fish caged far away. However, regardless of how close to effluent they were caged, the survivors performed similarly in aggression, startle-response, and dispersal behavioural tests. The distance to the wastewater treatment effluent outflow

did not influence any physiological measure in the invasive round goby; in contrast, native bluegill sunfish caged near the outflow showed an increase in oxygen consumption and metabolism, an expansion of gill surface, and a decrease in blood-O_2 affinity. These results suggest that there is a metabolic cost of living near effluent discharge. The distance from effluent outflow appeared to influence the wild fish community. Despite having low oxygen and high contaminant levels, many fish inhabited areas within the effluent plume and appeared to use it as a thermal refuge. The sites of intermediate distance from effluent outflow contained the highest diversity and abundance of native species. Our results highlight the importance of assessing animal responses to real-world complex contaminant mixtures found in municipal wastewater effluent and doing so at multiple levels of biological organization.

Selenium and C-starts: The impacts on and potential recovery of fathead minnow escape behaviours

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This study investigated the effects of dietary selenium on the escape behaviours of the fathead minnow (*Pimephales promelas*). Minnows rely on a fast-start escape response to evade the strike of a perceived threat. C-starts, the typical flight response for minnows, are a reflex where the fish bends its body into a "C" shape, allowing it to burst away from a potential predator. When selenium-rich effluent is released into a body of water from a mine or other anthropogenic development, serious impacts on fish health and community structure can be observed. Selenium exposure in minnows has been shown to alter their motor and cognitive function. A fish's ability to survive in the wild depends on an appropriate response to a predator. Minnows were administered either a control diet or one of two selenomethionine-spiked diets (15 $mg \cdot kg^{-1}$ or 30 $mg \cdot kg^{-1}$ nominal) for 35 or 70 days. Individual fish were placed in an arena where their response to a weight drop was recorded by high-speed camera. Velocity, acceleration, distance travelled, latency to respond, escape angle and body bend angle were calculated for each fish. These response variables were also tracked weekly for a period of 35 days post-exposure to determine if the impacts of a selenium-spiked diet were reversible. The impacts on the visual and chemosensory-based responses to predation threats were also carried out to achieve a holistic understanding of the impacts of dietary selenium on the antipredator behaviours of small freshwater fish. Altered escape performance in prey can influence established predator-prey equilibrium, and ultimately lead to a shift in the community dynamics of the system.

The effect of copper nanoparticles on olfaction and olfactory-mediated behaviour in rainbow trout (*Oncorhynchus mykiss*)

Parastoo Razmara[1], Effat Mohaddes[1], Ebi Lari[1], Greg Pyle[1]

[1]*University of Lethbridge*

Fish rely on olfaction for their survival, growth, and reproduction. Metal contaminants (e.g., copper) can impair fish olfaction. Although the copper ion (Cu^{2+}) has drawn the most attention in olfactory toxicology, the impact of copper nanoparticles (CuNPs) on fish olfactory systems has not been well determined. The objective of this study was to investigate time-dependent effects of CuNPs and Cu^{2+} on olfactory acuity and olfactory-mediated behaviours of rainbow trout. To establish CuNPs or Cu^{2+} induced olfactory-impairment thresholds, inhibitory concentration (IC) curves were determined. Fish were exposed to a geometric dilution series of CuNPs or Cu^{2+} for 24 hours, and fish olfactory acuity was measured using electro-olfactography (EOG). Afterwards, fish were exposed to CuNPs or Cu^{2+} at concentrations known to impair olfaction by 50% (322 and 6.8 $\mu g \cdot L^{-1}$ for CuNPs and Cu^{2+} respectively) for a 24-hour or 96-hour exposure period. The response of fish to a social cue (taurocholic acid) was studied using EOG and choice maze. Results of EOG revealed that while a 96-hour exposure to CuNPs caused a significantly greater impairment of fish olfactory function relative to 24 hours, fish olfactory acuity partially recovered after 96 hours under continuous Cu^{2+} exposure. Behavioural responses of rainbow trout to alarm cue supported the results of neurophysiological experiments. Although fish exposed to control water or Cu^{2+} for 96 hours had an avoidance response to alarm cue, those exposed to the CuNPs did not respond to the alarm cue. Over the same exposure periods, CuNPs caused progressive deterioration of olfactory acuity, whereas at least a partial olfactory recovery was documented for continuous Cu^{2+} exposure. The present study strongly suggests the mechanism of toxicity caused by CuNPs is distinct from that of Cu^{2+}.

Altered behaviour: The effect of early venlafaxine exposure on zebrafish (*Danio rerio*) development

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The rise of antidepressant prescriptions worldwide has resulted in an increased release of neuroactive compounds into the aquatic environment. Venlafaxine is one of the most highly prescribed antidepressants worldwide, resulting in levels of the drug in the $\mu g \cdot L^{-1}$ range in our waterways. As antidepressants are often designed to function at low doses, there is growing concern over how these drugs will affect non-target species that often share conserved pathways with humans. Venlafaxine acts as a serotonin and norepinephrine reuptake inhibitor in humans, suggesting that neuroamine-mediated pathways may be impacted during neural development in non-target animals. We assessed developmental changes in zebrafish (*Danio rerio*) in response to venlafaxine exposure during embryogenesis. Zebrafish embryos were microinjected with venlafaxine at the 1-4 cell stage, and assessed for disruptions in neural development and larval behavior. Embryo

exposure to this drug causes developmental defects, disrupts neurogenesis, and alters the behavioral response. The possibility of developing high throughput behavioral assays as a rapid screening tool for neurotoxicity will be discussed. Overall, exposure to environmental levels of venlafaxine disrupts early development and escape behavior in zebrafish. This study was supported by the Natural Sciences and Engineering Research Council of Canada Discovery and Strategic Grants to MMV.

Transgenerational effects of carbamazepine exposure on zebrafish (*Danio rerio*) behaviour

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The application of behaviour as an ecologically important indicator of sublethal toxicity has significantly grown in the last decade. Behavioural endpoints provide the advantages of non-invasiveness, high sensitivity and integrated outcome of underlying physiological and biochemical changes. We examined changes in parental male zebrafish (*Danio rerio*) courtship and aggressive behaviour and related those behavioural changes to levels of 11-ketotestosterone, after chronic treatment with 10 $\mu g \cdot L^{-1}$ carbamazepine (CBZ). CBZ is a frequently detected neuroactive pharmaceutical compound and possible anti-androgen. Offspring were reared in clean water and assessments of both behaviours and hormone levels continued in the F1-F3 offspring. CBZ exposure significantly altered male courtship, lowered aggression, and reduced 11-ketotestosterone levels in plasma, whole body and testes of the parents. The same effects were observed in the unexposed offspring up to the F3 generation. Interestingly, paternal exposure of carbamazepine was most important for offspring effects. Our research supports the employment of behaviour as a useful tool to study endocrine disruption and transgenerational effects and shows that complex behaviours can be altered by parental exposures to pharmaceuticals.

Environmentally relevant concentrations of diltiazem affect olfactory-mediated behaviour of fathead minnows (*Pimephales promelas*)

Andrew Rempel[1], Abraham Fischer[1], Malika Ganguli[1], Bryan Brooks[2], Greg Pyle[3], Chris Metcalfe[1], William A. Dew[1]

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Diltiazem is a calcium channel blocker used primarily for the treatment of angina and hypertension. The concentration of diltiazem in a waterway can be in excess of 100 $ng \cdot L^{-1}$ due to release via wastewater treatment plants. As diltiazem is a calcium channel blocker, it is possible that it will affect calcium channel function in a wide variety of tissues. One such tissue is the olfactory epithelium, which is in constant contact with the external environment and relies on calcium channels to function. Olfaction mediates a wide variety of essential behaviours including finding food and detecting predation/predators. Therefore, fish in environments containing diltiazem may have impairment of olfactory-related behaviours due to impairment of calcium channels in the olfactory epithelium. To

test if olfactory-related behaviours could be affected by diltiazem, we exposed fathead minnows (*Pimephales promelas*) to 0, 2, or 20 ng·L^{-1} nominal treatment levels of diltiazem for 10 minutes or 96 hours and measured the response of fathead minnows to olfactory cues. The results demonstrate that at either time point, 2 ng·L^{-1} diltiazem has no effect on antipredator response in fathead minnows; however, exposure to 20 ng·L^{-1} diltiazem completely blocks any response to antipredator cues. This study demonstrates that diltiazem at or below concentrations found in waterways receiving effluent from wastewater treatment plants can have a profound effect on olfactory-mediated behaviours in fish. Future studies are needed to understand the relationships among environmental exposure, internal dose, and olfactory function and behaviour in various fish species.

Endocrine Disrupting Chemicals: From Molecular Modes of Action to Physiological Consequences

Effects of endocrine disruptors on reproduction of aquatic organisms: Systematic knowledge review

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[1]Université TÉLUQ, [2]Université du Québec à Rimouski

Exposure to synthetic and natural chemicals is almost inevitable in our daily lives. Some of them raise concerns about the health effects of endocrine disruptors (ED). Our project aims to identify the data of the last 10 years on exposure to PE (polybrominated diphenyl ethers (PBDE), alkylphenols, bisphenol A (BPA), parabens, phthalates, perfluorinated compounds) in order to assess the validity of the presumptive effect of published data on the effects of these EDs on the reproductive health of aquatic organisms: changes in sex ratio, congenital malformations, sperm quality disruption, sexual inversion, alteration of plasma levels vitellogenin as well as anomalies of gonad development. Thus, after identifying and evaluating the quality of the 378 papers, these were subdivided according to the PE, the organism tested, the exposure window and the measured effect. When sufficient data were available, a meta-analysis was performed to compare the effects measured with exposure. The presentation will show how this systematic synthesis of knowledge sheds light on the methodological biases that sometimes lead to important differences in the results obtained.

Swim bladder inflation failure of larval Japanese medaka (*Oryzias latipes*): A comparison of pharmaceutical and signaling pathway inhibitor effects on gene expression

Zacharias Pandelides[1], Matthew Overturf[1], Erin Ussery[1], John Guchardi[1], Douglas Holdway[1]

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Inadequate swim bladder inflation can have serious long-term effects on fish populations. Many environmental contaminants can inhibit swim bladder inflation of larval fish. Previous studies in our laboratory have determined that embryonic exposure to pharmaceuticals including 17α-ethinylestradiol, levonorgestrel, and diclofenac, both alone and in mixtures, can impair swim bladder inflation of Japanese medaka (*Oryzias latipes*). This study established that embryonic exposure to 1µM IWR-1, a Wnt signaling inhibitor, as well as 5µM cyclopamine, a Hedgehog (Hh) signaling inhibitor, resulted in >95% inhibition of swim bladder inflation of Japanese medaka larvae. The effects of these inhibitors on the expression of medaka genes related to the Hedgehog (Hh) and Wnt pathways, as well as genes involved in the formation of the three cell layers that make up the swim bladder (epithelial, mesenchyme, and outer mesothelium) were determined at 80, 96, 101, 144, 180 and 216 hours post fertilization. Following this experiment, the expression of these key genes was measured following exposure to 17α-ethinylestradiol,

levonorgestrel, and diclofenac. We established that these pharmaceutical compounds significantly inhibited the expression of genes related to the formation of the three layers of the swim bladder, with levonorgestrel causing the most significant effects. Two of the pharmaceutical compounds, 17α-ethinylestradiol and diclofenac, were also found to disrupt Pbx1b, a gene involved in surfactant production.

Acute and chronic effects of multiple stressors on microRNA in zebrafish

Heather Ikert[1], Paul Craig[1]

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As the human population continues to grow, increased anthropogenic stress is placed on the aquatic environment. Pharmaceuticals such as venlafaxine (VFX), a heavily prescribed and readily detectable antidepressant, are found downstream of wastewater treatment plants. As a result of climate change, increased surface water temperatures and decreased dissolved oxygen levels have been observed. In this study, a multi-stressor approach was used to determine the cumulative, sublethal effects of the aforementioned stressors on microRNA (miRNA) in adult zebrafish (*Danio rerio*). MiRNA are small, conserved, non-coding RNA which act by decreasing mRNA translation. This impacts the functional responses of downstream targets and is a method of environmental and epigenetic regulation of phenotypic response. Adult zebrafish were exposed to control (27°C, 100% dissolved oxygen, 0 $\mu g \cdot L^{-1}$ VFX) or stressed (32°C, 50% dissolved oxygen, 1.0 $\mu g \cdot L^{-1}$ VFX) conditions for 24 hours or 21 days. RNA was extracted from liver, gonad, and muscle tissue and RT-qPCR was performed on specific miRNA related to proteins that respond to hypoxia, heat stress, or contaminants. This comparison between lengths of exposure showed differences between initial and acclimated responses. Due to the conserved nature of miRNA, this will improve our understanding of the effects that environmental stressors have on epigenetic regulation.

Comparing metabolomic responses in *Oryzias latipes* to environmentally relevant concentrations of the type-2 diabetes drug Metformin and its metabolite, guanylurea

Erin Ussery[1], Zacharias Pandelides[1], John Guchardi[1], Dario Bonetta[1], Douglas Holdway[1]

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Human pharmaceutical wastes threaten many natural processes of non-target aquatic organisms, through the introduction of such contaminants into the environment. One of the most prevalent contaminants is the type 2 diabetes drug metformin. Metformin has been measured in surface waters and wastewater effluents in the ng-$\mu g \cdot L^{-1}$ concentration range. Our recent research shows that Japanese medaka (*Oryzias latipes*) exposed to environmentally relevant concentrations of metformin from embryo through 28 days post hatch have a significant decrease in length (mm) and weight (mg) of both males and females when compared to control fish. We demonstrated using radiolabelled metformin that about 1% of the waterborne concentration of metformin is found inside both embryo and larval medaka after exposure windows ranging from 24 hours to 7 days. As >90% of

metformin is metabolized into guanylurea during the wastewater treatment process, its metabolite guanylurea is also a major concern. Guanylurea is found in the environment in higher concentrations than metformin, usually in the $\mu g \cdot L^{-1}$ concentration range in surface waters. Metabolomics is emerging as an efficient method for understanding sub-lethal effects on organisms by assisting in determining the biochemical mode of action in response to exposure to a particular contaminant. This study compares the effects of metformin and its metabolite guanylurea on fish early-life stages, in Japanese medaka, including length, weight, and changes to the fish's metabolome.

Complicating a stressful event: MicroRNA and DNA methylation regulates responses associated with BaP exposure in rainbow trout

Paul Craig[1], Ivan Cadonic[1], Christopher Kuc[1], Leslie Bragg[1], Mark Servos[1]

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Benzo[*a*]pyrene (BaP), a polycyclic aromatic hydrocarbon formed by the incomplete combustion of organic material found ubiquitously in the aquatic environment, is an established carcinogen and has been linked to multigenerational effects through alterations in DNA methylation patterns. The initial objective of this study was to examine the regulation of DNA methylation following acute (24-hour) and prolonged (14-day) exposure to low ($1 ng \cdot L^{-1}$) and high ($10 ng \cdot L^{-1}$) BaP in rainbow trout (*Oncorhynchus mykiss*). However, with the recent release of the rainbow trout genome, we were able to conduct a more detailed analysis regarding the regulation of the enzymes involved in DNA methylation, DNA methyltransferases (DNMTs). Specifically, we obtained the 3' untranslated region (3'UTR) of DNMT1 and DNMT3a, which are regulators of maintenance and *de novo* DNA methylation, respectively. Bioinformatic approaches were used to identify candidate microRNA (miRNA) that potentially binds to the DNMT1 and 3a 3'UTR and would likely inhibit the transcript abundance. Results indicated a significant decrease in the level of global methylation in both liver and muscle, with an associated decrease in total DNA methyltransferase activity and DNMT3a transcript abundance. Further, there was a significant increase in one specific candidate miRNA (miR29a) in both liver and muscle that was predicted to bind to DNMT3a. Moreover, we have demonstrated that miR29a is also found in circulation following a stressful event, which was additionally detected in the plasma of these fish. Taken together, this research establishes that miRNA acts as an essential mediator between the environment and DNA methylation patterns via DNMTs, which is further confirmed by a genomic regulatory mechanism that has been deeply conserved throughout evolution.

New Methods and Novel Approaches for Assessing and Monitoring Environmental Contaminant Mixtures or Individual Priority Substances

Passive samplers in surface water 101: An introduction to sampling low-level hydrocarbons

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Passive samplers are becoming more widely used to detect low-level hydrocarbons and/or integrate fluctuating hydrocarbon concentrations. Successful study designs must consider the limits of the samplers in the environments that they are deployed in, approaches that demonstrate sufficient conservatism and appropriate quality assurance measures. Hutchinson Environmental Sciences has conducted four multi-year studies of low-level natural gas products in rivers and tributaries in northeastern British Columbia gas fields. We have used passive samplers to measure benzene, toluene, ethylbenzene and xylenes (BTEX), petroleum hydrocarbons and polycyclic aromatic hydrocarbons (PAHs) in dynamic mountain rivers. From our experience I will share: (1) the site conditions and regulator concerns that made passive samplers preferable; (2) key aspects to consider in passive sampler surface water study designs; (3) how site-effects were differentiated from background conditions; (4) examples of sampler deployment and recovery methods; (5) lessons learned from field complications; and (6) measures of success. This talk is geared towards those wishing to learn more about the practicalities of deploying passive samplers in surface water.

Using passive sampling as a decision-making tool

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Passive sampling devices (PSDs) present many advantages over conventional sample collection methods for quantifying hydrophobic organic compound (HOC) availability in sediment, soil, surface water and stormwater in terms of cost and data quality. PSDs provide data to estimate contaminant bioavailability and toxicity to environmental receptors that is more accurate than conventional grab or mechanically-extracted samples, as it quantifies freely-dissolved contaminants. This presentation will highlight examples where PSDs have been used in the laboratory and in the field to make decisions on site investigation and remediation, as well as additional techniques and advancements to simplify and improve sampling and to lower costs. In terms of site investigation, we will demonstrate how we used a rapid (2-week) passive sampling deployment to evaluate polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbon (PAH) availability and risks at an active shipyard. A remedial treatability example will highlight the use of PSDs to evaluate the effectiveness of different levels of activated carbon, using the results

to optimize the remedy and save costs by avoiding excess carbon use. In a third example, we highlight the *ex situ* application of PSDs to evaluate the digestive availability of benzo[*a*]pyrene in soils contaminated with clay targets, using the results to derive an alternate human health risk-based cleanup levels that account for the low availability of clay target-associated benzo[*a*]pyrene. Additionally, the presentation will review our recent advances in deploying samplers without the use of scuba divers, optimizing *ex situ* passive sampling with small sediment and soil samples, and evaluation of new analytes.

Passive sampling options for non-polar contaminants and their relevance for ecotoxicity studies

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Passive sampling is gaining significant interest as either a stand-alone or companion method for monitoring a range of environmental contaminants in the field. Some of the advantages identified by users include simplified field work, data free from bias of sediments and colloids, time-integrated data and, often, significantly lower detection limits. These benefits make the technology an ideal tool for evaluating contaminant concentrations in support of ecotoxicity studies and site risk assessments. Several different technologies are gaining acceptance in North America for passive sampling deployments. This presentation will describe the different technologies commonly used for determination of petroleum hydrocarbons and other non-polar contaminants such as polycyclic aromatic hydrocarbons, pentachlorobiphenyls and volatile solvents. Key considerations will be described for selecting the appropriate device(s) for a given site, recommended lab methods for processing the devices after deployment, and appropriate approaches for performing the necessary data analysis. Finally, a comparison of typical data sets from the different technologies will be discussed in relation to each other and to similar data from both conventional chemical analysis approaches and other measures of toxicity and bioaccumulation.

Whole effluent toxicity testing: Is there a non-vertebrate approach?

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Royal Dutch Shell (Shell) employs animal testing for the hazard assessment of chemicals and products, research and development testing, and effluent discharge compliance. Some 84% of ~102,000 of the vertebrate organisms used in 2015 were fish employed in whole effluent toxicity (WET) tests for product stewardship and regulatory compliance applications. Of this, 30% of Shell's global fish use in 2015 was from its Canadian operations. In an effort to decrease the dependence on vertebrate testing for compliance, Shell is proactively seeking non-vertebrate (alternative) testing approaches to trial as potential replacements for traditional vertebrate methods currently used for WET testing. To identify potential alternative methods, an extensive literature review was

undertaken to identify and prioritize potential non-vertebrate alternative methods. This selection process identified trends in the bioassays applied regionally and elucidated alternative approaches that harmonize global testing requirements. The toxicity testing methods evaluated were selected from international standard organizations and WET methods previously used by Shell globally. These were further refined based on their applicability to effluent testing in the North American compliance framework. Priority was given to those methods that were available commercially in North America, were freshwater, and included apical endpoints in methods similar in duration and style to current compliance testing methodologies. Additional priority was given to methods which had the greatest similarity to fish. An initial list of 64 standard protocols from agencies from around the world were compiled and refined to a list of 7 that reflected five testing frameworks for further performance testing. The advantages and challenges associated with each method were investigated. An overview of Shell's approach to eliminating vertebrate testing will be presented, and will include a discussion of a combination of alternative *in vitro* and *in vivo* techniques. It may be that no single method or test by itself is sufficient to replace vertebrate testing, but using a weight-of-evidence approach could help to reduce vertebrate testing.

Development of a standardized toxicity test method using a native amphibian

Leana Van der Vliet[1], Bonnie Lo[2], Jennifer Miller[3], Paula Jackman[1]

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Although there is growing evidence of the sensitivity of amphibians to contaminants and 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. Working towards a standardized test method with a native amphibian species (*Lithobates pipiens*, Northern leopard frog) has provided many challenges over the last 10+ years. We will present some lessons learned from laboratory research and notable features of the test method, including: (1) how challenges faced during the research and development phase were mitigated to improve the test method, (2) the advantages of the exposure periods chosen, (3) why and how the goals of this testing are distinct from a short-term lethality test, and (4) the rationale for a change in reference toxicant test design. In addition, we will present some preliminary results from our inter-laboratory study with sodium chloride and thyroxine. Environment and Climate Change Canada's amphibian test method will continue along the usual path for validation of a toxicity test method, including additional inter-laboratory testing, development of quality control criteria, and improvement of methodology text. The result will be the first Canadian standardized test method using a native amphibian species.

Development and standardization of Environment and Climate Change Canada's threespine stickleback reference method

Jennifer Miller[1], Leana Van der Vliet[2], Lisa Taylor[2], Rick Scroggins[2]

[1]Miller Environmental Sciences Inc., [2]Environment and Climate Change Canada

As part of the 10-year review of the *Metal Mining Effluent Regulations* (MMER) and the development of mines in the Canadian far-North, a need was identified to develop new biological reference methods to measure acute lethality in saline effluent from mine operations discharging to the marine environment. One of these was a marine fish test method to be used for monitoring and compliance purposes as part of the amended MMER to substitute for the rainbow trout (*Oncorhynchus mykiss*) acute lethality test (EPS 1/RM/13) used for the assessment of acute lethality in freshwater mining effluents. A new reference method which specifies procedures and conditions for an acute lethality test with threespine stickleback (*Gasterosteus aculeatus*) has been developed and standardized and will be published by Environment and Climate Change Canada in 2017. The method is intended for use with effluent samples having a salinity of greater than 10 $g \cdot kg^{-1}$ discharging directly to estuarine or marine receiving waters. Procedures are also provided to evaluate different types of substances such as chemicals, formulated products or chemical mixtures. The new Reference Method (EPS 1/RM/10, second edition) replaces the first edition of Environment Canada's Generic Threespine Stickleback Biological Test Method, published in 1990 and amended in 2000. The revised method includes numerous updates such as: the conversion from "generic" to "reference" method, the revision of methods for salinity adjustments and preparation of artificial seawater, the requirement that test organisms be obtained from estuarine or marine waters or cultures, a narrowed size-range of fish recommended for use as test organisms, the revision of holding and acclimation guidance to reflect current and varied laboratory practices, and the review and revision of the recommendations and requirements of the test method. The scope of these changes will be discussed along with key components of the test procedure and rationale for selection of test specifications.

Development of a new regulatory test method for the marine copepod *Acartia tonsa*

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A review of the Canadian *Metal Mining Effluent Regulations* (MMER) has recently identified the need for a compliance test method suitable for assessing the acute lethality of saline effluent discharged into marine or brackish environments. The freshwater invertebrate *Daphnia magna*, currently used to monitor acute lethality of mine effluents, is inappropriate for testing in brackish and marine waters as *D. magna* is intolerant of salinities in excess of 4 parts per thousand (ppt). To fill this gap in MMER regulatory testing, ECCC launched an initiative to develop a test method using the marine copepod *Acartia tonsa* to measure the acute lethality of effluents. Research is currently in progress, and includes: determination of the conditions needed to establish in-house cultures of

Rhodomonas salina (an algal food source) and *A. tonsa*; toxicity testing with a wide range of salinities to determine the salt tolerance range; reference toxicant testing with nickel chloride ($NiCl_2$); development of test methodology (including test endpoints and validity criteria); and management of an inter-laboratory study to validate the proposed test method. Establishment of an *R. salina* feed source included axenic starter cultures as well as larger-scale feed cultures. For both cultures, algae were found to grow well under conditions of 1800 lux, a 12:12 light/dark cycle, salinity 30 ± 2 parts per thousand (ppt) and a temperature of 20 ± 2ºC. However, when up-scaling to the feed culture, algal populations crashed without the addition of gentle aeration to the larger solution volumes. Subsequent addition of continuous gentle aeration enabled the algae to overcome the growth limitations and achieve a stable population density of approximately 1.5 million cells·mL^{-1}. Both the starter and feed cultures were successfully maintained in natural and artificial (Instant Ocean and ESAW media) seawater. *A. tonsa* were found to grow well under conditions of 400-500 lux, a 16:8 light/dark cycle, salinity 30 ± 2 ppt, a temperature of 20 ± 2ºC, and continuous gentle aeration. Copepods housed in clear 1L LDPE culture containers fed daily with *R. salina* achieved population densities of approximately 300 individuals per litre, which were stable between culture days 14 and 28. Culture health criteria are still under evaluation. The proposed test method will be based on a 48-hour exposure starting with the egg stage, whereby newly laid eggs are obtained from breeding male and female copepods. The test endpoint will be survival, with specific details related to this endpoint being investigated as part of the ongoing research efforts. Preliminary exposures of the copepods to a range of salinities (i.e., 5-35 ppt) using the proposed test method suggest that sudden transfer to/or exposure to lower salinities may impact hatching success but not the survival of hatched organisms. This testing not only provided a first look at the ability of copepods to withstand rapid changes in salinity, but also insight as to reasonable expectations for control organism survival. The next steps currently in progress are reference toxicity tests and the initiation of an inter-laboratory study—both focused on assessing the acute toxicity of *A. tonsa* to $NiCl_2$ and Canadian toxicology laboratory training sessions once the method is finished.

A new alternative for reference toxicant testing in longer-term biological test methods

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Standardized biological test methods published by Environment and Climate Change Canada (ECCC) require the conducting of routine reference toxicant tests to assess the sensitivity of the test organism population over time. However, the requirement to conduct routine reference toxicant tests becomes challenging when the test design requires a prolonged exposure period to capture sublethal or life-cycle-type effects (e.g., reproduction inhibition). For these longer tests, there is currently a requirement to run a reference toxicant test of a shorter duration than the definitive tests. This calls into question the value of these results, since definitive tests are of longer duration and focus on different endpoints. To directly address this issue, in tests which run longer than 14 days, ECCC has decided to allow reference toxicant testing to be conducted by one of two options in new

and amended test methods: (1) multi-concentration tests run twice yearly; or (2) a single concentration test run with each definitive test to serve as a positive control. The positive control option is defined as an exposure of test organisms run the same as a negative control (i.e., same replication, organism requirement, test vessels, etc.) except exposed to a single concentration of a known toxicant and would be included as a separate treatment within each definitive test. The endpoint for a positive control would be determined as the mean response (e.g., weight, number of young, abnormal development of individual replicates) subtracted from the mean response in the negative control, divided by the mean negative control response and multiplied by 100 to provide a percent inhibition. To use this approach, each laboratory will be required to define the positive control response and determine acceptability limits. Results from five reference toxicant tests are recommended to determine the appropriate partial effect response (e.g., 40% inhibition) and acceptable range (e.g., >30% and <50%, with a coefficient of variation ≤40%). Advantages of the positive control approach to meet standard method reference toxicant obligations will be outlined, as well as ECCC's plans for introduction of this testing option.

Application of PRSTM, DGT and CaCl2 as tools to approximate plant accumulation and toxicity to nickel and cobalt alone and in a mixture

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Metal contamination in soils most often occurs as mixtures of more than one metal. Although there have been numerous studies on accumulation and toxicity of single metals, implications of multiple metals of concern remains largely unknown. Tools for risk assessment of mixtures of metals in soils are lacking. One possible tool is the use of ion exchange resins as proxies for plant accumulation of metals and growth. Plant root simulator (PRSTM) probes have their origins in testing for soil nutrient bioavailability, but they have been adapted for use in metal bioavailability. Diffusive gradients in thin film (DGT) were originally created for measuring bioavailability of metals in water, but were soon adapted for use in sediments and then soil. The application of these methods to metals in soil is not yet universal, and the implications of the multiple metals for these tools are yet to be investigated. If these tools are acceptable for this application, they may be applied as a screening tool for use in risk assessment when more than one metal of concern is present. To this end, a fixed-ratio ray design was used to compare uptake and toxicity in barley to Ni and Co as singles and in a mixture that included nickel (Ni), copper (Cu) and cobalt (Co). Plant accumulation and response were compared to PRSTM, DGT, CaCl2 extraction and pseudo total metal concentrations. Single metals were more bioavailable than in the mixture at low concentrations for both Ni and Co. However at higher concentrations, Ni became more bioavailable in the mixtures. The range of concentrations studied for Co was lower, so this effect may not have been captured if it were present. Plant accumulation was most closely mirrored by PRSTM and CaCl2, while DGT more closely mirrored plant toxicity as represented by growth parameters for both metals. These results illustrate a notable concentration dependence on metal bioavailability in mixtures. At low concentrations, metals are adsorbed to specific binding sites on soil and are not removed

by the presence of additional cations. However, at higher concentrations adsorption is largely attributed to exchange reactions. Furthermore, plant uptake is metal-specific at lower concentrations, while uptake at higher concentrations is not. PRSTM and DGT show promise as tools to assess the interaction between metals in mixtures in plant uptake and toxicity. This information will be critical in the application of these tools to risk assessment of metals mixtures in soil.

Assessing the effects of soil and waterborne contaminants on wetland vegetation and associated soil fungi

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Water quality impairments impinge on valued wetland ecosystem functions through their impacts on wetland vegetation and associated soil fungi (mycorrhizal fungi). To maintain, enhance and restore wetland ecosystem function, quantifying and monitoring plant and fungal responses to soil and waterborne contaminants is necessary. We are developing and utilizing a suite of laboratory, greenhouse and field-based assays to monitor and assess the effects of soil and waterborne contaminants on wetland plants and mycorrhizal fungi. Our assays include established response variables (seed germination, mortality, biomass, resource partitioning) and less frequently used responses (root and shoot morphometrics, mycorrhizal inoculum potential, mycorrhizal colonization, spore germination, hyphal growth, and plant fungal signaling responses). Using growth room and field-based studies of mining impacts at Giant and Tundra Mines (NWT), assays have shown widespread impacts of arsenic exposure on plant and mycorrhizal growth and development and on plant community structure. Assessments of petroleum hydrocarbon remediation efforts at Colomac Mine (NWT) indicated high mycorrhizal inoculum potential and plant community structure comparable to non-impacted areas. At environmentally relevant concentrations of the ubiquitous antimicrobial triclosan, controlled-exposure studies demonstrated a significant reduction in seed germination, plant growth and mycorrhizal colonization. Using continuous flowthrough exposure systems, effects on mycorrhizal colonization and plant morphology have also been detected in several wetland plants exposed to orthophosphorus at or below provincial guidelines. Since existing approaches utilizing greenhouse/growth room bioassays do not capture temporal heterogeneity in water quality, and soil/sediment/water chemistry analyses do not reflect bioavailability of a given compound, we are developing a series of field-deployable assays (FDAs) to obtain a direct assessment of soil/water toxicity using species of known providence and behavior. These FDAs permit the assessment of a range of wetland plant species, from emergent to submerged macrophytes. Results of the FDAs are site-specific, reflect bioavailability, and circumvent many of the known drawbacks associated with existing field- and laboratory-based assessments. When used in conjunction with existing approaches, the FDAs provide a more comprehensive assessment of contaminant effects.

Quantification and profiling of retinoids in fish by liquid chromatography–tandem mass spectrometry

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Retinoids (vitamin A) are a class of compounds essential for a number of physiological processes including vision and immune system function. They are stored as biologically inactive esters which are mobilized and converted to active retinoic acid (RA) according to physiological requirements. RA is a key component in gene expression and embryonic development, particularly in fish. Disruption of the retinoid system has been observed in several species by exposure to xenobiotics and contaminants from various sources including mine tailings and pulp mill effluent. Retinyl esters are usually analyzed by ultraviolet spectrophotometry, but these methods lack specificity and require authentic standards for quantification which can be expensive or impossible to obtain. RAs are present in fish at $pg \cdot g^{-1}$ levels and have not been adequately quantified to date. A streamlined extraction method has been developed to generate separate fractions of storage and active retinoids from biological tissues to be simultaneously analyzed using a tandem mass spectrometry (MS/MS) platform. A high-sensitivity triple quadrupole instrument was used to quantify retinoic acids while a quadrupole time-of-flight instrument was used as a tool to quantify and profile stored retinoids with a high degree of specificity using full-scan accurate mass MS/MS data. The need in aquatic toxicology for sensitive and robust methods to probe the distribution and function of retinoids in fish as well as to investigate the ultimate effects of perturbations to this essential yet poorly understood biological system will be discussed. Preliminary results will also be presented from a small-method application comparing two populations of fish sampled at a wastewater treatment plant to identify potential effects on retinoid status.

Protein and metabolite responses in rainbow trout (*Oncorhynchus mykiss*) exposed to TBBPA-DBPE

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The Chemical Management Plan (CMP) has identified Tetrabromobisphenol A bis(2,3-dibromopropyl ether) (TBBPA-DBPE) as a replacement flame retardant with the potential to enter the Canadian aquatic environment. There is concern that TBBPA-DBPE might also pose a risk of disrupting the endocrine systems of exposed organisms. The present experiment was designed to assess the sub-lethal effect(s) of TBBPA-DBPE in juvenile rainbow trout (*Oncorhynchus mykiss*) in order to identify potential sub-lethal mechanistic pathways. The fish were exposed to waterborne TBBPA-DBPE in a flow-through design. TBBPA-DBPE was delivered to the tanks by peristaltic pumps to maintain consistent concentrations. Nominal exposure concentrations were: 0 µM; 0.001-; 0.01-; 0.032-; and 0.1-µM. There were three separate exposures: one for 2 days, another for 10 days, and the last for 21 days. Eight fish were housed per tank, with three replicate tanks per exposure concentration for a grand total of 225 fish. We performed non-targeted analysis of plasma

proteins and targeted analysis of plasma metabolites on fish from all exposures using liquid chromatography–tandem mass spectrometry (LC-MS/MS). In total, the expression of more than 500 proteins and 300 metabolites in rainbow trout plasma was significantly affected by waterborne exposure to TBBPA-DBPE. Of those, 29 proteins and metabolites were differentially expressed after all three exposure durations. In this talk, we shall present a synthesis of the data from our multi-omics approach to gain insights onto the potential mode of action of TBBPA-DBPE via the gluconeogenesis pathway in fish.

Individual and mixture exposure to Zn and Ca alter mitochondrial bioenergetics in rainbow trout

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Aquatic environments are impacted by stressors that may affect organismal health and biodiversity at the ecosystem and species levels. One of the problems associated with human activities is metals pollution. Interaction between these metals could be antagonistic, additive or synergistic, causing a variety of effects on aquatic organisms. Among the metals of concern to the aquatic environment, zinc (Zn) is commonly found at elevated levels in aquatic environments. Moreover, Zn in aquatic species have been found to be mitigated by calcium (Ca) at the organismal levels; therefore, studying their interactive effects at internal target sites such as cellular metabolism is necessary for the development of more sensitive biomarkers of toxicity. It was hypothesized that Ca would protect against Zn-induced bioenergetic impairment and Zn would utilize Ca uptake pathway (mitochondrial calcium uniporter: MCU) to enter mitochondria. The individual and binary effects of Zn and Ca on mitochondrial respiration, reactive oxygen species (ROS) and membrane potential ($\Delta\Psi mt$) were evaluated. The study examined whether the uptake of these cations into mitochondrial matrix is a prerequisite for their deleterious effects on mitochondrial function. Only in combination, low and high doses of the two cations synergistically suppressed and promoted mitochondrial ROS, respectively. Individually, Zn was more potent than Ca in stimulating ROS production while Ca more potently dissipated the $\Delta\Psi mt$. Independently of the MCU, Zn caused an immediate depolarization of $\Delta\Psi mt$ that was associated with relatively slow enhancement of H_2O_2 release. In contrast, an equitoxic dose of Ca caused transient depolarization, and stimulation of both respiration and H_2O_2 release, effects that were completely abolished when the MCU was blocked. Overall, my study highlights that the mitochondrial uptake for Ca, but not Zn, is exclusively through the MCU and their mitochondrial responses vary with the mitochondrial functional metric.

Fish behaviour and nonlinear mixed models: A new approach for testing contaminants and contaminant interactions

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Animal behaviour has potential to be a powerful endpoint in toxicology and ecological risk assessment. Behavioural alterations onset by exposure to contaminants can alter activity patterns in risky and safe environments, providing ecologically relevant information regarding predation risk, food acquisition, survival, and fitness. Characterizing various behavioural phenotypes in response to environmental stimuli so as to quantitatively describe a change in activity, the speed at which activity changes, and total and maximum activities, would improve the use of animal behaviour in ecotoxicological research. We present a novel, nonlinear mixed-model approach with two behavioural endpoints at two different lifestages of the zebrafish (*Danio rerio*). We validate the suitability of two nonlinear mixed models for analyzing: (1) changes in zebrafish embryo photomotor responses, and (2) alterations of activity patterns in zebrafish larvae light:dark trials. We exposed embryos and larvae to sedatives, stimulants, and sedative-stimulant mixtures. We found two nonlinear functions that, when optimized with mixed modelling, performed excellently in describing these embryonic and larval behavioural phenotypes. Most importantly, they were capable of distinguishing among various treatments and identified mixture interactions using classical and easily recognizable statistical approaches. The successful application of the nonlinear modelling on behavioural data, particularly in the context of a mixture, will be most useful to researchers of all disciplines working with animal behaviour.

In vitro genotoxicity of surface water and wastewater from Lake Ontario in freshwater mussels (*Elliptio dilatata*)

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A variety of chemicals can induce damage to the DNA of animals, which, if not repaired, can lead to a cascade of biological effects at any level of biological organization. First developed in the 1980s, the single cell gel electrophoresis assay—or Comet assay—is used to visualize and quantify cellular DNA damage. In recent years, a number of studies have used this approach to evaluate DNA damage from exposure to specific chemicals (e.g., PAHs), as well as from complex mixtures of contaminants. Several regions of the Great Lakes have suffered from considerable habitat degradation due to the influence of intensive industrial, agricultural and urban development. In collaboration with the Ontario Ministry of the Environment and Climate Change, we developed an *in vitro* Comet assay to assess DNA damage in the hemocytes of the freshwater mussel *Elliptio dilatata* exposed to surface water and wastewater influent and effluent extracts from three sites in Lake Ontario: Hamilton Harbour, Toronto Harbour and Humber Bay. These extracts induced modest DNA damage after a 4-hour *in vitro* exposure. Surface water extracts from Hamilton Harbour slightly induced DNA damage compared to the control, particularly surface water from the

west end of the Harbour (12.5% and 25% extracts only), and an Index Station located in the middle of the Harbour (100% extract). These results were somewhat expected, considering that sediments in the harbour are highly contaminated with genotoxic compounds. Significantly increased DNA damage was observed in mussel hemocytes exposed to three of the Toronto Harbour wastewater influent samples (12.5%, 25% and 50% extract) collected in June 2015, but these results were not consistent with the same samples collected in October 2015. The Humber Bay extracts did not appear to be particularly genotoxic, with the exception of the 100% extract for the Humber River surface water plume collected in October 2015. In the case of the Toronto Harbour data, the results of the present study indicate that wastewater treatment may successfully reduce the genotoxicity of the influent. Multivariate analyses are underway to investigate potential relationships between the induction of DNA damage and chemical (i.e., metals, PCBs, pharmaceuticals, antibiotics) concentrations in the samples examined.

Mining, Environment, and the Evolution of Environmental Effects Monitoring

Atmospheric deposition of metal-bound dusts at mine sites: Understanding the contribution of snowmelt as a source of metals into lakes

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[1]Natural Resources Canada

Recent studies in some mine sites have shown a significant contribution of metals associated with particulate matter entering watersheds due to localized wind erosion of waste piles, dust from roads and atmospheric deposition during snow/rainfall events (Timoney and Lee, 2009; Kelly *et al*, 2010, Huntsman-Mapila *et al*, 2014). Collecting particulate matter during snowfall events provides an opportunity to quantify loading to specific geographic locations. It is also of particular interest as metals accumulated over the winter would result in a significant metal pulse into the water column during freshet. This presentation outlines the results of snow sampling in the Val d'Or mining region and the use of multi-disciplinary techniques, including scanning electron microscopy, sequential extractions, and toxicology to better understand the contribution of snowmelt as a source of metals into local lakes. By using a weight-of-evidence approach we were able to investigate the various factors and mechanisms affecting metal mobilization in snow. Understanding, on a watershed scale, the contribution of metals from atmospheric particulates is important if we are to gain further insight into the fate and effect of metals in aquatic systems.

Assessing the toxicity of lake sediments contaminated by historic gold mining activities

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Mining operations can be a driver of economic success for a community, but once operations cease, a legacy of contamination is typically left behind. Such is the case at Giant Mine in Yellowknife, NWT. Operating from 1948-2004, Giant Mine released 20,000 tonnes of particulate arsenic trioxide to the atmosphere, which was consequently deposited onto the landscape surrounding the mine-lease territory. In this study, Pb-210 dated lake sediment cores are used to characterize the extent of contamination at the site. Novel methods in paleo-ecotoxicology are employed to reconstruct conditions in local lakes prior to the onset of mining operations, in an area where pre-impact bio-monitoring data are not available. Metal concentrations in sediment profiles were determined for cores spanning a 20 km distance downwind of the roaster stack. Profiles of multiple metals, including arsenic, antimony, and lead, track a peak in contamination during the height of mining operations, which decreases with distance. Toxicity tests in *Daphnia* indicate that lake water collected 1 km from the mine decrease *Daphnia* survivorship, despite the mine's

having been inactive for over 10 years. Principal Component Analysis (PCA) of metals in the sediment dated prior, during, and after the cessation of mining activities show clustering of chemically similar lakes prior to mining, with divergence during operations, and little change occurring since the mine closure. These results suggest that lakes in the Yellowknife region were contaminated from mining operations at Giant Mine, and that the aquatic ecosystems have not returned to pre-mining conditions. Future work examining multi-trophic level responses to contaminant exposure in dated sediments will increase our understanding of the impact of mining operations on the landscape, and will help predict what conditions are needed to expect a recovery in this ecosystem.

Monitoring, impact and risk assessment, and remediation: Mount Polley Mine, British Columbia

Carolyn Anglin[1], Colleen Hughes[2], Luke Moger[2], Lee Nikl[3], Chris Kennedy[4], Trish Miller[5], Pierre Stecko[6]

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The Mount Polley Mine is located in the Cariboo Regional District in central British Columbia, approximately 56 kilometres northeast of Williams Lake. In the early hours of August 4, 2014, a layer of glaciolacustrine clay underlying the perimeter embankment of the tailings storage facility (TSF) failed, causing the embankment to slump and breach, releasing approximately 25 million cubic metres of supernatant and interstitial water, tailings and embankment construction materials (rock, engineered fill, and till). The resulting debris flow extensively scoured and deposited material along the approximately 9 km valley of Hazeltine Creek and deposited material in Polley Lake and Quesnel Lake. Emergency response, remediation and rehabilitation activities began immediately, in parallel with a comprehensive, and ongoing, monitoring program. Initial channel stabilization work was completed in the Hazeltine Creek channel within five months of the TSF failure. Two post-event environmental impact assessments have been completed, and human health and ecological risk assessments are nearing completion. The post-event environmental impact assessments found several indicators of effects to aquatic and terrestrial organisms associated with physical mechanisms (e.g., scouring and smothering), but limited evidence of effects related to chemical mechanisms. As part of the impact assessment, a geochemical characterization program was undertaken to assess the metal leaching and acid rock drainage potential of the tailings in both subaqueous and subaerial environments. Results of these studies have consistently indicated the released materials to be non-acid-generating and that the leaching potential in the subaerial environment is low. In addition, the potential for oxidative and reductive dissolution in the subaqueous environment is considered to be negligible. Ongoing monitoring of Quesnel Lake and Polley Lake indicates that the metal concentrations in the water column overlying the submerged tailings are not increasing. Risk assessments are being carried out to determine the significance of environmental conditions resulting from the breach to human health and the environment. A conceptual site exposure model was developed based on investigations of soil, sediment, groundwater and surface-water monitoring data. Potential exposure

pathways to contaminants were evaluated based on the physical and chemical properties of the possible contaminants and additional data, including plant, invertebrate and fish tissues. A number of potential human health exposure scenarios were evaluated, including the site worker and subsistence and traditional land user. No contaminants of concern were found for human health in soil, sediment or surface water, including drinking water. The ecological risk assessment is using multiple lines of evidence including: (1) chemistry, (2) toxicity testing, and (3) *in situ* biological community assessment. The weight of evidence supports that the physical changes to the habitat are the primary stressors to ecological receptors. Preliminary results of the risk assessments indicate that risks to terrestrial and aquatic receptors from potential contaminants related to the breach are low and meet risk-based standards. Extensive remediation has already been completed, and the results of the risk assessments will be incorporated into a final remediation plan which is presently in development.

The importance of country foods studies in environmental monitoring programs

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Proposed mining and milling developments have the potential to impact environmental media and thus impact ecological and/or human health in nearby communities. Assessments of country foods are often overlooked as a source of information during environmental monitoring programs despite their potential to complement conventional environmental data collections and enhance community engagement. Whenever possible, consideration of country foods should be included in the baseline monitoring program. This can provide information on the types of food consumed and the concentrations in these food items. Frequency and consumption rates of country foods can vary widely depending on the geographical location, species available, and traditional values of a community, particularly for Indigenous communities. Without obtaining site-specific consumption rates, risk assessments must rely on generic information to estimate exposure and potential impacts. For several projects and regions across Canada, the available literature may not reflect the consumption rates of certain traditional foods. Including analysis of country foods in baseline programs prior to development will document naturally occurring parameter concentrations in the region. This can be extremely useful for data comparisons once the development is operational. This information is also used directly in risk assessments. It may be possible to harmonize aspects of traditional environmental monitoring programs with country foods, allowing a transparent link between traditional knowledge and environmental monitoring. For example, a greater emphasis may be placed on culturally-relevant fish species. This does need to be defined in the initial stages of the project to allow for the maximum benefit to be realized to the community and the proponent.

A statistical approach to developing a rationalized long-term monitoring program for the closed Island Copper Mine

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The Island Copper Mine Pit Lake in British Columbia, Canada, serves as a biogeochemical treatment system for metal leaching and acid rock drainage collected from the upland waste dumps. At the time of closure, this treatment system was unproven, and the implementation of this approach in a closed mine treatment system was considered experimental. However, 20 years later, the Pit Lake treatment system has proven to be extremely effective with concentrations of metals in the Pit Lake surface water consistently achieving discharge criteria. There are now 20 years of data from which to evaluate Pit Lake performance and to determine the most effective monitoring program going forward. The Island Copper closure plan recognizes that monitoring will be required on the site for many decades. In order to determine the most efficient and effective monitoring program, statistical analyses were conducted on existing water quality data. Monitoring parameters were selected based on the frequency and magnitude above guidelines and/or their necessity to evaluate geochemical changes. Autocorrelation analyses were used to determine the frequency of monitoring that would provide unique information, and linear time-series models were used to determine when samples should be collected to ensure that minimum and maximum values of key parameters would be captured. Correlation analyses were used to rationalize the depths to be sampled in the Pit Lake to ensure unique information is collected. A recommended long-term monitoring program was developed which married the core aspects of the monitoring program with a data quality management plan, environmental response plans, and reporting requirements and schedule.

DNA barcoding approach to identify benthic invertebrates from a mine site in Ontario

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Biomonitoring requires the collection and identification of specimens to establish baseline characteristics of the biological community and track changes over time and space. Accurate taxonomic identification is a foundational element of biomonitoring studies, which is traditionally done by experts using morphological keys. Several factors can limit accurate morphological identification, including life-stage of the specimen, preservation history, partial or damaged specimens, adequacy of taxonomic keys, and expertise of the taxonomist. New DNA-based approaches to specimen identification such as DNA barcoding offer attractive solutions because they are more objective and repeatable, can be applied to all life stages and to fragments, can be automated, and are more rapid and scalable than morphological approaches. Successful identification using DNA barcoding requires a robust reference library against which the sequence of an "unknown" can be matched and subsequently identified. The purpose of this study was three-fold: (1) to assess the ability to recover DNA barcodes from formalin-fixed morphological voucher specimens collected

from a mine monitoring program, (2) to assess the maturity of the existing Biodiversity Institute of Ontario's barcode reference sequence library in providing matches to the sequences derived from the study specimens, and (3) to assess the congruence of specimen identifications derived from morphology and barcoding approaches. In addition, this study demonstrates to regulators the use of DNA barcoding for taxonomic verification in a federally required environmental effects monitoring program. The results of this pilot study will be presented along with recommendations for follow-on work, including development of a more suitable preservative to maintain DNA integrity and allow for morphological identification, development of standardized field-collection procedures, potential enhancement of the DNA barcode reference library, and investigation of metabarcoding as a potential time-saving approach. This work was supported by Ontario Genomics through funding to R.H. Hanner, with in-kind collaboration and support from Alamos Gold and Stantec.

The use of genetic analysis to aid in Arctic environmental effects monitoring fish studies

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The Raglan Mine is a nickel mine located on the Ungava Peninsula in the Nunavik territory of northern Quebec. Under the *Metal Mining Effluent Regulations* (MMER) of the federal *Fisheries Act*, the mine is required to conduct environmental effects monitoring (EEM) studies to determine whether mine effluent is causing effects on fish health, fish habitat, fish food, and usability of fisheries resources (Environment Canada 2012). Arctic charr (*Salvelinus alpinus*) is the only fish species that occupies freshwaters within the mine watershed. This watershed is typical of Arctic watersheds in that the Arctic charr population exhibits relatively low productivity (e.g., slow growth), they do not spawn annually, and there is phenotypic variability (i.e., diadromy) within the population. As a result, the second and third EEM fish surveys at Raglan Mine implemented a standard non-lethal sampling approach to limit impacts of lethal sampling to the fish population. However, limitations of aging fish non-lethally, as well as the inability to distinguish sex, had potentially constrained the determination of whether there are real effects of mine effluent on Arctic charr within the mine receiving waters. Because it is well documented that male and female growth rates differ, it was hypothesized that the differences in growth rates documented in the second and third EEMs was potentially related to different proportions of male and female fish collected from the respective effluent-exposed and reference study areas. In the most recent EEM (Phase 4), Raglan Mine modified its non-lethal survey to include a genetic analysis to determine Arctic charr sex, thus allowing fish population endpoints to be calculated separately for males and females. The Phase 4 EEM study showed that significantly lower Arctic charr growth and condition that were suggested between the effluent-exposed and reference areas based on evaluation of the population as a whole in the previous EEM studies were no longer evident with the implementation of a sex-specific evaluation. It is likely that the inability to differentiate between sexes contributed to the significant differences in growth and condition between

the areas in the earlier EEM studies. Raglan Mine determined that the use of genetic analysis to separate males and females within a non-lethal fish survey was an efficient and cost-effective tool to further their understanding of potential mine-related influences on the Arctic charr population in the mine receiving environment.

Using ultrasound as a non-lethal method for measuring fish liver size for environmental effects monitoring

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Canada's environmental effects monitoring (EEM) program evaluates potential impacts of metal mining and pulp mill effluents in aquatic receiving environments. The EEM program recommends lethal sampling of 20 male and 20 female fish of 2 different species to study body condition, liver size (hepatosomatic index-HSI), and gonad size (gonadosomatic index-GSI). Repeated lethal EEM sampling in aquatic ecosystems with populations of endangered or low productivity fish species can have negative impacts and field sampling may be discouraged or prohibited. The need for alternative, non-lethal methods for EEM programs has been gaining prominence. Ultrasound is a non-invasive tool that can be used to study HSI or GSI in fish and has had only limited use to date. Our objective is to develop and validate ultrasound as a non-lethal EEM method first with HSI in fish with varying liver structures, followed by GSI in future projects. Accuracy and sensitivity analyses using a laboratory treatment (food withdrawal for 2 weeks) was conducted to compare HSI in rainbow trout (*Oncorhynchus mykiss*) using traditional lethal gravimetric versus novel ultrasound methods. From the ultrasounds, we generated 3-dimensional (3D) constructs of livers from fed versus food-withheld rainbow trout to calculate liver volumes and generate HSI. Results from the lethal gravimetric method and non-lethal ultrasound method revealed a significant difference in HSI between fed versus food-withheld rainbow trout ($p<0.05$ in t-test, n=8-13/treatment; 16% change in HSI). In addition, these findings provide empirical evidence for the accuracy of ultrasound as a method to compare liver volumes without having to euthanize fish. We also conducted a species comparison laboratory study to correlate the accuracy of ultrasound versus gravimetric method to estimate liver volume and HSI in fish with varying liver structures. We compared the accuracy of ultrasound in rainbow trout, which have a compact liver, versus lake sturgeon (*Acipenser fulvescens*), which have a diffuse and highly irregular liver. Despite irregular structure, we found a good correlation in lake sturgeon between HSI estimated by ultrasound versus gravimetric method ($r=0.51$), and a much stronger correlation in compact trout livers ($r=0.96$). Findings of our sensitivity analysis and species comparison study suggest that ultrasound can accurately evaluate changes in HSI in fish with a compact liver such as rainbow trout. Findings of our laboratory study confirm the overall feasibility of using ultrasound in fish with different liver structures as a reliable tool for non-lethal EEM programs.

Advanced research in subcellular metal partitioning in aquatic organisms: Towards a tool to better estimate the risk of toxicity linked to metal exposure in the environment?

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Environmental exposure to metals may lead to their accumulation in aquatic organisms and cause toxicity. Since 2002 in Canada, metal mining companies subject to the *Metal Mining Effluent Regulations* (MMER) have been required to conduct environmental effects monitoring (EEM) studies. Typical EEM studies include the collection of data on life-history characteristics of two sentinel fish species and benthic invertebrate communities upstream (reference) and downstream of a discharge. If a biological effect(s) is observed (the regulatory definition being a statistically significant difference) and confirmed in two consecutive phases of the program, then companies must undertake an Investigation of Cause (IOC) study to determine the cause of effect(s), as differences between exposure and reference areas may not be caused by metal exposure but rather by other environmental factors such as habitat differences (i.e., food resources). IOC studies are not prescriptive and little guidance has been provided by Environment Canada. Current approaches usually involve relating total metal concentrations in organisms or in their internal organs to observed effects; however, metal toxicity depends on the behaviour of metals once they enter a cell. Once inside the cell, metals may bind to sensitive components and cause deleterious effects, but metals can also be detoxified by binding to molecules designed to sequester them; thereby limiting their toxic effects. The current work aims at developing a tool using subcellular metal partitioning for IOC to distinguish metal-induced effects from effects related to environmental variables other than metals, by (1) assessing to which intracellular ligands metals bind in organisms and (2) relating metal accumulation in specific subcellular fractions to toxicity. To this end, mature male and female white suckers (*Catostomus commersoni*) were collected in three lakes downstream of a metal-mining effluent discharge and in a reference area. Metal and metalloid subcellular partitioning (As, Cd, Cu, Ni, Pb, Se and Zn) among potentially metal-sensitive fractions (MSF) and detoxified metal fractions (BDM) in white sucker livers was determined after differential centrifugation and heat-denaturation steps. In parallel, several effects biomarkers were measured (activity of enzyme indicators of antioxidant, metabolic or biosynthetic capacities) to assess whether the linking of metal accumulation in particular subcellular fractions to effects will provide better estimates of the risk of toxicity than the current approach of using total tissue metal accumulation in fish. Based on these results, we will discuss how the determination of metal subcellular partitioning might be incorporated into an IOC tool for determining whether biological effects observed downstream from an effluent discharge point are metal-related.

Selenate versus selenite: Uptake into algae and trophic-transfer to *Daphnia magna*

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[1]Natural Resources Canada

The uptake of selenium (Se) from water to primary producers has generally been accepted as the most important stage in the toxicity of Se, due to accumulation and conversion to the organic form Se-methionine. The objective of this study was to determine whether speciation of Se in water is important in determining trophic-transfer and toxicity. This presentation will provide a comparison between selenate- and selenite-exposed algae and the effects of dietary exposure to the cladoceran *Daphnia magna* over two generations. An algal diet, consisting of both *Chlorella kessleri* and *Pseudokirchneriella subcapitata* was spiked in a dose-dependent manner with selenite and selenate over a 7-day growth period resulting in a dietary-Se exposure of 5, 10, 20, 40 and 80 $mg \cdot kg^{-1}$ Se (nominal concentrations). First generation *D. magna* were fed over a 21-day period to assess survival and reproduction, offspring were collected and either exposed to dietary-Se or a control diet for a further 21-days. Tissue concentrations of both first and second generation *D. magna* were assessed along with visual inspection of the daphnids for any potential deformities. A comparison of reproduction, survival and tissue Se concentrations, including trophic-transfer factors, will be presented.

Acute and chronic toxicity of four rare earth elements to fish (*Salmo trutta*, *Oncorhynchus mykiss* and *Pimephales promelas*)

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Rare earth elements (REEs) are used in a variety of manufactured products and interest in their extraction from mineral ores has increased recently in Canada. This group of elements, which is comprised of yttrium, scandium and the lanthanides, has insufficient toxicological data to generate environmental benchmarks for long-term exposure. Chronic toxicity tests involving 28-day early life-stage tests with brown trout (*Salmo trutta*) and 7-day tests using fathead minnows (*Pimephales promelas*) were conducted with lanthanum (La), neodymium (Nd), yttrium (Y) and cerium (Ce). The 28-day brown trout test resulted in chronic effects on dry weight (IC10 values) of <7.9, <8.0, 21.1, and 90.8 $\mu g \cdot L^{-1}$ for dissolved Ce, La, Nd and Y, respectively. IC10 values for biomass in the 7-day fathead minnow tests were determined to be 15.7, 19.8, 27.9, and 12.9 $\mu g \cdot L^{-1}$ for dissolved Ce, La, Nd and Y, respectively. In addition to the chronic tests, acute testing with rainbow trout (*Oncorhynchus mykiss*) and fathead minnows in various water types revealed information on REE solubility and the role of water hardness as a potential toxicity modifying factor. The presentation will include discussion regarding acute-to-chronic ratios, possible modes of toxic action and recommendations for future REE testing.

Biological treatment solutions to ecotoxicological problems in mining

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Active and historical mining operations can pose significant potential toxicity risks to nearby aquatic and terrestrial receptors associated with water discharged from mine sites. This presentation will discuss the use of biological treatment processes to provide toxicity reduction of mining-related contaminants including cyanide, ammonia, nitrate, heavy metals and selenium before release to the environment or before reaching sensitive ecosystems. Pre-design and post-implementation monitoring requirements of biological treatment systems for mining-related chemicals will be discussed, including which types of analyses are key to understanding the 'health' of the biological cultures. Baseline monitoring, pilot test data, and operating system data all inform the effectiveness of the treatment and are essential to interpretation of treatment-system data. Through maintenance of specific redox conditions, optimization of the biological culture(s) can be obtained in order to degrade or sequester mining-related contaminants and reduce toxicity releases to the environment. This presentation will discuss the type of biological treatment systems that can be used to treat specific mining-related contaminants and several case studies will be presented.

Real-time environmental monitoring of mining effluents using a microbial fuel cell (MFC)- based sensor

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Natural resources exploitation creates a significant disturbance to ecosystems with a risk of residual materials propagating outside of the designated mining areas. Accordingly, extensive environmental monitoring is of great importance during the active mine life and following mine closure, sometimes in perpetuity. While in-situ high-frequency observations are crucial to uncover the variations that could occur at the site, real-time site monitoring methods are lacking. Short-term variations in contaminant concentrations are largely unknown and contaminant propagation is often detected with significant delays. This study describes an environmental biosensor, which exploits high sensitivity of microbial fuel cells (MFCs) to variations in environmental conditions, such as the presence of electron donors and acceptors (e.g., organic materials, metal sulphates, ammonium, etc.). Our experiments established fast MFC voltage response to changes in mining water composition (e.g., iron, and zinc concentrations). Therefore, MFC electrical performance could be inferred with the concentration of a target contaminant, thus enabling a low cost and low maintenance biosensor capable of detecting abrupt changes in the environmental conditions.

Hartnell's time machine: 170-year-old nails shed light on health of the Franklin Expedition crew

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Much mystery surrounds the ultimate fate of Franklin's crew, who died in their quest to find the Northwest Passage in 1845. An exhumation of three crew members took place in the 1980s. In this manuscript, we present data from our studies of nail samples from one of these crewmembers, John Hartnell. We have used synchrotron micro-XRF mapping, laser ablation ICP-MS, and stable isotope analysis on the big toenail and thumbnail to examine the distribution of metals, and nitrogen and carbon isotopic changes in the nails. Keratinous tissues such as nails readily bind metals circulating in the blood; therefore, as they grow they provide a temporal record of changes in metal exposure. We focused our research on lead (Pb), since lead has been the subject of much speculation and study in previous work on the Franklin expedition. We have also added new insight into dietary change and nutritional health of the expedition by studying the distribution of copper (Cu) and zinc (Zn), which have not previously been studied. Our research provides a new perspective on the circumstances surrounding this ill-fated mission. By combining our results from Hartnell's nails with information from past research on lead in the crew's tissues and historical accounts of the Expedition, Inuit testimony, and general toxicology, we present three new key discoveries regarding the fate of the Franklin Expedition.

Perspectives on proposed amendments to the *Metal Mining Effluent Regulations*

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On May 13, 2017, Environment and Climate Change Canada (ECCC) published proposed amendments to the *Metal Mining Effluent Regulations* (MMER) in the *Canada Gazette*, Part I, for a 60-day public comment period. ECCC is currently proceeding with finalizing the amendments and plans to publish them in the *Canada Gazette*, Part II in the spring of 2018. The amended regulations would apply to diamond mines as well as metal mines, and would include lower limits for some substances. The amended regulations would also include a limit for un-ionized ammonia. The Mining Association of Canada (MAC) has been challenging the scientific basis for this particular limit. Once the amendments come into force, there would also be a new requirement that effluent not be acutely lethal to *Daphnia magna* as well as rainbow trout (*Oncorhynchus mykiss*). ECCC is introducing flexibility to use a different fish species in cases where saline effluent is deposited into saline environments. MAC has strongly supported this amendment. The amendments will also bring significant changes to the environmental effects monitoring (EEM) requirements of the regulations. These amendments are intended to make the EEM program more effective and efficient. One of the most notable amendments would be the removal of the requirement to determine the magnitude and geographic extent of effects. This would allow sites with confirmed effects to proceed more quickly to the Investigation of Cause.

The amendments would also introduce a new requirement to monitor concentrations of selenium in fish tissue at sites meeting conditions to be specified in the regulations.

Evaluation of proposed amendments to the metal mining Environmental Effects Monitoring program related to determining an effect on biological endpoints

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Environmental effects monitoring (EEM) is a science-based performance measurement tool used to evaluate the adequacy of the *Metal Mining Effluent Regulations* (MMER) in protecting fish, fish habitat, and the use of fisheries resources. In 2012, a 10-year review of the MMER was initiated by Environment and Climate Change Canada (ECCC), and following multiple years of stakeholder consultations, proposed amendments were published in Canada Gazette, Part I in May 2017. In this talk, we will evaluate the proposed MMER amendments related to the definition of an effect and the triggers for mines to move into subsequent EEM monitoring phases. The metal mining EEM program stipulates endpoints used for determining effects in benthic invertebrate communities and fish populations in areas exposed to mine effluent. An effect is defined as a statistically significant difference in any endpoint between the exposure area and a reference area. In the proposed MMER amendments, the definition of an effect remained unchanged. The use of critical effect sizes (CESs) was proposed for determining triggers into subsequent biological monitoring phases; however, there are numerous effect endpoints for which CESs are not provided, and in these cases the traditional definition of an effect will be applied. Case studies will serve to illustrate the importance of ensuring all endpoints used to determine effects are meeting the intended objectives of the EEM program and the MMER amendments. There has been extensive discussion regarding the use of baseline and relevant regional data in metal mining EEM studies dating as far back as the 1996 Assessment of the Aquatic Effects of Mining in Canada report. The importance of using multiple reference areas and a before-after-control-impact (BACI) study design in determining effects as part of the EEM program will also be discussed using case studies. One of the drivers identified by ECCC when undertaking the 10-year MMER review was to focus monitoring efforts by mines on sites with higher environmental risk, and the successful completion of this objective hinges on appropriate determination of an effect in the aquatic receiving environment.

Scientific perspectives on the development of a new coal-sector effluent regulation for Canada

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In 2012, Environment and Climate Change Canada (ECCC) initiated a review of the *Metal Mining Effluent Regulations* (MMERs), which have not been updated since their initial promulgation in 2002. The review included a number of proposed revisions to the MMERs,

including changes to effluent limits, addition of regulated parameters (e.g., selenium, ammonia), and modifications to toxicity testing and environmental effects monitoring (EEM) requirements. The review was followed by a two-year multi-stakeholder consultation process, which included input on key scientific issues and debate on scientific and social aspects emerging from the new proposed regulation. One of the outcomes of the review and consultation processes, and the unique characteristics of coal effluent vs. effluents of metal and diamond mining, has been a proposal by ECCC to develop and establish a coal regulation separate from the MMERs. Recently, ECCC released an initial consultation document, accompanied by Canada-wide consultations, addressing the proposed coal regulation, with an aim to promulgate the new Canadian coal regulation in 2019. This presentation reviews the fundamentals of coal effluents and key considerations in the management of their potential environmental impacts in the context of the proposed coal regulation for Canada. Key aspects of the regulation, with a specific focus on the use of current science and best industry practices in the development of the regulation, will be outlined and discussed.

Is There a Problem Here? Integration of Ecotoxicology and Hazard/Risk Assessment

Using Geographical Information Systems (GIS) for spatial risk assessment and landscape ecotoxicology

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Modelling the impacts of chemical pollution across a landscape is challenging due to the spatial heterogeneity and complexity of ecological systems. Within the field of ecotoxicology there is an acknowledged need to include spatial analyses to assess risk. As a result, the field of landscape ecotoxicology emerged, which is the study of the spatial distribution of contaminants across a landscape and the impact this has on ecological systems. While landscape ecotoxicology and spatial analyses can theoretically be useful for risk assessment purposes, they have yet to become a common practice because of the lack of standardized methodology. We proposed that a geographic information system (GIS) is the ideal platform for the integration, analysis, and visualization of ecotoxicological data. We demonstrate the utility of a GIS and the landscape ecotoxicological approach through the synthesis and evaluation of biomonitoring data collected under the Joint Oil Sands Monitoring (JOSM) project. Data from the five separate wildlife health monitoring projects conducted under JOSM were compiled into a relational geodatabase. The data were analyzed using spatial methods such as Getis-Ord's G, Moran's I, and spatial densities to assess spatial patterns of various contaminants included in the biomonitoring data. These results and spatial patterns are intuitively communicated through maps. Ultimately, this synthesis project can be used for ecological risk assessment and will help to further develop and strengthen a long-term monitoring plan in the region.

Caffeine and paraxanthine in aquatic systems: Global exposure distributions and probabilistic risk assessment

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This study presents one of the most complete applications of probabilistic methodologies to the risk assessment of emerging contaminants. Perhaps the most data-rich of these compounds, caffeine, as well as its main metabolite (paraxanthine), were selected for this study. Information for a total of 29,132 individual caffeine and 7,442 paraxanthine samples was compiled, including samples where the compounds were not detected. The inclusion of non-detect samples (as censored data) in the estimation of environmental exposure distributions (EEDs) allowed for a realistic characterization of the global presence of these compounds in aquatic systems. EEDs were compared to species sensitivity distributions (SSDs), when possible, in order to calculate joint probability curves (JPCs) to describe the risk to aquatic organisms. This way, it was determined that

unacceptable environmental risk (defined as 5% of the species being potentially exposed to concentrations able to cause effects in more than 5% of the cases) could be expected from chronic exposure to caffeine from effluent (28.4% of the cases), surface water (6.7% of the cases) and estuary water (5.4% of the cases). Probability of exceedance of acute predicted no-effect concentrations (PNECs) for paraxanthine were higher than 5% for all assessed matrices except for drinking water and ground water; however, no experimental effects data was available for paraxanthine, resulting in a precautionary deterministic hazard assessment for this compound. Given the chemical similarities between both compounds, real effect thresholds – and thus risk – for paraxanthine would be expected to be close to those observed for caffeine.

Assessing the toxicity and risk of salt-impacted winter road runoff to the early life-stages of freshwater mussels

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In temperate urbanized areas where road salting is used for winter road maintenance, the level of chloride in surface waters has been increasing. This is a concern for salt-sensitive freshwater mussels because many species, including species at risk, have ranges limited to southern Ontario, Canada's most road-dense region. While the early-life stages of freshwater mussels are known to be particularly sensitive to salt, the direct toxicity of salt-impacted winter road runoff to Ontario freshwater mussels had not been examined. This study examined the acute toxicity of field-collected winter road runoff (14,400 mg $Cl \cdot L^{-1}$) to two mussel early life stages; glochidia (i.e., larvae; 48-hour exposure, *Lampsilis fasciola*) and newly-released juvenile mussels (<1 week old; 96-hour exposure, *Lampsilis siliquoidea*). To characterize potential responses in different receiving environments, acute sensitivity to salt-impacted road runoff was examined in waters of different hardness. The effect of a chronic exposure (28 days) to winter road runoff was also assessed using older (7-12 months old) juvenile *L. siliquoidea*. The 48-hour EC_{50}s for *L. fasciola* glochidia exposed to different dilutions of road run-off were similar in moderately hard (~100 mg $CaCO3 \cdot L^{-1}$) water (EC_{50} 7.8% runoff water, or 1177 (95% Confidence Intervals 1011-1344) mg $Cl \cdot L^{-1}$) and very hard (~250 mg $CaCO3 \cdot L^{-1}$) synthetic water (EC_{50} 7.0% runoff water, or 1032 (739-1324) mg $Cl \cdot L^{-1}$). The 96-hour EC_{50}s for newly released (< 1 week old) *L. siliquoidea* were 13.8% runoff water (2276 (1698-2854) mg $Cl \cdot L^{-1}$) in moderately hard water and 20% runoff water (3159 (2206-4112) mg $Cl \cdot L^{-1}$) in very hard water. These effect concentrations correspond with the acute toxicity of chloride reported in other studies, indicating that chloride is likely the driver of toxicity in salt-impacted road-runoff rather than other compounds (e.g., metals, polycyclic aromatic hydrocarbons). The EC_{50} from the 28 day static renewal exposure in moderately hard water with 7-12 month old *L. siliquoidea* was 10.6% runoff water (1810 (1429-2190) mg $Cl \cdot L^{-1}$). Toxicity data from the current study and the literature, along with concentrations of chloride in the surface waters of Ontario, were used to conduct a probabilistic risk assessment of chloride to early-life-stage freshwater mussels. The assessment indicated that acute toxicity of mussel early life-stages during the months for which chloride data are available (March to November) is

unlikely to occur; however, there is a lack of field chloride data for the winter months when the majority of salt-impacted road runoff occurs. In addition, the risk assessment exercise revealed that chronic exposure to elevated chloride levels could pose a risk to juvenile freshwater mussels and that further investigation is warranted to ensure that the most sensitive organisms are protected.

Pesticides in the Environment

Toxicity of environmentally relevant neonicotinoid pulses to aquatic invertebrates

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Neonicotinoids are a group of pesticides commonly used in agriculture which, due to their high water solubility, can be transported to aquatic systems. Transport is driven by precipitation, with heavy rain events causing spikes or pulses in neonicotinoid concentrations many times higher than background levels. Due to a conserved mechanism of action between agricultural insect pests and aquatic invertebrates—especially aquatic insects—these non-target species have the potential to be negatively impacted by neonicotinoid contamination. A monitoring program during the summer of 2015 by the Ontario Ministry of the Environment and Climate Change revealed pulses of neonicotinoids into freshwaters near agricultural lands of approximately 2.7 $\mu g \cdot L^{-1}$ imidacloprid, and 2.3 $\mu g \cdot L^{-1}$ thiamethoxam at peak; each pulse lasted for approximately 24 hours. Our study investigated the effect of exposures emulating these field pulse doses to four species—*Chironomus dilutus* (midge), *Hyalella azteca* (scud), *Neocloeon triangulifer* (parthenogenic mayfly), and *Hexagenia* spp. (burrowing mayfly)—with chronic tests. We exposed each species in separate bioassays to nominal treatment concentrations of 2.5 $\mu g \cdot L^{-1}$ (average peak concentration), 5.0 $\mu g \cdot L^{-1}$ (2x peak), and 10.0 $\mu g \cdot L^{-1}$ (4x peak) of either imidacloprid or thiamethoxam, along with a negative control. After 24 hours in the pulse exposure, which mimicked the pulse of neonicotinoids into surface waters, individuals were moved to control conditions and monitored over their partial (*H. azteca* [42-day], *Hexagenia* spp. [21-day]) or full (*C. dilutus* [≤56-day], *N. triangulifer* [≤30-day]) life cycle. Endpoints included chronic survival, growth, and for *H. azteca* and *C. dilutus*, reproduction. This study connects environmentally relevant patterns of neonicotinoid exposure and chronic aquatic toxicity testing, with the goal of furthering our knowledge of non-target neonicotinoid toxicity.

Risk posed by pesticides to native freshwater mussels in the Great Lakes basin

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Canada supports 55 of the approximately 300 Unionidae species found in North America, with 40 species found within the Great Lakes basin. Freshwater mussels contribute important ecological functions to aquatic systems. The water filtered by mussel assemblages can contribute to improved water quality and the filtered material deposited in sediment provides a link between pelagic and benthic food webs. In addition, mixing of sediments by burrowing mussels can improve oxygen content and release nutrients. However, nearly 70% of global freshwater mussel species are listed as either endangered, threatened, or in decline. In Ontario, 28 species are in decline or in need of protection. Even though freshwater mussels are considered extremely sensitive to contaminants, little is

known about the risk pesticides pose to the most sensitive life stage: glochidia. *Villosa iris*, rainbow mussel, is currently listed as "special concern" in Ontario. A potential risk to the recovery of freshwater mussel species is the presence and persistence of pesticides and pesticide mixtures in the Great Lakes basin. Acute (48-hour) toxicity tests were performed with glochidia to determine the effect on viability following exposure to azoxystrobin, boscalid, metalaxyl, myclobutanil, carbaryl, clothianidin, imidacloprid, thiamethoxam, rotenone, malathion, prochloraz, chlorpyrifos, flupyradifurone, and cypermethrin. Glochidia were also exposed to environmentally relevant binary mixtures of pesticides to determine effects on viability. The study found that in general glochidia were relatively insensitive to the pesticides tested and LC_{50}s ranged from 594 to >17400 $\mu g \cdot L^{-1}$. All neonicotinoid LC_{50}s were greater than the highest concentration tested. The pesticides tested likely represent a *de minimis* risk to the viability of glochidia in Ontario streams where pesticide concentrations are considerably lower than those tested in this study.

Tadpole susceptibility to parasitic infection after exposure to a neonicotinoid: clothianidin

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Relatively little is known about the impact of neonicotinoid insecticides on vertebrate wildlife. Amphibians are excellent vertebrate bioindicators as they are sensitive to environmental stressors and may be particularly vulnerable to neonicotinoid exposure during their aquatic life stage. In previous studies we found no detrimental effects of chronic exposure to neonicotinoids on conventional tadpole life-history traits such as survival, growth and development, which can affect amphibian fitness. However, there is some evidence to suggest more subtle immunotoxic effects could be of concern for non-target organisms, such as amphibians, exposed to neonicotinoids. Other studies have found that certain chemicals, including pesticides, can make tadpoles more susceptible to parasitic infection through mechanisms such as immunosuppression and reduced anti-parasite behaviour. The objective of our study was to assess whether neonicotinoid exposure affected tadpole susceptibility to parasitic helminths. We chronically exposed recently hatched northern leopard frog (*Lithobates pipiens*) tadpoles to environmentally relevant concentrations of technical grade clothianidin for 2 weeks. We then exposed the tadpoles to the free-living infectious stage (cercariae) of a helminth parasite, the trematode *Echinostoma trivolvis*, for 2 days and assessed for levels of parasitism and leukocyte profiles. We found no significant differences between control treatments and the 1 or 100 $\mu g \cdot L^{-1}$ treatments in prevalence, abundance or intensity of echinostome cysts or in tadpole leukocyte profiles (GLMM, all p-values > 0.05). We also conducted independent cercariae-clothianidin exposures and confirmed that clothianidin was not toxic (i.e., no effects on survival or activity) to these free-living infective parasitic stages. Therefore, exposure to the neonicotinoid clothianidin during the larval amphibian stage does not affect susceptibility to parasitism in northern leopard frogs. We recommend that further research be conducted on the other neonicotinoids, both individually and as mixtures, to better assess environmentally relevant exposures and effects on cercariae and on tadpole

susceptibility to parasitism. Finally, the susceptibility of other species of frogs or parasites to neonicotinoids would be of interest to more fully understand the effects of neonicotinoids on amphibians as non-target wildlife at risk of exposure.

Fate and effects of neonicotinoid insecticide thiamethoxam in shallow model wetlands

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Thiamethoxam is a neonicotinoid insecticide that can reach wetlands in agro-ecosystems primarily through runoff. The effects of thiamethoxam on non-target organisms in shallow wetland ecosystems have not been well characterized. To this end, two separate mesocosm studies were conducted to characterize the response of invertebrates. The first study investigated the effect of a single-pulse application of thiamethoxam (0, 25, 50, 100, 250, and 500 $\mu g \cdot L^{-1}$; n=3) on zooplankton communities in prairie wetland mesocosms over eight weeks. The mean half-life of thiamethoxam among the different treatments was 4.0 days in the water column, with dissipation attributed to photodegradation and biodegradation. The concentration of thiamethoxam was < 0.08 $\mu g \cdot L^{-1}$ in the majority of mesocosms by 56 days. There were no statistically significant differences in zooplankton abundance or diversity between control and treated systems. The second study examined more chronic exposure through a single application of coated canola seeds at three treatment levels based on recommended seeding rate (i.e., 6 kg/ha; 1x, 10x, and 100x seeding rate) on both zooplankton and emergent insects over ten weeks. The mean half-life of thiamethoxam in the water column of mesocosms was 6.2 days. Significant differences in zooplankton community structure and emergent insect abundance between control mesocosms and the two greatest treatments were observed. Consequently, data generated from these mesocosm studies indicates acute and chronic exposure to thiamethoxam at environmentally relevant concentrations likely does not represent a significant ecological risk to wetland zooplankton and emergent insects, in terms of abundance and community structure.

Pesticide exposure and effects on migratory birds: Past and current threats

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Over the last 50 years, agriculture has undergone dramatic changes worldwide, with consequences for many farmland-associated migratory birds. The relationship between agricultural intensification and avian declines has been established in Europe, but links and mechanistic studies for North American species have received less study. Through analysis of trends in bird populations associated with farmlands and grasslands in North America, we found declines occurred with 57 of 77 (74%) species exhibiting decreases from 1966 to 2013. The greatest concurrence in multiple species declines occurred during 1960s-1980s,

a period with high agrochemical use and widespread conversion of grasslands to cropland. The most severe declines occurred in aerial insectivores (average decline of -38.3% from 1966 to 2013), followed by grassland birds (21.5%) and shrub species (-16.0%). Evidence suggests pesticides are one of the strongest drivers of farmland bird declines through mortality and sublethal effects, and indirectly through reduced food supplies. Mechanistic captive and field studies examining pesticide effects to wild migratory birds are rare. Here, we present a case-study with captive, seed-eating white-crowned sparrows (*Zonotrichia leucophrys*) caught at a stopover site in Saskatchewan, Canada, to assess and compare the effects of oral exposure to two common and widely used insecticides: imidacloprid (neonicotinoid) and chlorpyrifos (organophosphate). We used funnel trials to measure migratory orientation and activity, and found that birds exposed to low concentrations of imidacloprid reduced food consumption, experienced significant mass loss, and stopped orienting correctly in behavioural trials, whereas control birds maintained body mass and a seasonally appropriate northward orientation. Birds exposed to chlorpyrifos also showed effects on migratory orientation, but without acute effects of mass loss. Farmland landscapes make up a significant proportion of the land cover and bird habitat in North America where the use of toxic pesticides is still increasing, so further work is urgently needed to evaluate the magnitude of pesticide effects to migratory birds across their range.

Assessing mesocosm-derived effects of imidacloprid on aquatic invertebrate community function

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To support their re-registration of imidacloprid, Bayer Crop Science (Bayer) submitted to the US EPA and Canadian Pest Management Regulatory Agency (PMRA) a probabilistic ecological risk assessment (ERA) on the effects of acute and chronic exposure of aquatic invertebrates to imidacloprid. In that ERA, chronic effects derived from mesocosm studies were used to create a taxon-sensitivity distribution (TSD) based on no observed effects concentrations (NOECs) for abundance, emergence and mortality. While these taxonomic-level endpoints are relevant for the given population in question, they may not be as relevant for the overall aquatic community. To expand upon the initial TSD assessment, sub-lethal community endpoints such as diversity, feeding rate and abundance as they related to the overall community structure, not just effects grouped by taxonomic hierarchies, were examined. This community-approach involved re-evaluating higher-tier mesocosm studies previously identified as acceptable based on transparency and data quality with an eye for the above-mentioned community endpoints and compiling a dataset of their respective NOECs. The resulting dataset was organized based on organism taxonomy (i.e., class, subclass, family or subfamily), community effect, and the sampling method (i.e., emergence traps, artificial substrate samplers, Ekman sediment samplers and leaf packs). For each grouping, the most sensitive acceptable NOEC from each study was carried forward to calculate a geometric mean NOEC. Each NOEC was converted to a time-weighted average (TWA) to account for differences in study durations, retreatments and environmental breakdown of imidacloprid in mesocosms. Results of this community

assessment suggest that the structure and diversity of the emerging insect community (based on endpoints such as similarity indices, principal response, relative abundance and diversity index) is the most sensitive to imidacloprid (TWA NOEC between 0.39 and 2.47 $\mu g \cdot L^{-1}$) compared to other community components for which data were available (e.g., macrozoobenthos and zooplankton; TWA NOEC from 2.0 $\mu g \cdot L^{-1}$ to 18.67 $\mu g \cdot L^{-1}$). This range in community-level effects is relatively the same as the range of TWA NOECs for organism-level effects (0.58-13.7 $\mu g \cdot L^{-1}$) used to derive the TSD from a previous study. Therefore, the overall conclusion of this analysis is that imidacloprid NOECs for sub-lethal community endpoints derived from mesocosm studies as they related to the overall community structure are similar to NOECs grouped by a less holistic level of organization (i.e., the taxon level). Endpoint selection for regulatory decision-making should rely on environmentally relevant mesocosm studies when available and necessitated by lower-tier risk-assessment conclusions. This work demonstrates that effects on one, or a few, sensitive species or sensitive life-stages do not necessarily translate to adverse effects on the aquatic invertebrate community structure or function. Therefore, regulatory authorities should consider the use of toxicity thresholds based on community-level effects for regulatory decision-making.

An ecological risk assessment for thiamethoxam in Canadian surface waters: A weight-of-evidence approach

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The neonicotinoid insecticide thiamethoxam is widely used in agriculture across North America. It is detected more and more frequently in surface waters, in part due to increasing adoption by farmers to control crop pests. As a result, questions related to the ecological risk of thiamethoxam to aquatic ecosystems need to be addressed. To move our understanding forward, we used a weight-of-evidence approach to quantify the risk posed by thiamethoxam. All available toxicity data in the open literature were scored for their strength of experimental methods and the ecological relevance (survival, growth, development, and reproduction) of the responses reported. Numerical scores were assigned for strength and relevance. The means of the scores were then used to weigh the evidence for thiamethoxam contributing to ecologically significant responses. Our focus was on Canada, where the vast majority of measured concentrations are in the $ng \cdot L^{-1}$ range, with $\mu g \cdot L^{-1}$ observed under certain circumstances (e.g., downstream of greenhouses). There were also acute data of sufficient quality to create species sensitivity distributions (SSDs) for insects, generally seen as most sensitive, as well as less sensitive invertebrates (e.g., zooplankton). Together, the weight of evidence and SSDs revealed little likelihood of adverse outcomes for insects from acute exposure to thiamethoxam in terms of apical endpoints in most regions. There was effectively no risk observed for zooplankton, fish, and amphibians. Chronic exposures were less well characterized, and remain a point of significant uncertainty. We do know rapid removal (on the order of days) is expected for thiamethoxam, barring continuous inputs. We recommend that chronic exposure in the

environment be better characterized, and recognize the need for well-conducted mesocosm studies to reduce uncertainty for pesticide risk assessors.

Toxicity of diquat dibromide in aquatic vertebrates

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Aquatic herbicides are a group of pesticides used to control aquatic weeds and invasive plants in surface waters, but are poorly studied with respect to their adverse effects on non-target aquatic wildlife compared to agricultural-use pesticides. Diquat dibromide is a commonly used non-selective contact herbicide used to control crops (potato, cotton, and other seed crops), but also aquatic submerged and floating weeds. The toxic mode of action of diquat dibromide is due to the production of superoxide generated during photosynthesis, which damages plant cell membranes and cytoplasm, leading to desiccation. Although diquat strongly adsorbs to soil, organic matter, and plants in surface water, it is highly water soluble and is resistant to microbial degradation. Therefore, due to its complex nature and application methods, it not only has the potential for acute but also chronic exposure scenarios for non-target aquatic organisms. The present study examined the toxicity of diquat dibromide in a commercial formulation (Reward®) during acute, chronic and pulse waterborne exposure experiments in multiple life-stages of the fathead minnow (*Pimephales promales*) and rainbow trout (*Oncorhynchus mykiss*) and in larval stages of the Northwestern salamander (*Ambystoma gracile*). One of the main findings was that the early larval northwestern salamander was the least sensitive of these three species during acute exposures (LC_{50} between 33.7 and 152 $mg \cdot L^{-1}$), but during chronic exposures exhibited similar sensitivity compared to early life-stage fathead minnows and rainbow trout. Comparisons to the peer-reviewed literature show that the most acutely sensitive species is larval walleye (*Stizostedion vitreum*; 96-hour LC_{50}=0.75 $mg \cdot L^{-1}$); however, very few chronic exposure studies to diquat dibromide in fish and none in amphibians are reported. Therefore, this research presents novel chronic toxicity exposure data in two model fish and a poorly studied amphibian. These data will further aid in the development of risk assessment plans for managing invasive plant species, while mitigating effects on non-target aquatic wildlife.

Alternative lampricide treatments: Assessing effectiveness and physiological effects on lake sturgeon

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The lampricide 3-trifluoromethyl-4-nitrophenol (TFM) is used to control sea lamprey populations in the Great Lakes. Typical treatments involve the application of TFM in lamprey nursery streams at the minimum lethal concentration (MLC) of the sea lamprey ammocoetes (99.9% mortality over 9 hours). Lake sturgeon, a culturally important fish in the Great Lakes, are particularly sensitive to TFM during early development (juveniles <15-

20 cm). Since sturgeon can detoxify TFM via glucuronidation, we proposed that their detoxification capacity is overwhelmed during TFM treatments, leading to mortality. Therefore, we investigated the effectiveness of an alternative TFM regimen, where fish were exposed to a lower concentration of TFM for a longer time, to decrease the burden on their detoxification capacity. Consequently, ammocoetes and lake sturgeon were simultaneously treated with either the 9-hour or 24-hour MLC of the lamprey, over 9 and 24 hours, respectively. Lamprey mortality was 100% over the two treatments, while sturgeon mortality decreased from 65% during exposure to the 9-hour MLC, to <5% during exposure to the 24-hour MLC. In addition, the lake sturgeon were able to detoxify TFM three times more efficiently during the long and low TFM regimen compared to the 9-hour MLC exposure, typical of a TFM field application. This study revealed that when lamprey were exposed to the long and low TFM regimen, they exhibited a limited detoxification capacity of the lampricide. Analysis of mRNA expression of the glucuronidation gene UDP-glucuronyl transferase (UDP-GT), UDP-GT protein activity and tissue-specific energy reserves (glucose and glycogen) will offer insights into the TFM detoxification ability and energy allocation in both species and how these factors are influenced by exposure to TFM. Our current study has shown that the "long-and-low" TFM regimen is an effective treatment alternative that eliminates lake sturgeon mortality without compromising the effectiveness of the lampricide, and it has offered insights into the ability of lake sturgeon and sea lamprey to detoxify TFM.

Patterns in pesticides in Manitoba surface waters

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The widespread use of pesticides, herbicides, insecticides, and fungicides in a variety of agricultural and urban settings has been a concern for decades, owing to a large body of research detailing negative environmental effects to exposures. Since 1972, the Government of Manitoba (in collaboration with other stakeholders such as Environment and Climate Change Canada) have carried out pesticide monitoring in surface waters. Since 1995, Manitoba has collected and analyzed over 3,300 water samples for approximately 90 pesticides from over 200 sites in the province. The objective of the current study is to summarize a comprehensive analysis of existing provincial and regional patterns of pesticides in Manitoba surface waters. More specifically, this study aims to identify the pesticides that occur most frequently in Manitoba rivers and lakes, and describes the locations of known pesticide hotspots. Pesticide concentrations are compared to environmental quality guidelines for source drinking water, protection of aquatic life, and agriculture (i.e., irrigation and livestock water), after spatial and temporal trends (including seasonality) have been investigated to determine factors influencing their concentrations. Specific challenges and limitations can impact knowledge and understanding of monitoring pesticides in Manitoba surface waters (e.g., changing methodologies, dealing with concentrations below method detection limits, incorporating multiple levels of 'censoring' the data set, dealing with metabolites and isomers, and

pesticide mixtures). Recommendations are proposed to improve the efficiency and efficacy of Manitoba's pesticide monitoring, interpretation, and reporting program.

Direct analysis of glyphosate in wine using the QSight® 210 Laminar Flow Tandem Mass Spectrometer

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Glyphosate is a common herbicide used on crops to kill weeds. Due to its wide usage, it is not surprising that glyphosate has been detected in variety of foods. Recently, the International Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans". In lieu of regulatory bodies setting limits on glyphosate in food, it becomes imperative to develop robust and sensitive analytical methods for glyphosate detection. We present a study of glyphosate analysis in wine using triple quadrupole mass spectrometer with a unique "Stay Clean" interface. This allows for the direct analysis of wine with no sample preparation, detecting glyphosate in the low parts per billion and showing minimal signal loss (RSD ≤10%) over 300 injections, highlighting instrument robustness.

Detecting the imidacloprid from honeybee (*Apis mellifera*) exposure in the environment

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Honeybees (*Apis mellifera*) play a vital role in pollination of plants and crops, especially for wild plants in mountain areas in China. However, there are increasing reports on the decline of honeybees in many regions of China, especially in the past several years. To better understand the possible factors affecting honeybee health, we collected *Apis mellifera* samples from 14 provinces and detected the concentration of imidacloprid (IMD) residue in the honeybee under natural conditions. The results showed that imidacloprid can be found in samples from all provinces, and the levels of IMD from Zhejiang bee samples were significantly higher than those from Sichuan. The highest concentration of IMD was found in the samples from Zhejiang province, up to 34.28 $\mu g \cdot kg^{-1}$ per bee, while the lowest concentration was 18.86 $\mu g \cdot kg^{-1}$ per bee in samples from Sichuan province. These results indicate that pesticides should be considered as one of the factors affecting honeybee health, and that more attention should be paid to this possibility in the future.

Exploring the toxic mechanisms of pyrethroids on honeybee, *Apis mellifera ligustica* Spinola

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Free calcium ions in the neuroplasm of insects are important intracellular secondary messengers which play an important role in regulating the release and biological activity of neurotransmitters, as well as in regulating insect behavior. They are a target of many neurotoxic pesticides, and as we know, honeybees are sensitive to many insecticides whose toxic mechanisms are not clear. This study investigated the effect of deltamethrin (DM) on calcium channel in the brain nerve cells of adult workers of *Apis mellifera ligustica* Spinola that were cultured *in vitro*. The results showed that the intracellular calcium concentration was significantly elevated even with a very low concentration of deltamethrin (3.125×10^{-2} $mg\cdot L^{-1}$). Further testing revealed that the target of deltamethrin toxicity to honeybee is the T-type voltage-gated calcium channel, while it has no significant effect on the L-type voltage-gated channel, N-methyl-D-aspartate receptor-gated calcium channels, and calcium store. As the only low-voltage-dependent calcium channel in mammals, the T-type calcium channels can be activated at a very low membrane potential (-60 mV), resulting in depolarization of the membrane. This leads to the opening of the T-type calcium channels and influx of calcium, further inducing activation of multiple enzymes and opening of channels that outbreak action potential and nerve impulses, which in higher animals impairs neurological function: for example, epilepsy and Parkinson's disease in humans. The latest studies showed that abnormalities of T-type calcium channels will affect learning and memory-related activities in animals. Combining the results of past studies, it was found that DM may cause swarming, feeding, learning acquisition and other behavioral abnormalities, suggesting that the DM may act on the T-type calcium channel in brain cells of honeybees and result in abnormalities. These results highlight the protective measures existing or needed to protect honeybees against pyrethroid poisoning.

Water Quality Guidelines: Policy, Bioavailability, and Case Studies

A framework for assessing guidelines from alternative jurisdictions

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The Canadian Council of Ministers of the Environment (CCME) has been the primary interjurisdictional forum for development of Canadian environmental quality guidelines (CEQG). CEQGs for the Protection of Aquatic Life are currently developed under the guidance of the Guidelines Project Team of the Water Management Committee. There is growing recognition that timelines for guideline development are lengthy, while resources are limited. In order to be more responsive to policy needs, the Guidelines Project Team engaged a contractor to undertake an Alternate Water Quality Parameter Assessment, which reviewed guideline development protocols from alternate jurisdictions and developed a framework for assessing these guidelines for their similarity to CCME-derived guidelines. This presentation will provide an overview of the protocol assessment, the framework and key decision points which are relevant for assessing guidelines from alternate jurisdictions.

Guideline development with genomic data: How to determine data quality and what to do with it all

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Genomics-derived data provide a potential wealth of relevant information for the development of water quality guidelines. More and more species' genomes are being sequenced and transcriptomes analyzed through DNA microarray, RNA sequencing (RNA-seq), and quantitative real time polymerase chain reaction (qPCR) methods. Population compositions and species distributions are now being analyzed using metagenomics and environmental DNA (eDNA) methods. In order to get the most out of these powerful techniques, it is essential to be able to effectively evaluate genomic data quality and the appropriateness of interpretation. Specific examples will be used from aquatic wildlife to explore the concept of developing a checklist of requirements for reviewing genomics-based toxicity data for the eventual inclusion in water quality guideline derivation.

Characterization of metal toxicity with respect to interspecific diversity in the *Hyalella azteca* cryptic complex

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Cryptic species complexes, which are prevalent in freshwater invertebrates, present a challenge to water quality guidelines because they introduce uncharacterized interspecific variability. Classification on the basis of morphological similarities has erroneously grouped divergent taxa as the same species. However, modern molecular methods have improved the speed and reliability to identify such groups. *Hyalella azteca* is a freshwater amphipod broadly distributed across North America, and due to morphological similarities was thought to be a single species until recent molecular studies identified over 85 provisional species. This previously unrecognized species complex has been used in toxicity tests for over 25 years, and only recently has there been interest in investigating the effect of interspecific variability. Currently, the protocol for the use of *H. azteca* in water-only and sediment toxicity tests relies on morphological identification of species, neglecting molecular methods such as DNA barcoding. The application of toxicological investigations on this amphipod to water quality guideline derivation in Canada and the United States could result in values that are either too conservative or not conservative enough. The concern of cryptic species is particularly relevant to toxicity studies with *Hyalella* because they are among the most sensitive of model organisms. This presentation will summarize the responses of two broadly distributed *H. azteca* species to four metals. As *H. azteca* has historically been and continues to be used in toxicity tests, consideration must be given to potential species-level differences that could reduce the efficacy of water quality guidelines.

Implementation of bioavailability-based water quality guidelines that are time-variable

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Bioavailability-based approaches for establishing water quality guidelines (WQGs) incorporate the best tools (e.g., biotic ligand models [BLMs] or multiple linear regressions [MLRs]) for understanding the effects of water chemistry conditions on chemical bioavailability, but they present challenges in a regulatory context that is accustomed to representing WQGs as single values. For some metals, WQGs are hardness-based, reflecting that as hardness increases, toxicity decreases. When WQGs are based upon a single variable, it is reasonable to use a mean or geometric mean of that variable (e.g., hardness) to determine a WQG. When multiple water chemistry variables are used to derive a WQG, this does not necessarily make sense, because basing the WQG on mean values (or a percentile of each explanatory variable) does not necessarily represent an actual exposure condition. Instead, it makes sense to evaluate the WQG at each water chemistry condition and evaluate the potential for exceedance to establish a benchmark that represents the maximum concentration that would be considered compliant within the appropriate

regulatory context. We have developed an approach to do this for the purpose of deriving site-specific benchmarks that implement time-variable biotic ligand model (BLM)-based WQC in the United States. Recognizing that the same questions regarding implementation of time-variable WQGs will be relevant for Canada, especially if WQGs for metals move toward the BLM, we have explored the appropriateness of this approach for use in Canada. The fixed monitoring benchmark (FMB) approach is a probabilistic approach to establishing a single numeric target that can be used to evaluate compliance or attainment of WQGs or to establish site-specific water quality objectives (WQOs) that may be useful in deriving effluent permit limits. While this evaluation is exploratory in nature, it demonstrates that it is important to consider approaches for implementing time-variable WQGs or WQOs that are dependent upon multiple water chemistry characteristics that may vary in a site-specific manner (e.g., with various degrees of correlation). Utilizing an implementation approach that appropriately accounts for the time-variable nature of the WQG or WQO and the concentration of the constituent of interest preserves the scientific basis for using an approach such as the BLM for derivation of WQGs.

Pharmaceutical uptake and toxicity in larval zebrafish: Ionized and non-ionized drugs are differentially influenced by pH and dissolved organic carbon

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Human and veterinary pharmaceuticals have been observed in natural aquatic environments around the world, and many also impact fish health. While water composition plays an important role in the bioavailability and toxicity of contaminants such as metals, whether components of natural waters influence pharmaceutical interactions with fish is largely unknown. This study examined the impacts of pH and dissolved organic carbon (DOC) on the acute toxicity and bioavailability of waterborne pharmaceuticals in larval zebrafish (*Danio rerio*), a surrogate species. Drugs included sertraline (selective serotonin reuptake inhibitor; SSRI), fluoxetine (SSRI), diclofenac (nonsteroidal anti-inflammatory drug) and ethinyl estradiol (estrogenic). At pH of 6-8, sertraline (weak base, pKa=9.9), fluoxetine (weak base, pKa=9.8) and diclofenac (weak acid, pKa=4.0) would exist primarily (>98%) as ionized molecules. In contrast, ethinyl estradiol (weak acid, pKa=10.3) would be primarily un-ionized (>99%) at pH of 6-8. The un-ionized form is thought to be more bioavailable. The acute toxicities of sertraline, fluoxetine and diclofenac were influenced by pH over an environmentally realistic range (pH 5.8, 7, 8.2), and the 96-hour LC_{50} was related to the predicted concentration of un-ionized compound. For example, the sertraline 96-hour LC_{50} was 942 $\mu g \cdot L^{-1}$ at pH 5.8 and 229 $\mu g \cdot L^{-1}$ at pH 8.2, while the predicted percentage of un-ionized sertraline at each pH was 0% and 2.0%, respectively. In addition, terrigenous and autochthonous DOCs protected against sertraline toxicity. At environmentally realistic pharmaceutical concentrations (0.1 $\mu g \cdot L^{-1}$), sertraline uptake by larvae was 5.4-fold higher at pH 8.2 compared to pH 5.8, while DOC decreased sertraline uptake. In contrast, the uptake of ethinyl estradiol (0.1 $\mu g \cdot L^{-1}$) was not influenced by pH or DOC; ethinyl estradiol would be primarily un-ionized in those water chemistries. The impacts of these emerging contaminants of concern on fish may be

dependent on the water body into which they are discharged; however, the influence of pH and DOC on pharmaceutical uptake and toxicity appear to be predictable based on the physicochemical properties of the drug. These findings will improve environmental risk assessment and facilitate the development of site-specific water quality guidelines for pharmaceuticals.

Saturation binding kinetics of chronic exposures of metal mixture (Ni, Cu, Cd) to aquatic plant provides mechanistic insights

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Risk assessments for metal-elevated waters typically use environmental quality standards derived from studies of single-metal toxicity thresholds. However, geologic co-occurrence in the feed-stocks means that most metal-elevated sites are a mixture of metals. Extensive research into the mechanisms of interactions of trace metals with water chemistry factors (pH, major cations) has been done to develop the Biotic Ligand Model (BLM); however, little knowledge of interactions among co-existing trace metals exists. Here, we aim to gain mechanistic insights into the impact of mixtures on metal–BL binding in aquatic plant *Lemna minor* under alkaline conditions (pH 8.3). Metal accumulation in tissues from single versus ternary mixture exposures was characterized using saturation binding principles derived from enzyme kinetics. The basis is that a fixed number of BL binding sites exist, which become saturated and can be fitted to a Michaelis–Menten saturation curve. The occupancy of BL binding sites by a metal may be limited, or not, by the co-occurrence of another metal. No prior studies have exploited saturation binding to explore metal–BL binding for phytotoxicity; hence the importance of the present work for ecotoxicological risk assessment of mixtures. Results showed that Ni binding to the BL was independent of co-exposure to other metals, while Cu experienced anticompetitive inhibition (Cd or Ni binds to Cu–BL complex only) and Cd experienced noncompetitive interaction (Cu or Ni binds to BL and Cd–BL complex). In addition, copper inorganic complexes (hydroxides, carbonates) played a role in metal bioavailability in single metal exposure but not in mixtures.

Fluorescence-based methods for metal (Cu, Ni) speciation in marine systems: Estimation of bioavailability

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Risk assessments and regulatory criteria for metals are ultimately dependent on measured effects concentrations. These effects are potentially mitigated by ligands in the specific receiving water. Such ligands potentially complex metals and decrease their bioavailability and associated toxicity. Ultimately, the toxicity of dissolved metals depends on the specific chemical form of the metal, i.e., metal speciation. Prediction of metal speciation and associated toxicity/risk in salt water using software developed for fresh

water, i.e., the Windermere Humic Aqueous Model (WHAM) is not trivial because not only does the higher ionic strength affect chemical reactivity (i.e., large activity corrections) but marine organic matter is not necessarily chemically the same as organic matter of terrigenous origin. To address this potential modelling gap experimentally, a fluorescence-based method was developed in order to predict free ionic forms of metals in the presence of marine dissolved organic matter at full strength salinity. The method initially involves scanning excitation and emission wavelengths simultaneously to generate a 3-D fluorescence map of each source water. The major features of this map are then captured using variable angle synchronous spectra "slices" through the surface. As Ni or Cu is titrated into the sample at fixed pH, the change in fluorphore intensity is monitored. This change, either enhancement or quenching, can be fit to a metal complexation model embedded in an inorganic speciation "side-reaction" model to determine logK and binding capacity ($\mu mol \cdot mg^{-1}C$) values for the organic complexation. In turn these values can be used to determine the free ion for any conditions of total metal and dissolved organic carbon (DOC) in a site-specific manner. This method has been applied to samples from the east and west coasts of North America and the resultant speciation data, i.e., free ion estimates, were compared to the EC_{50} for rotifer and embryo (mussel and urchin). The results show that at the EC_{50} the free ion in the various samples is the same even though the total metal-based EC_{50}s are highly variable. In addition, the free ion estimates at the EC_{50} of samples containing organic matter match the calculated free ion EC_{50} value for artificial seawater control samples. These results demonstrate that bioavailability-based corrections are possible using a very simple analytical method in a site-specific manner. These results can be used to calibrate metal-specific seawater speciation models for future use as a bioavailability estimation tool.

Development and evaluation of a method for estimating missing chemical parameters in the Biotic Ligand Model

Kelly Croteau[1], Robert Santore[1], Adam Ryan[1]

[1]*Windward Environmental*

The Biotic Ligand Model (BLM) has been widely used as a means for explaining and predicting how water chemistry affects metal toxicity to aquatic organisms. Recent versions of the BLM have added an option to estimate full ion chemistry from several simple chemistry parameters. The full list of chemistry parameters required for the BLM includes pH, dissolved organic carbon (DOC), major cations (Ca, Mg, Na, and K), major anions (SO_4 and Cl) and alkalinity. The simple chemistry inputs use hardness in place of all of the individual ion concentrations and estimates alkalinity from pH and atmospheric CO_2 (g). The method employed to convert the simple chemistry into full chemistry utilizes ion ratios (calcium by each of the other 3 cations, and sulfate by chloride) and the partial pressure of CO_2 (g) in the atmosphere to estimate all six major ions and alkalinity from hardness and pH using basic charge- and mass-balancing concepts and carbonate equilibria relationships. In the absence of an approach for estimating missing chemistry measurements, the lack of complete chemistry for some water bodies can become an implementation hurdle for water quality guidelines that are based on the BLM. This

estimation method expands the applicability of the BLM by making it possible to run the model when some or all of the detailed ion information is missing, while providing a standard for chemistry estimation to ensure some consistency between users. The advantage of this approach is that it can provide chemical inputs to the BLM that are geochemically reasonable and mimic the distribution of ions for a given region. Although there is a clear conceptual basis for this estimation method, and the final model results are generally close to those using full chemistry, the application of the BLM to water quality standards (WQSs) warrants taking a closer look at the uncertainties and errors that can arise when this method is used. Among the questions that will be addressed in this presentation are how WQS calculated with the estimation method compares with WQS developed from full chemistry, whether there is a systematic bias, and suggestions for improvement of this method.

Revisiting the chemosensory-based Biotic Ligand Model (BLM) for copper: How much can we borrow from the gill-based BLM?

William Dew[1], Greg Pyle[2]

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

The current copper Biotic Ligand Model (BLM) was designed to prevent fish mortality by predicting safe copper concentrations using site-specific water quality measures. Research has demonstrated that there are nonlethal effects of copper at concentrations far below those that cause lethality. One of these effects is a loss of olfactory function and olfactory-dependent behaviours. Attempts have been made to produce a chemosensory-based BLM (cbBLM) designed to predict safe copper concentrations that are protective of chemosensory function. These attempts were based on the gill-based BLM (gbBLM), and assumed that the water quality parameters important to prevent lethality would also protect against loss of chemosensory acuity. Unfortunately evidence does not support this assumption, and there are still gaps in the literature that need to be filled. The question that arises, then, is how much of the gbBLM is applicable to the cbBLM? The purpose of this talk is to parse out the various elements of the gbBLM and see if they also apply to a cbBLM based on the literature, as well to chart a path from what we currently know to what we need to know if a cbBLM is to be considered useful.

An equilibrium partitioning theory sediment guideline based on the Biotic Ligand Model

Robert Santore[1], Adam Ryan[1], Kelly Croteau[1]

[1]Windward Environmental

The Biotic Ligand Model (BLM) is a computational framework used for predicting the effects of water chemistry on metal bioavailability. In this presentation we describe the development of an extension of the BLM framework that can be applied to mixtures of multiple metals (the mBLM). The mBLM was used to predict toxicity in chronic exposures with *Hyalella azteca*. Calibration of the mBLM to *H. azteca* was accomplished by

comparison with water-only toxicity tests. Equilibrium partitioning theory predicts that the toxic effect that may result from exposure to sediment pore-water is similar to effects resulting from exposure to the water column. The calibrated model was then used to predict whether field sediments from several sites characterized by elevated metal concentrations would be toxic to *H. azteca*. Predicted toxicity was compared with measured toxicity in chronic tests with *H. azteca* from several field sites and high predictability was observed. The mBLM analysis allows sediments to be ranked to identify which samples would be most likely to cause toxicity. The mBLM can also identify which metals in the mixture of metals are most likely to be responsible for any observed toxicity. The overall good performance of the mBLM suggests that it is a powerful predictive tool for use in assessing risk from metal concentrations in field sediments and could be used as part of a general methodology in the development of sediment guidelines.

Update on British Columbia copper water quality guidelines for protection of aquatic life

Ali Azizishirazi[1], Angeline Tillmanns[1], Robert Santore[2], Kelly Croteau[2]

[1]BC Ministry of Environment and Climate Change Strategy, [2]Windward Environmental

The first (and current) copper Water Quality Guideline (WQG) for the protection of aquatic life in British Columbia was published in 1987. Since then, a great deal of research has been conducted on copper toxicity and the factors that influence copper toxicity. Therefore, an update to the copper WQG based on the most recent science is required. Following the British Columbia Ministry of Environment (BC ENV) protocol for WQG development, a thorough literature review was conducted to compile a toxicity database for B.C. species. As bioavailability-based models have shown promise in predicting toxicity across different water chemistry conditions, BC ENV retained Windward Environmental to develop a modified version of the Biotic Ligand Model (BLM) to enable the calculation of bioavailability-based guidelines. The BC ENV BLM is designed to calculate a water chemistry-specific copper WQG that follows BC ENV's policy to protect the most sensitive aquatic species at the most sensitive life stage. The BC ENV BLM is based on several toxicity-modifying factors and calculates chronic and acute WQGs values. It includes a "simplified" feature which requires only water temperature, DOC, pH and hardness, and estimates the remaining toxicity-modifying factors based on BC-specific ion ratios. The BC ENV copper WQG is in the test phase and will be released for review in the near future.

Case study: Derivation of site-specific water quality objectives and setting an industrial discharge criterion for formaldehyde

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The Ontario Ministry of the Environment identified elevated concentrations of formaldehyde in the effluent of an industrial facility that exceeded the provincial water quality objective (PWQO) of 0.8 $\mu g \cdot L^{-1}$; median effluent concentrations were 245 $\mu g \cdot L^{-1}$. The

Province required the discharger to set a limit for formaldehyde in the effluent to protect the receiving environment and requested that the operator propose a discharge criterion for formaldehyde and a site-specific water quality objective (SSWQO). The criterion was to be guided by a scientifically defensible chronic water quality objective (WQO) and incorporate an understanding of site-specific factors that may modify the risk to aquatic life. This objective posed a challenge as insufficient chronic studies had been conducted to derive a Type A or Type B guideline following Canadian Council of Ministers of the Environment (CCME) standard protocols for deriving WQOs, and the local receiving environment had not been well characterized. Our approach combined guidance from both the CCME and the United States Environmental Protection Agency (USEPA) for deriving SSWQOs and chronic WQOs using an acute to chronic ratio (ACR). First, an acute SSWQO was derived using a species sensitivity distribution model (SSD Master Version 3) with 12 fish species, 4 amphibians and 7 aquatic invertebrate species. This acute SSWQO was used as a limit to ensure that effluent was not acutely lethal to aquatic life at the point of discharge. The ratio of acute to chronic toxicity was calculated for species for which both acute and chronic studies had been conducted. No chronic studies had been conducted on fish so a conservative ratio proposed by the USEPA was applied. The final chronic SSWQO was derived dividing the acute SSWQO by the geometric mean of the ACRs. This chronic SSWQO was set as a maximum objective for the receiving environment. Field investigations between 2013 and 2016 determined the magnitude of formaldehyde loss from between the last point of control and the receiving environment under varying conditions. Results from those investigations indicated that formaldehyde decreased on average by 55%; reductions ranged from 11% to 80% and were highest in wet weather. An understanding of formaldehyde's environmental fate directed a Kendall rank correlation coefficient analysis of other measured water quality and abiotic factors, indicating dilution and volatilization via increased turbulence were the predominant predictors of formaldehyde concentrations in the receiving environment. The proposed discharge criterion was conservatively set to protect against acute lethality and to meet the derived chronic SSWQO. This introduces an additional degree of protection for aquatic life as formaldehyde concentrations are expected to decrease in the effluent prior to reaching the aquatic environment.

Issues related to developing a water hardness-based water quality guideline

Sarah Bogart[1], Eric Stock[1], Greg Pyle[1]

[1]*University of Lethbridge*

An abundance of literature has shown that an increase in salinity, or the total ionic content of water, can be toxic to freshwater aquatic life, and anthropogenic salinization of freshwaters is increasing. More recent research has shown that shifts in the balance of major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO^{3-}, SO_4^{2-}, Cl^-) in water can also be toxic, meaning that salinity alone does not fully describe risk to aquatic life. Despite this, and although a Ca water quality guideline (WQG) exists for the protection of agricultural water uses, of these major ions, only WQGs for the protection of aquatic life exist for Cl- and $SO4^2$-. Yet, increases in the Ca^{2+} and Mg^{2+} content of water alone (i.e., water hardness) can likewise be toxic to aquatic organisms. Anthropogenic sources that have the potential to increase water

hardness in receiving waters to potentially toxic concentrations include effluent from coal mining and soda ash production, and produced waters from (for example) oil and gas extraction, agricultural drain water, and the use of Ca- and Mg-based road de-icers. The development of a water hardness WQG for the protection of aquatic life is therefore appropriate. To this end, relevant literature was acquired, reviewed, and classified for potential inclusion in a WQG following federal and provincial protocols for WQG development. During this process, several major data gaps in and issues with the currently available literature were identified. These challenges will be discussed in the context of how other jurisdictions have regulated related, and often confounding, water quality parameters (e.g., total dissolved solids (TDS) or salinity via conductivity), the need to consider additional confounding factors (e.g., alkalinity), potential regulatory options for a water hardness WQG, and what is required in terms of future research and improved data consistency.

Marine sediment quality guideline for PCBs: Updating Guidelines Using Multiple Lines of Evidence

Angeline Tillmanns[1], Justin Lo[2], Frank Gobas[2], Ali Azizishirazi[1]

[1]BC Ministry of Environment and Climate Change Strategy, [2]Simon Fraser University

Polychlorinated biphenyls (PCBs) are a group of legacy contaminants that were banned from commercial production in the 1970s. The high persistency and hydrophobic properties of PCBs cause them to biomagnify and high levels of PCBs have been detected in marine mammals along the coast of British Columbia. Given the chemical properties of PCBs, they are most highly associated with sediments in the marine environment. The current B.C. marine sediment quality guideline (SQG) is designed to be protective of benthic invertebrates, however the biomagnification of PCBs in marine mammals was not specifically considered. Recent scientific studies suggest the B.C. marine SQG is significantly higher than the PCB concentrations in sediments which result in the accumulation of PCBs in marine mammals to levels which are detrimental to animal health. These conclusions were based on both ecological models and empirical calculations using a biotic sediment accumulation factor approach. However, studies have also shown that ambient sediment concentrations in some areas of the B.C. coast already exceed concentrations considered to be protective of marine mammals. Other jurisdictions, such as Washington and California, have adopted ambient PCB concentrations rather than guideline values that are below ambient concentrations to guide resource management. This talk will review the multiple lines of evidence that can be used to update a PCB marine sediment quality guideline for British Columbia.

Drafting a guide for developing water quality objectives and effluent quality standards for quartz mining projects in the Yukon

Brendan Mulligan[1], Bill Slater[2]

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The Yukon Government, with input from Yukon First Nations, Yukon non-governmental organizations (NGOs), Yukon assessment and regulatory agencies, and industry, is presently undertaking the Mine Licensing Improvement Initiative (MLII), which involves the development of technical guidelines to improve administrative and operational practices for regulating and licensing quartz mines in Yukon. Through this process, a draft "Yukon Guide for Developing Water Quality Objectives and Effluent Quality Standards for Quartz Mining Projects" (the "draft Guide") has been developed. The draft Guide provides technical and scientific guidance about approaches and procedures for developing Water Quality Objectives (WQOs) for freshwater ecosystems in Yukon. WQOs provide narrative and numerical definitions of acceptable water quality conditions in specific receiving waters that may be affected by a project. The Guide identifies three water management approaches (non-degradation, use-protection, use-restoration) and associated narrative WQOs. A water management approach is selected based on the social, economic and environmental value, and condition of the receiving waters. It not only defines the narrative WQO, but also forms the foundation for selecting procedures for developing numerical WQOs. The Guide describes five such procedures for developing numerical WQOs for specific Contaminants of Potential Concern (COPCs): adoption of generic water quality guidelines as WQOs, the background concentration procedure, the recalculation procedure, the water effect ratio procedure, and the resident species procedure. The Guide also describes methods for using the WQOs to derive Effluent Quality Standards (EQSs). EQSs are the maximum concentrations of COPCs that a project is authorized to release in liquid effluent, usually specified in water licences issued by the Yukon Water Board. EQSs are calculated based on the dilution available in the receiving environment and consideration of the portion of the assimilative capacity that may be allocated to a specific discharge. Assimilative capacity is the difference between pre-development background water quality and the most sensitive use-protection water quality objective. Allocation of assimilative capacity considers a range of factors, including existing and anticipated future uses and needs. In Yukon, it has also been used to address treaty rights of First Nations that relate to water quality. The development of the draft Guide was completed by Slater Environmental Consulting, based on technical content and recommendations provided by MacDonald Environmental Sciences Ltd., and a project team composed of representatives from Yukon Government (Departments of Environment and Energy, Mines and Resources), the Yukon Water Board Secretariat, and the Yukon Environmental and Socio-economic Assessment Board. The draft Guide integrates principles and guidance on development of WQOs from several sources, including the British Columbian Ministry of Environment and the Canadian Council of Ministers of the Environment. A peer review of the draft Guide has been conducted by Minnow Environmental Inc. The Guide has also been reviewed by Yukon First Nations, NGOs, and industry.

Behavioural toxicology in water quality guideline development

Greg Pyle[1]

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A considerable amount of research attention has recently been devoted to understanding how environmental toxicants affect aquatic animal behaviour. However, there has been some resistance in accepting behavioural endpoints for consideration in water quality guideline development. Some reasons for this resistance include a lack of standardized methods for collecting behavioural data, behavioural data are inherently variable, and only a few studies attempt to link laboratory-based behavioural results to natural populations, among others. However, behavioural toxicology has advanced considerably in recent years owing to the development of high-throughput, automated, and in some cases standardized techniques, advances in our understanding about the physiological basis of contaminant effects on chemosensory-mediated behaviours, and increasing evidence that behavioural results generated under laboratory conditions can be observed in natural environments. In this presentation, I will summarize the state of the science and discuss both the pros and cons of behavioural endpoints for consideration in water quality guideline development using an adverse outcome pathway framework.

When is Clean, Clean Enough? A Discussion of Wastewater Treatment Technologies in Relation to Aquatic Organism Health

Our last discharge: Quantifying funeral home sewer discharges

Mark Payne[1], Sonya Kleywegt[2]

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Municipally-owned sewage treatment plants (STP) are widely viewed as point sources for emerging contaminants to surface water bodies. Provincial and federal regulators typically focus on these plants to reduce loadings to surface water bodies. This has been an effective approach to traditional contaminants, but emerging contaminants are not as easily reduced or removed during typical wastewater treatment processes. The present wastewater treatment infrastructure represents a significant public investment and works very well to prevent nutrients and heavy metals from entering watersheds, but its efficacy at removing emerging contaminants is much less defined. In general the discharge of contaminants from industrial, commercial and institutional (ICI) facilities in the province is regulated at the municipal level of government. Each municipality establishes their own sewer-use bylaw limits for ICI facilities that discharge directly to the receiving STP. Due to cost of treating emerging contaminants, it may be more cost effective to review onsite treatment of select ICI facilities to reduce their impact to the receiving sewershed and ultimately, the receiving water bodies. As of 2014, there were more than 700 funeral homes operating in the province of Ontario, with 20 located in the Regional Municipality of York (York Region). During the body embalming process, embalming fluids are used to replace the blood and bodily fluids of the deceased, and the bodily fluids are washed down the drain. This survey examined the direct wastewater discharged from 8 funeral homes in York Region. A total of 50 samples were collected in 2016 during the embalming process from the funeral homes' lotline sewer maintenance access hole. Samples were analyzed for a suite of contaminants including nutritive, metals, formaldehyde, legacy pesticides, PCBs, pharmaceuticals, nonyl phenols and other emerging contaminants. Surprisingly, none of the funeral homes had any form of on-site wastewater treatment. The lack of treatment prior to discharge allows contaminants to flow freely into the sewer system. Select legacy pesticides, pharmaceuticals, and other emerging contaminants were detected at most sites, and metals were detected below current sewer-use bylaws. Formaldehyde, a key contaminant in embalming fluids was detected at levels known to be toxic to select aquatic organisms, reaching maximum concentrations of 561 $mg \cdot L^{-1}$. The average funeral home yearly loading of formaldehyde to the sewershed was estimated to be 125 Kg. Given the level of formaldehyde and other contaminants measured, it is possible that small sewersheds may experience significant impacts at their STP that could reduce plant efficacy, and that onsite pretreatment of funeral homes' discharges may be a risk management option for municipalities.

The impact of toxicity on wastewater

Cary Morris[1]

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Through ecotoxicology, we have the ability to observe the impact that chemicals will have on ecosystems. Toxicity testing is a form of monitoring used widely across the globe as a check on that impact. There are many methods of measuring toxicity ranging from simple bacteria *Daphnia*, to higher life forms such as fish. These methods can be used as biological early warning systems. Here, we will focus on bacterial toxicity testing, specifically focusing on that of wastewater monitoring. Wastewater monitoring can help in many ways, not only to assure regulatory compliance of your effluent, but also monitor the impact that the influent can have on your system, and the impact your discharge will have on the environment. Using the Microtox® range of products, wastewater can be screened quickly and accurately at all points in the system. There are many options with these products, including the completely portable DeltaTox II, the lab based Model 500, and the continuous monitoring system, Microtox CTM. We will discuss these technologies and how they can help optimize wastewater monitoring programs across different applications and industries.

We can measure what is in municipal wastewater, but do the fish care?

Joanne Parrott[1], Sean Backus[1], Vince Pileggi[2], Shahram Tabe[2], Wayne Parker[3]

[1]Environment and Climate Change Canada, [2]Ontario Ministry of Environment and Energy, [3]University of Waterloo

Fathead minnow lifecycle exposures have been conducted in our lab for several individual pharmaceutical compounds (ethinylestradiol [EE2], propranolol, venlafaxine) and their mixtures. We have also assessed several complex municipal wastewater effluents (MWWEs). In some cases, MWWE exposures caused decreased survival, growth, and breeding success. However, many MWWEs that still contained measureable concentrations of many pharmaceuticals and personal care products (PPCPs), did not impact fish survival, growth, or reproduction. It is clear that EE2 in minute quantities can affect fish reproduction and wild fish populations. Relationships for other PPCPs are not as clear, with most compounds in our lifecycle lab fish tests showing no impacts when exposure concentrations are environmentally relevant. In cases where MWWEs affected fish reproduction, it was often difficult to separate out overt toxic effects (from ammonia exposure, for example), from effects caused by low concentrations of PPCPs. If MWWEs can be identified that cause no effects in fish, perhaps we can live with the low concentrations of PPCPs, especially if the fish seem to be ok with them?

Utility of different tests to detect the reproductive effects of municipal wastewater effluents

Glen Van Der Kraak[1], Cory Schilling[2], Mark Servos[3]

[1]University of Guelph, [2]Integrative Biology, University of Guelph, [3]University of Waterloo

We conducted lab-based experiments to determine the potential of effluents from different municipal wastewater plants to affect the reproduction of adult zebrafish (*Danio rerio*). The endpoints measured included whole-animal reproductive performance based on the numbers of eggs spawned over a 7-day exposure period and biomarkers of reproductive function, including the levels of sex steroid hormones levels in ovarian tissue and the expression of selected genes in the ovary. In studies conducted in 2013 and 2015, a 50% dilution water collected immediately downstream of the wastewater treatment plant in Waterloo, Ontario, resulted in a significant reduction (50 and 85% in the two years) in the numbers of eggs spawned. In contrast, effluent collected downstream of two different sewage treatment plants (Kitchener and Guelph, Ontario), an upstream reference site on the Grand River (Hespler) and the laboratory control water had no effects on spawning success. Measurement of ovarian testosterone and 17β-estradiol levels did not reveal an effect for fish exposed to the Waterloo effluent whereas zebrafish exposed to the Guelph effluent had lower levels of 17β-estradiol but only for the 2013 study. Expression of genes involved in steroid hormone biosynthesis including steroid acute regulatory protein, aromatase and the luteinizing hormone receptor also failed to reveal a consistent pattern of response. A reduction in aromatase expression was seen in fish exposed to the Waterloo effluent in 2013 but not 2015, whereas the other genes were not affected. Similarly, fish from the Guelph site showed a reduction in aromatase in the 2013 but not in the 2015 studies. We detected high concentrations of ammonia in the Waterloo effluent, which we suspect is responsible for the reproductive impairment. Indeed other studies in which zebrafish were exposed to ammonia led to impairment in spawning success but inconsistent effects on steroid hormone levels or the expression of genes involved in steroid hormone biosynthesis. Collectively these studies suggest that the inclusion of endpoints of whole-animal performance such as spawning success are a far more robust screen of reproductive toxicants than measurements of hormone levels or gene expression, particularly when the underlying mechanisms of reproductive impairment are unknown.

Municipal wastewater treatment plant effluent-induced effects on freshwater mussels

Patricia Gillis[1], Joseph Salerno[1], Shane de Solla[1], Mark Servos[2], Rodney McInnis[1], Erin Leonard[3]

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

The relationship between municipal wastewater treatment plant (WWTP) effluent exposure and biological responses within aquatic ecosystems remains poorly understood, especially at the population level. To examine the effect of WWTP effluents on sentinel invertebrates, freshwater mussels were assessed in the Grand River, Ontario, in populations associated with the outfall of a major municipal WWTP. This watershed has a diverse community of twenty-five species of mussels, including nine Species at Risk, and is representative of many habitats that receive WWTP effluents. Population surveys were

conducted to assess the presence and species richness of freshwater mussels. In total, 55 sites downstream of the WWTP were examined using timed visual searches with one or two hours of effort spent searching 100 m long river segments. Although seven species of mussels were found in moderate abundance (mean of 8 mussels per hour of searching across two sites) immediately upstream of the WWTP outfall, no live mussels were observed for 7.0 km downstream of the WWTP. Long-term water quality monitoring data indicate that ammonia and nitrite concentrations along with large seasonal declines in diel dissolved oxygen were associated with the extirpation of mussels downstream of the WWTP. The first live mussels found downstream were below the confluence with a major tributary indicating that in addition to an improvement in water quality to a state that enables mussels (and/or their fish hosts) to survive, a nearby mussel refuge in the tributary may have facilitated the recolonization of the depauperate WWTP-impacted zone.

Assessing the health of fish exposed to municipal wastewater across levels of biological organization

Mark Servos[1], Meghan Fuzzen[2]

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

Municipal wastewater represents one of the largest sources of effluent to Canadian aquatic ecosystems. These effluents contain a diversity of contaminants, including those than can alter endocrine function and reduce reproductive performance of fish. Recent studies in the central Grand River have demonstrated that a wide variety of endocrine active compounds (e.g., natural estrogens, ethinylestradiol, triclosan) are released into the environment from municipal wastewater outfalls. Fish populations, including rainbow darter (*Etheostoma caeruleum*), associated with these outfalls have shown a variety of biological changes including altered gene expression (i.e., transcriptomes), physiology (e.g., steroid production), energy storage and reproductive success (e.g., egg survival). Of particular note has been the extremely high incidence and severity of intersex (developing eggs in testes tissue) below the outfalls. The Region of Waterloo is currently investing hundreds of millions of dollars to upgrade several treatment plants in the watershed. More efficient aeration of secondary clarifiers, increased retention time, and a switch from chlorine to UV disinfection in the Kitchener treatment plant led to an increase in nitrification and decreased estrogenicity of the effluent. In the three years since these changes, a recovery of several biological endpoints in fish, including steroid production and intersex, has been observed. Some effects, such as gene expression and low incidence of intersex, remain evident and are likely due to residual chemicals, upstream urbanization and other wastewater effluent outfalls. However, these subtle changes are difficult to separate from natural variability. This leads to the question of, “How clean is clean enough?”

Panel discussion: When is clean, clean enough?

Mark Servos[1], Meghan Fuzzen[2]

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

Wastewater effluents from municipal and industrial sources are known to contain complex mixtures of chemicals, including some that are endocrine disrupting. Endocrine disrupting compounds (EDCs) have the potential to have severe consequences for populations of aquatic organisms, as demonstrated by the collapse of the fathead minnow population in a lake dosed with the active agent in the birth control pill (Kidd et al. 2007). In addition to removal of pathogens and organic waste, treatment of effluent has been shown to remove some EDCs. Advanced treatment (secondary or tertiary processes) can remove an even larger proportion of EDCs. Some countries are moving to implement tertiary treatment which has additional infrastructure and operational costs and challenges. How do we determine when additional improvements are necessary? Are there criteria or a philosophy that should be applied? Can we monitor for effects of interest and if so at what level of biological organization and how do we assess significant change? The question posed by the chairs of this session is, "When is clean, clean enough?" The panel discussion will include a representative from each level of government (municipal, provincial and federal) and academia.

Assessing Natural Variability: How Different is Too Different?

A structured framework for interpreting monitoring triggers from long-term programs

Kelly Munkittrick[1], Tim Arciszewski[2]

[1]Canada's Oilsands Innovation Alliance (COSIA), [2]Alberta Energy Regulator

Environmental effects monitoring is a form of adaptive monitoring that is more than 25 years old. While there is generic guidance for designing monitoring programs, there is less formal guidance for interpretation. Although there has been a lot of work over the last five years in advancing the statistical approaches for triggers for initiating shifts in tiers of monitoring, there is not any structured formal guidance for decision-making beyond simple numerical comparison. As chemical analytical approaches become more sensitive, and as research and monitoring efforts intensify around the oil sands, the increased sensitivity and power improve our capacity to detect change. The capability to detect change needs to be balanced by more rigour in interpreting the information, especially in long-term programs where overpower in designs is a concern. A structured, rigorous framework can be used to meaningfully inform environmental monitoring programs, and needs to consider potential exposure and adverse outcome pathways, potential ecological relevance, and relative risks to focus efforts. Understanding industry activities and processes as well as decision contexts is important for orienting the research in a way that can drive change in environmental performance. This presentation will use case studies of recently published studies to evaluate an interpretation framework that could be used to focus monitoring and improve the translation of monitoring information into meaningful change.

Developing and applying normal ranges of flow-corrected residual concentrations for water chemistry variables in the Athabasca River

Tim Arciszewski[1], Rod Hazewinkel[2], Kelly Munkittrick[3], Bruce Kilgour[4]

[1]Alberta Energy Regulator, [2]Alberta Environment and Parks, [3]Canada's Oilsands Innovation Alliance (COSIA), [4]Kilgour & Associates

Normal ranges of variation are more commonly being used to evaluate change in environmental monitoring programs. In an Adaptive Monitoring program normal ranges are delineated by thresholds and operationalized in general surveillance as a trigger to focus scientific attention and employ more specific and detailed diagnostic procedures. Ideally, sensitivity of the thresholds can be increased by accounting for known sources of variation, such as the influence of discharge on water chemistry. Using discharge as a covariate and regression techniques suitable for censoring, several types of thresholds (annual and 6-year occurrence rates computed for the observed exceedances of the 2.5, 50, and 97.5 percentiles and unusual sequences of data) were applied to residual concentrations of 4 selected water chemistry variables measured routinely in the lower Athabasca River: chloride (Cl^-), sulphate (SO_4^{2-}), total vanadium (V_t), and total aluminum (Alt). The normal ranges suggest the chemistry of water in the Athabasca River has been changing since 2010. Among the multiple thresholds used, Alt has shown changes at a site

downstream of oil sands development since 2010, but V_t has not, suggesting an influence of mining dust. SO_4^{2-} showed the most compelling results. Several hypothetical sources of SO_4^{2-} are possible, including changes in local groundwater chemistry and discharge, sulphur emissions from upgrader stacks, export from wetlands, or basin-wide changes. Trends within the baseline period also suggest the changes detected here began prior to 2010. Regardless of the specific origin of the changes, the results here demonstrate the utility of flow-corrected thresholds and multiple analytical techniques to detect change in water chemistry.

Robust regression to estimate water quality triggers

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Water quality inevitably changes over time, by season, with flow, etc. Depending upon how natural variability is defined, these naturally varying confounding factors (structural variables) may or may not be accounted for when estimating water quality triggers. If it is deemed that some structural variables should be acknowledged when estimating triggers, the data may be simply subdivided and simple quantile estimators be used to estimate triggers. One weakness of this approach is that subdividing data is only possible for discrete structural variables such as seasons. Another is that the smaller sub-data sets will lead to less precisely estimated triggers than triggers estimated using methods that allow for control of structural variables. The effects of structural variables can be modeled using general linear models (GLMs). Commonly used GLMs such as ANOVA and ANCOVA have particular assumptions (normality, lack of censoring, lack of correlation) that are not likely to be met when modelling water quality data. Robust GLMs sidestep some of these issues. Robust GLMS are used to estimate measures of central tendency while controlling for season.

Basal plasma proteomic variation and perturbation responses in white sucker (*Catostomus commersonii*) from various Canadian ecosystems

Denina BD Simmons[1], James Sherry[2]

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The white sucker (*Catostomus commersonii*) is a freshwater cypriniform fish that is nearly ubiquitous in all middle-to-northern regions of North America. White sucker feed by sucking up organic matter, plants, algae, and invertebrates from the benthos of rivers and streams. Because of these features, white sucker is commonly used as an organism in Canadian environmental effects monitoring programs. Throughout the course of multi-year field collections and multiple laboratory exposure studies, we have collected an extensive proteomic dataset for white sucker plasma. Plasma has many advantages for use in bio-monitoring, particularly given its potential for non-lethal sampling, and as a circulating bio-fluid, it is representative of whole organism responses. A drawback of plasma, however, is that there is higher innate variation among individual protein levels. In this presentation,

we will discuss plasma proteome variation in white sucker by comparing field versus laboratory, control/reference versus exposed, and male versus female fish. We will also describe how these data can have utility for future monitoring programs that include omics studies using white sucker plasma.

Using a conductivity-alkalinity relationship as a tool to identify surface waters in reference condition across Canada

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The underlying natural relationship between conductivity and alkalinity was used to identify surface water-quality monitoring sites that are in a "reference" or minimally disturbed condition. Data from over 40,500 surface water samples from 1,230 stations were combined for the time period 2005-2015 from various federal, provincial, and joint federal-provincial/territorial freshwater monitoring programs (e.g., Freshwater Quality Monitoring and Surveillance Program, Ontario's Provincial Water Quality Monitoring Network). Of the samples, 30,357 provided conductivity and alkalinity data. Surface water samples with a measured conductivity that deviated (by more than 41 $\mu S \cdot cm^{-1}$) from the predicted conductivity calculated from the sample's alkalinity were deemed to be non-representative of a reference condition, while samples within 41 $\mu S \cdot cm^{-1}$ of the predicted value were deemed representative of a reference condition. The 41 $\mu S \cdot cm^{-1}$ cutoff value was determined using signal detection theory. This approach to identifying reference condition was validated by demonstrating that samples producing 'reference' samples were typically from catchments that had minimal anthropogenic disturbances. The approach will be useful when establishing normal (background) range criteria for inorganics in freshwater, which represent acceptable or unimpaired conditions within a specified management area.

An alternative to the statistical determination of natural variability: a proposed three-pronged approach using regional environmental assessment, community knowledge and paleolimnology

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Accurately characterizing baseline conditions, including natural variability, is an essential component of understanding the existing environment, what has changed in it and defining appropriate responses to those changes. We make comparisons to baseline conditions to help direct future development and management decisions. Whether the data collected accurately reflect the system is a question approached by scientists, and the scientific answer is defended through statistical arguments. Baseline studies are often relatively short, only encompassing between 1 and 3 years of data. Comparisons to those data are only able to identify relatively large-magnitude changes with statistical confidence. Monitoring programs capable of accurately characterizing baseline conditions and

statistically distinguishing a subtle project-related change in the environment from natural variability, with reasonable power, are often beyond the reach of proponents. Even populated areas thought of as "data rich" often have significant gaps in available data, which impair statistical descriptions of natural conditions. An effective scientific study would be costly and require far more samples over a longer term than is usually possible. Discussions of the significance of observations are reserved for statisticians digressing into nuance and whose conclusions are beyond the grasp of most decision-makers or potentially affected parties. This often results in an incomplete understanding for decision-makers to act upon, and for comparisons of environmental conditions to benchmarks rather than pre-existing baseline conditions, limiting our ability to determine whether the environment has drifted outside the range of natural variation and, if so, what the causes of drift may be. We propose a three-pronged approach to characterize baseline conditions and natural variability as a supplement to statistically-directed project-specific sampling initiatives common to development projects. Regionally, variability present at project-specific study areas would be compared to data generated over an expanded geographic focus to understand whether local variability is within the range of regional natural variation. This is possible through a broad regional assessment approach. The frequency and approximate magnitude of environmental extremes would be characterized through interviews with local populations, broadening the scope of consultation from understanding and approval of a project, to data generation and increasing their understanding of project interactions. These interviews would help characterize the short-term environmental changes characteristic of the statistical "tails" of natural variability often missed by traditional monitoring programs. Finally, a paleolimnological record would be used to understand whether the conditions observed in the scientific record and within living memory are characteristic of conditions over the long term (i.e., beyond 50-100 years). A case study where this approach has been applied will be discussed.

Toxicity and Exposure of Wildlife to Polycyclic Aromatic Hydrocarbons (PAHs)

Comparing wood frog (*Lithobates sylvaticus*) tadpoles and semipermeable membrane devices for monitoring polycyclic aromatic compounds in boreal wetlands in the oil sands region of northern Alberta, Canada

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Recent studies have reported evidence that surface mining operations in northern Alberta's oil sands region are contributing to the atmospheric deposition of metals and polycyclic aromatic compounds (PACs) in the vicinity of major bitumen upgrading facilities in the oil sands development area. As part of the Canada-Alberta Joint Oil Sands Monitoring Program, we examined the accumulation of PACs in small shallow boreal wetlands at varying distances from oil sands industrial activities through use of semipermeable membrane devices (SPMDs) and collection of wood frog (*Lithobates sylvaticus*) tadpoles. SPMDs were deployed in shallow lentic waterbodies adjacent to wood frog egg masses and were retrieved approximately 35 to 40 days later. Late stage tadpoles were retrieved from the wetlands at the same time as the SPMDs. Wetlands south of the Athabasca oil sands industrial area accumulated fewer PACs than wetlands situated north of Fort McMurray, Alberta; the latter wetlands were inside the oil sands surface mineable region. The highest concentrations of PACs were detected in SPMDs deployed within a 25 km radius of surface mining activity, consistent with snow deposition studies of PACs in the region. In wetlands located in the surface mineable region, PAC profiles from SPMDs and wood frog tadpoles were dominated by C1-C4 alkylated PACs, including dibenzothiophenes; these compounds represented 87-97% and 91-97% of PACs detected in SPMDs and tadpoles, respectively, which is strongly indicative of petrogenic sources. Total PACs in whole-body wood frog tadpoles ranged from 111 to 195 $ng \cdot g^{-1}$ wet weight. Contrary to differences seen in the SPMD PAC concentrations, there were no obvious differences in the sum of wood frog tissue PAC residues between wetland study sites. Alkylated fluorenes and dibenzothiophenes were found to be higher in tadpoles collected from a wetland located within 10 km of two bitumen upgrading facilities. The use of SPMDs in tandem with biomonitoring organisms offers considerable value when assessing the potential exposure of aquatic organisms to PACs; however, further validation is required to determine whether SPMDs can be utilized as surrogates for tadpoles for the monitoring of PAC environmental exposure.

Polycyclic aromatic hydrocarbons as endocrine-disrupting chemicals in free-ranging river otters (*Lontra canadensis*): From poo to populations

Philippe Thomas[1], Cornelya Klutsch[2], David Eickmeyer[3], Linda Kimpe[3], Laura Graham[4], Ifeoluwa Idowu[5], Wesley Johnson[5], Olga Francisco[5], Gregg Tomy[5], Laurie Chan[3], Jules Blais[3]

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Oil sands bitumen is heavily and severely biodegraded crude oil dominated by alkylaromatic hydrocarbons and hetero-compounds, some of which are likely oxidized, increasing their water solubility and potential for bioaccumulation and biomagnification in wildlife. The Athabasca River and the Peace-Athabasca Delta may be impacted by industrial development related to bitumen extraction occurring in the upstream region of the oil sands. There have been few studies on the exposure of free-ranging terrestrial mammals to polycyclic aromatic compounds (PACs). As such, the ecological significance and the effects of this exposure are poorly understood, especially in long-lived species where long-term survival and lifetime productivity may be adversely affected. Given the paucity of information on the levels and effects of PACs on reproductive success, immune function, and population-level responses, further investigative studies in terrestrial apex predators are warranted. In this project we assessed PAC contaminant burdens in liver tissues of river otters (*Lontra canadensis*) collected from traditional land users and registered trappers. A collaborative non-invasive sampling program involving the collection of otter feces at latrine sites at two different trap lines was undertaken. Analysis of the samples collected allowed us to determine chemically-induced stress responses in the animals as well the measurement of various fecal hormone metabolites (including progesterone, testosterone, thyroid hormones and corticosteroids). DNA was extracted from the same scat samples and used for a population genetic assessment of river otters to monitor population structuring in the vicinity of development sites. Local populations of river otters are responding to anthropogenic disturbances, as evidenced by altered fecal hormone metabolite levels and reduced local effective population sizes with correspondingly higher hepatic PAC burdens.

Comparing the photo-induced toxicity of polycyclic aromatic hydrocarbons in two amphibian species

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Aquatic organisms are rarely exposed to only one stressor in their environment. Chemical stressors such as contaminants along with wavelengths of solar radiation can interact to increase toxicity of contaminants. Polycyclic aromatic hydrocarbons (PAHs) are one class of organic molecules that are lipophilic and readily bioaccumulate. Certain PAHs exhibit photo-induced toxicity with ecologically relevant intensities of ultraviolet (UV) light, resulting in increased mortality, reduced fecundity, and behavioural effects in aquatic organisms. Early life-stages of amphibians may be particularly susceptible to PAH photo-induced toxicity as they are translucent, have permeable skin and undergo embryo and

larval development in shallow ponds. Limited studies have investigated the potential photo-induced toxicity of PAHs in larval amphibian species, making it difficult to predict their impact. The objective of the present study was to evaluate and compare the sensitivity of a laboratory model amphibian (*Xenopus laevis*) and an ecologically native species (wood frog, *Lithobates sylvaticus*) to the photo-enhanced toxicity of PAHs. 96-hour tests were performed in which tadpoles were exposed to individual PAHs (anthracene, naphthalene, benzo[*a*]pyrene) for 8 hours, transferred to clean water, and then exposed to UV light for 12 hours. Results were compared using mortality, growth, body burden, and whole body transcriptomic responses as endpoints. In the presence of UV light, anthracene and benzo[*a*]pyrene were found to increase mortality, and decrease total length, of both larval *Xenopus* and *Lithobates*. Based on mortality data, *Xenopus* was found to be the more sensitive species, suggesting the model organism might be protective of Ranids with regards to the photo-induced toxicity of PAHs. Body burden analyses at 8 hours will identify whether differences in uptake of PAHs contributes to the observed species-specific photo-induced toxicity. Transcriptomic analyses are underway in an effort to further understand the mechanism of toxicity of photo-induced PAHs in larval amphibians by examining genome-wide changes in gene expression following exposure to UV light and benzo[*a*]pyrene. This study demonstrates that UV exacerbates PAH effects in developing amphibians and that photo-induced toxicity of PAHs is species-specific. Overall, it is important to consider UV as an environmental stressor when assessing the toxicity of PAHs to larval amphibians.

Examining reproductive effects of dilute bitumen exposure in wild fathead minnows from the IISD-Experimental Lakes Area

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The International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) is embarking on a comprehensive program of study to examine the fate, behaviour and potential impacts of diluted bitumen (dilbit) in the freshwater aquatic environment as well as strategies to enhance recovery of impacted freshwater ecosystems. During the 2017 field season, baseline data and initial studies to refine methods were conducted in preparation for model spill studies in subsequent years. Here we report initial results from exposures of wild adult fathead minnows (FHM) (*Pimephales promelas*), captured from lakes at the IISD-ELA, to dilute concentrations of dilbit water-accommodated fractions (WAFs). Adult FHM (n=50m/100f) were exposed to high energy mixing WAFs at concentrations of 1:100,000 or 1:1,000,000 for 21 days. An unexposed reference group was also included. After the exposure period, FHM from each treatment were relocated to 10L breeding chamber aquaria so that 1 male and 2 females from a treatment were housed together in a breeding triplet (n=12 triplets per treatment) and ambient temperature was incrementally raised to 22°C to induce spawning activity. Video analysis of time spent in the breeding area and total number of spawning events was used to determine reproductive activity, and reproductive output was determined by the total number of eggs produced over a 14-day breeding period. Ongoing analysis of egg diameter,

hatching success, and mean larval length and weight at landmark developmental time points will be used to determine potential effects of exposure to the dilute WAFs among progeny of exposed FHM.

Reproduction, health, and exposure to polycyclic aromatic hydrocarbons and other contaminants in nesting tree swallows (*Tachycineta bicolor*) at Randle Reef, Hamilton Harbour

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Situated in western Lake Ontario, Hamilton Harbour was designated in 1987 under the Canada-United States Great Lakes Water Quality Agreement as one of 43 Great Lakes Areas of Concern due to local environmental degradation that had severely impacted the area's ability to support aquatic life. Historical discharges of pollutants from local industrial and municipal sources impaired water quality and contaminated sediment in the Harbour, and several contaminants of concern, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and several metals, were identified. Remediation work has begun in Randle Reef, an area in the Harbour where sediment is known to be highly contaminated with PAHs and other pollutants. This study examines reproduction, health, and contaminant exposure in a wildlife sentinel, the tree swallow (*Tachycineta bicolor*), as part of an assessment of pre-remediation environmental conditions at Randle Reef from 2013-2016. This species feeds on emergent aquatic insects in close proximity to its nesting site and has been used widely as an indicator of local sediment contamination in the Great Lakes. Twenty-five nest boxes were installed at two locations in Hamilton Harbour at Randle Reef and Bayfront Park (3 km upstream of Randle Reef) and at Long Point on Lake Erie (reference site). Additional monitoring was initiated in 2015 when nest boxes were installed at three locations upstream of the Harbour (but within the Area of Concern) in Cootes Paradise. Various measures of reproductive success were generally high at Hamilton Harbour and Cootes Paradise study locations. Concentrations of PCBs and polybrominated diphenyl ethers (PBDEs) were higher in eggs from the two Hamilton Harbour locations compared to eggs from the Long Point reference site and Cootes Paradise locations. In contrast, mercury concentrations in eggs were comparable among Hamilton Harbour and Cootes Paradise locations. As a measure of PAH exposure, air monitoring was conducted from 2013-2015 using polyurethane foam (PUF) disk samplers that were installed near nest boxes. Concentrations of PAHs in air were consistently higher at the two Hamilton Harbour locations compared to Cootes Paradise locations and the Long Point reference site. While PAH burdens were not higher in liver of nestling chicks from the two Hamilton locations compared to the reference site in 2013 and 2014, significant spatial variation in EROD activity (induced by PAH exposure and responsible for PAH metabolism) was found in liver of chicks among the three study locations. Additional EROD results are pending for chicks collected in 2016 to examine this pattern further. Several biochemical health endpoints associated with contaminant exposure, e.g., corticosterone (stress hormone) and immune and thyroid function, were also studied in chicks in 2015 and 2016.

Influence of polycyclic aromatic hydrocarbon exposure and diet on metabolomic responses of double crested cormorants (*Phalacrocorax auritus*)

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Earlier research indicated that airborne exposure to polycyclic aromatic hydrocarbons (PAHs) increased the incidence of mutations in double-crested cormorants (*Phalacrocorax auritus*) that were breeding in Hamilton Harbour relative to those from a Lake Erie reference site; further, alterations in gene expression in the putative p53 (tumor suppression gene) pathway were observed. We examined the metabolomics responses from cormorant chicks from each colony, using three tissues (blood, liver, lung). Over 200 metabolites, including acylcarnitines, amino acids, glycerophospholipids, hexose, sphingolipids, and biogenic amines, fatty acids, and bile acids, were measured by flow injection- or liquid chromatography-tandem mass spectrometry. Although all three tissues easily differentiated the three colonies, the majority of differences observed were between the Lake Erie colony and the other two Hamilton Harbour colonies. Based upon estimates of diet using regurgitates and stable isotopes (δC,δN), most of the differences in metabolomics responses appear to be due to diet, as opposed to either airborne PAH exposure, or dietary exposure to persistent organic pollutants (POPs). Metabolomics are a useful tool in determining responses of wildlife in field studies where there are multiple, sometimes confounding, stressors including contaminant exposure and ecological drivers.

Metabolomics endpoints in fathead minnow embryo-larval exposures to polycyclic aromatic hydrocarbons from oil sands sediments

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Water and sediment from the oil sands represent a complex mixture of organic and inorganic contaminants of potential harm to the ecosystem including polycyclic aromatic hydrocarbons (PAHs). Apical end points of toxicity, such as survival and growth, are traditionally used to understand the effects of exposure and to inform regulatory limits. More recently, molecular markers such as gene expression (transcriptomics), protein expression (proteomics) and changes in endogenous metabolites (metabolomics) have shown great potential in measuring earlier indications of potential toxicity, at lower exposure levels, and in informing the mode of action of these complex pollutant mixtures. These biological perturbations can be used to elucidate adverse outcome pathways connecting exposure and effect. Omics data can provide valuable insight into prioritizing and refining future contaminant measurement strategies and have the potential to inform regulatory approaches earlier than toxicological tests focused on apical end-points. In this study, we used targeted metabolomics in fathead minnow larvae exposed to sediments from the oil sands to study whether this approach could be used to link oil sands PAH exposure information and apical measures of toxicity such as survival, growth and

deformities. Fathead minnow embryos and larvae were exposed to low concentrations of three oil sands sediments (1-3 $g \cdot L^{-1}$) for 21 days. Sediment PAH (parent and alkylated) levels ranged from low concentrations in upstream sediments from outside the oil sands area (with 9 and 3 $ng \cdot g^{-1}$, for total EPA priority PAH and total alkylated PAHs, respectively) to high concentrations in downstream river sediments close to bitumen deposits (with 170-275 $ng \cdot g^{-1}$ EPA PAHs and 4500-6200 $ng \cdot g^{-1}$ alkylated PAHs). Larval fish were sampled at 16 days post-hatch and pooled bodies were assessed for a suite of 217 tissue metabolites including 43 amino acids and biogenic amines, 13 bile acids, 17 fatty acids, and 144 phospholipids and acylcarnitines (all measured in $ng \cdot g^{-1}$ tissue). Pooled larvae were extracted in methanol and chloroform, spiked with an isotopically labeled internal standard mix and concentrations of the metabolites were measured using a series of LC-MS/MS and flow injection (FI)-MS/MS analytical runs. Metabolomics endpoints were related to apical endpoints in fish tests (survival, growth, percentage deformities) and to exposure concentrations of PAHs and alkylated PAHs using univariate and multivariate statistical approaches such as ANOVA, PCA and Orthogonal Least Squares Partial Discriminant Analysis (O-PLSDA). Preliminary results show that tissue concentrations of the various metabolites in fish were detected in 36/43, 1/13, 11/17, and 129/144 cases, respectively, for each of the metabolite groupings above indicating appropriate assay performance.

Thermal Discharges: Research and Regulations

Management of the effects of thermal discharge: Environment and Climate Change Canada's perspective

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Because Canada is blessed with so much water, many industries use this natural resource for cooling and discharge large volumes of heated water back to the environment. Canada's nuclear power plants utilize once-through cooling technology that relies on a large water body as heat sinks. Once-through cooling is an efficient, low cost method of condensing steam back to liquid in the power generation industry. However it is becoming apparent that there may be ecological risks associated with the often extensive thermal plumes created by the large volumes of cooling water discharged by nuclear power plants. Environment Canada has responsibilities under Section 36(3) of the *Fisheries Act* which prohibits the deposit of substances or water that is changed by heat into water so that it is rendered or is likely to be rendered deleterious to fish or fish habitat. In assessing the effects of cooling water, Environment Canada has taken the approach of examining the biology of the fish species utilizing the waters impacted by the thermal plume, the thermal plume behaviour throughout the water column compared against acute and chronic temperature thresholds and modeling of survival of the most thermally sensitive life stages of a thermally sensitive fish species. Environment and Climate Change Canada is in the process implementing updated thermal guidance that considers physiological responses to thermal stress. This paper gives an account of how these management approaches have been used in the context of nuclear power plants in Canada.

Monitoring thermal effects in fish

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Various biological processes are temperature dependent, or temperature sensitive, and different species have optimal temperature ranges over which life functions occur. Temperatures outside these optimal ranges can have varying effects, depending on life history stage, length of time exposed, maximum temperature of exposure, and the rate of change in temperature relative to ambient conditions. In some instances, thermal releases associated with industrial discharges (i.e., process effluents, once-through cooling waters) can have an effect on resident and/or migratory biota. This presentation considers potential thermal effects on different species and life history stages of fish. There are various thermal assessment methods, including the upper incipient lethal temperature, the critical thermal methodology, maximum weekly average temperature, and delta temperature. These methods, the factors that affect their measurement, and their application will be discussed.

The effects of once-through cooling waters on whitefish embryogenesis

Joanna Wilson[1], Richard Manzon[2], Christopher Somers[2], Douglas Boreham[3]

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Once-through cooling waters from nuclear generating stations represent a possible source of thermal, radiological and chemical stressors to the near shore aquatic environment. These stressors may impact fish physiology and development. Of particular concern are species of whitefish; whitefish spawn in the near shore area in fall and have a long period of development lasting until ice break up in spring. Lake whitefish (*Coregonus clupeaformis*) are both commercially and culturally important, while round whitefish (*Prosopium cylindraceum*) are thought to be a thermally sensitive species and have suffered large population decreases in the Great Lakes. For the past five years, we have been studying the impacts of thermal stress, morpholine, and radiation on lake and round whitefish development. Chronic embryonic thermal stress results in higher mortality, smaller size at hatch, and earlier hatch in both species. Higher incubation temperature appears to result in hatching at an earlier developmental stage, based on fin definition and jaw morphology. Chronic morpholine (an anti-corrosive chemical) exposure during development increases mortality and alters growth in both species; although at doses that were higher than would be expected in the once-through cooling waters. Chronic, low dose radiation appears to increase growth early in development and advanced hatch. Collectively, our research suggests that the thermal stressors from once-through cooling are the most important for fish development and that round whitefish are the more thermally sensitive species.

Effects of fixed and fluctuating temperature on hatch of round whitefish and lake whitefish eggs

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Temperature-response information for use in evaluating thermal discharges is often over 30 years old and published in the non-peer-reviewed literature, especially for round whitefish (*Prosopium cylindraceum*) and lake whitefish (*Coregonus clupeaformis*) exposed to nonlethal, elevated, and variable temperatures. Egg incubation experiments on round whitefish collected in Lake Ontario and lake whitefish collected in Lake Huron were carried out from December 13, 2011, to April 7, 2012. Experimental treatments included ambient baseline control conditions as well as fixed and fluctuating (variable) temperature increases of 1, 2, 3, and 5°C above ambient baseline conditions. For both species, the window for hatching for all experimental temperature treatments was variable (range, 10-38 days for round whitefish and 11-44 days for lake whitefish), and the hatching windows tended to be greater as temperatures increased. Our results indicated that both fixed and variable incremental increases in temperature above ambient baseline conditions have a statistically significant effect on 50% hatch, and hatch occurs earlier with higher incremental temperature increases. The ecological significance of advanced hatch, such as

indirect mortality and food source availability, was evaluated. Additionally, the results of this study were also compared to other advanced hatch literature.

Impacts of thermal discharges on the development and fitness of lake whitefish (*Coregonus clupeaformis*)

Meghan Fuzzen[1], Christopher Somers[2], Richard Manzon[2], Douglas Boreham[3], Joanna Wilson[1]

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Lake whitefish (*Coregonus clupeaformis*) are a commercially and culturally important fish species in the Great Lakes. The population size of lake whitefish in the Great Lakes is declining due to many factors. The role of thermal discharges from industrial facilities in this decline is unknown. Early life-stages of whitefish are vulnerable to thermal discharges due to their relatively shallow depth of spawning (10 m) and the timing and duration of embryonic development (November to May). Previous studies have demonstrated that lake whitefish reared at elevated temperatures have reduced survival and earlier time to hatch. Additionally, early hatched larvae are smaller and are not as developed as later hatched larvae. It is hypothesized that larvae reared at elevated temperatures will have reduced fitness due to the smaller size and delayed developmental stage at the time of hatching. To test this, lake whitefish were reared at constant temperatures (0.5, 2, 5, or 8°C) during embryonic development and monitored for survival until hatch. Development was assessed by comparing morphometric features. The fitness of larvae was assessed by monitoring the transition to feeding and growth rate. Larvae reared at higher temperatures had a higher mortality rate during all periods of development, hatched earlier, and had a longer duration in the period between hatching and first feeding. It is predicted that the delay in feeding will be related to smaller jaw size and a slower growth rate. If this is found, it would suggest a lower recruitment of fish exposed to elevated temperatures during embryonic development.

The heat shock response in embryonic and young of the year juvenile round whitefish (*Prosopium cylindraceum*)

Andrea Murillo[1], Lori Manzon[2], Douglas Boreham[3], Joanna Wilson[1], Christopher Somers[2], Richard Manzon[2]

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Thermal stress can be a consequence of a fish's natural environment or a result of anthropogenic activities such as industrial thermal pollution. The production of heat shock proteins (hsp) is an important and highly conserved cellular response to thermal and other stressors. In this study, we assessed the kinetics and ontogeny of the heat shock response (HSR) by quantifying changes in gene expression of hsp70, hsp47, hsp90alpha, hsp90beta, and heat shock cognate 70 (hsc70) in embryonic and young of the year (YOY) juvenile round whitefish (*Prosopium cylindraceum*; RWF). For the kinetics experiment, RWF were subjected for 1-4 hours to three different heat shock (HS) temperatures: +3, +6, or +9 °C for embryos and +6, +9, or +12 °C for YOY juveniles. Embryo and YOY juvenile RWF were

allowed to recover at control temperatures of 4 °C and 10 °C, respectively, for 0-24 hours prior to sampling and quantifying hsp mRNA levels using RT-qPCR. Of the three typically inducible hsps, only hsp70 was increased consistently in embryos following heat shock. By comparison, both hsp70 and hsp47 increased in YOY juveniles. mRNA levels of the inducible hsp90alpha, the constitutive hsp90beta, and hsc70 did not vary significantly in comparison to controls. For the ontogeny experiment, embryos of different ages were subjected to a 2-hour HS of +6, +9, or +12 °C and allowed a recovery period at a control temperature for 2 hours. As expected, basal levels of hsp70 and hsp90alpha mRNA increased significantly throughout development. Basal hsp47, hsp90beta, and hsc70 mRNA levels increased significantly early in development (8 to 22 days post fertilization [dpf]) and stabilized later in development. RWF embryos upregulated hsp47 and hsp70 mRNA levels in response to HS as early as 8 and 22 dpf, respectively. The HSR differed in an age-specific manner throughout embryogenesis. A better understanding of the impact of thermal fluctuations which can arise from thermal effluents or climate change on key life-history stages of a sensitive cold water species may aid in predicting and mediating anthropogenic influences on these and other near-shore spawning fishes.

Mixing zones and application for industrial discharges

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The primary focus of monitoring and assessment of industrial discharges to surface waters is typically associated with chemical constituents. However, in some instances, thermal releases associated with these discharges can have significant effects on resident and/or migratory biota. The Ontario Provincial Water Quality objective for temperature is such that the temperature at the edge of the mixing zone must have an ambient water temperature of less than 10°C compared to background. This presentation outlines a multi-season field-based thermal plume delineation in the Welland Canal that utilized interpolation to determine the ambient water temperature at the edge of the mixing zone for the purpose of supporting an Environmental Compliance Approval amendment application. The potential implications of thermal loadings to surface water systems are discussed.

Guidance for design of fish impingement and entrainment programs at Class I nuclear facilities

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The first edition of Canadian Standards Association N288.9 Guidance for Design of Fish Impingement and Entrainment Programs at Class I Nuclear Facilities will be finalized in 2018. This Guidance is part of the CSA N288 Suite of standards on Environmental Management for nuclear facilities. This standard provides guidance for the design and implementation of fish impingement and entrainment monitoring and assessment

programs at Class I Nuclear Facilities, but may also be used by any facilities (i.e., nuclear facilities that are not Class I, or non-nuclear facilities) with high water use or species at risk concerns. The primary objective of an impingement and entrainment monitoring and assessment program is to quantify fish impingement and entrainment. Compliance with the Guidance will assist facilities in assessing effects of water use on fisheries by incorporating best practices from Canada and internationally. This presentation describes the Canadian Standards Association process for developing the guidance, and the key elements of the guidance. Key elements included are guidance on sampling design, data collection, and data analysis for both impingement and entrainment.

Harmful Algal Bloom Toxins in Biota of the Great Lakes

Harmful algal bloom modelling: Running before we can walk? A critical evaluation of the current state of knowledge

George Arhonditsis[1], Yuko Shimoda[1]

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In the context of aquatic biogeochemical modelling, there is an increasing pressure to explicitly treat the functional diversity of biotic communities. The first part of my presentation evaluates the capacity of 124 aquatic biogeochemical models to reproduce the dynamics of phytoplankton functional groups. The analysis reinforces earlier findings that aquatic ecosystem modellers do not seem to consistently apply conventional methodological steps during the development of their models. There is also significant uncertainty with respect to the mathematical representation of key physiological processes (e.g., growth strategies, nutrient kinetics, settling velocities) as well as group-specific characterizations typically considered in the literature. Harmful algal blooms (HABs) are a major concern for water industries as they represent high risk for human health and economic costs for drinking water treatment, and thus one of the outstanding challenges is to offer credible modelling tools that can serve as early warning systems to assist with the operational control of cyanobacteria dominance. Using cutting-edge machine-learning and Bayesian inference techniques, I will illustrate a methodological framework that aims to: (1) identify the factors that regulate the occurrence of HABs; (2) provide predictions of HABs under different climate scenarios; and (3) obtain a probabilistic mapping of areas around the Great Lakes that are characterized by an excessively high risk of HAB formation ("hot-spots"). The application of Bayesian inference techniques provides a convenient means to address the urgent need for novel modelling tools that can support water quality management throughout the Great Lakes region.

Development of a prognostic tool for forecasting harmful algal booms (HABs) and cyanotoxin violations

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Phosphorus control has been historically regarded as the most effective management action for the reduction of harmful algal blooms (HABs) in lakes and rivers. Despite extensive restoration efforts, however, HAB occurrence is on the rise around the Great Lakes in recent years, posing risks to human health. The objective of this study is to identify potential environmental conditions, where a set of variables significantly increases the frequency of HABs and the likelihood of cyanotoxin violations in waters used in drinking water treatment plants (i.e., Microcystin> 1.0 $\mu g \cdot L^{-1}$). Our study site, the Bay of Quinte, a Z-

shaped embayment at the northeastern end of Lake Ontario, experiences frequent and spatially extensive algal blooms and predominance of toxic cyanobacteria. We identified several variables (i.e., total phosphorus, ammonium, temperature, chlorophyll a, wind-speed, and light) that are connected with the likelihood of Microcystis blooms during the growing season in the bay. The integration of Bayesian hierarchical frameworks with regression modelling allowed us to delineate the role of different abiotic factors on the spatiotemporal variability of the phytoplankton composition and to quantify threshold values of environmental variables that may increase the likelihood of Microcystin violations. Findings suggested that there is a significant temporal and spatial heterogeneity with respect to the role of the factors examined and the potential impacts of dreissenid mussels that may have contributed to the shifts in the composition of phytoplankton assemblages. Thus, total phosphorus alone cannot always explain the year-to-year variability of Microcystis and the toxin productions in the system. This spatially explicit approach is useful in identifying "hot spots", where more intense monitoring is required, thereby allowing us to examine toxin productions at macroscales.

Development of a method for the analysis of non protein-bound microcystins in fish from Lake Erie by liquid chromatography-triple quadrupole mass spectrometry (LC-MS/MS)

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Microcystins are cyclic heptapeptide hepatotoxins produced by cyanobacteria found in freshwater environments. They are toxic to higher organisms, causing sickness or even death. Even though only one particular variant is currently regulated under the Ontario *Safe Drinking Water Act* (microcystin-LR, 1.5 $\mu g \cdot L^{-1}$), over 100 different microcystins variants have been reported to date. In fish, microcystins are in equilibrium between the covalently protein-bound and not protein-bound (free) forms. Most recent developments for microcystin analysis in fish are based on the Lemieux oxidation of all the cyanotoxins to the common fragment 2-methyl-3(methoxy)-4-phenylbutyic acid (MMPB). Even though these methods can quantitatively determine the total amount of microcystins, they can't differentiate between the different variants originally present in the fish. The present study describes the development of a method for the analysis of 12 different variants of free microcystins in fish based on aqueous extraction (5% ammonium hydroxide), solid phase extraction cleanup and liquid chromatography-triple quadrupole mass spectrometry instrumental analysis with matrix-matched calibration standards. Ten yellow perch (*Perca flavescens*) and ten walleye (*Sander vitreus*) samples were analyzed with the validated method (fillets and livers analyzed separately). As expected, concentration in liver was significantly higher than in fillets, since these compounds are hepatotoxins. In yellow perch liver, concentration of total free microcystins ranged from <20 to 331 $ng \cdot g^{-1}$, where microcystin LA and dmLR were present in a 1:1 ratio. In walleye liver, concentrations ranged between <20 to 4829 $ng \cdot g^{-1}$, where LA accounted for 98% of the total microcystins.

Proteome responses of microcystin-exposed *Daphnia magna*

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Microcystins are toxins produced by some freshwater cyanobacteria, and their global presence in surface waters is increasing, particularly as the climate warms, resulting in altered nutrient profiles and increased algal bloom events. In vertebrate organisms, microcystins are well-established hepatotoxins. There is less known about the mechanism that causes toxic effects after microcystin exposure in invertebrates. To address this, we exposed *Daphnia magna* to live, laboratory-grown blue-green algae, *Microcystis aeruginosa,* (as a food source) that is high in microcystin toxins for 24 hours. We then performed shotgun proteomic analysis on whole homogenates of pooled daphnids from each in an exposed treatment and a control treatment. A unique feature of this method was the inclusion of *D. magna* whose proteome was entirely labelled with stable 13C and 15N isotopes, which allowed direct relative quantification and comparison between the control and exposure treatment for each replicate. We will present the preliminary proteomic results from this study, with an emphasis on what biological functions in *Daphnia* were affected by exposure to microcystins in order to determine a toxicological mechanism of action.

Analytical tools in Ontario's algal bloom response protocol

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Algal blooms have received increased attention in recent years due to increased public awareness after a number of high-profile events and concern over the effects of a changing climate and landscape. The Ontario government has a protocol in place to respond rapidly to reports of suspected blooms. The protocol provides clarity on the roles and responsibilities of the government agencies involved, ensuring that bloom incidents are managed effectively. This presentation will describe the analytical tools that support the protocol and how the information is used, focusing in particular on the role of algal identification. Using information collected by the algal identification services within the protocol, the presentation will also describe some of the recent trends in bloom occurrence and distribution.

Impact of Air Contaminants on Ecological Health

Organophosphate ester sources, concentrations, emissions and fate in Toronto

Miriam Diamond[1], Tim Rodgers[1], Jimmy Truong[1], Liisa Jantunen[2], Paul Helm[1], Congqiao Yang[1]

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Flame retardants (FRs) have been used to mitigate health and financial risks due to fires. The widespread use of additive FRs (not chemically bonded) that are semi-volatile organic compounds (partition between gas/dissolved and condensed phases) has resulted in their global distribution and occurrence in biota and humans. Concerns over the hazardous nature of some brominated flame retardants (BFRs), have resulted in increased use of organophosphate esters (OPEs). Now some OPEs are also coming under regulatory control due to concerns over human health toxicity. Concerns for aquatic toxicity may also arise due to elevated levels of some OPEs in urban surface waters which see the greatest usage of OPEs, and FRs in general. This paper discusses the indoor sources of OPEs in products (e.g., electronics, upholstered furniture) and building materials (e.g., polyurethane foam insulation), their emissions to the indoor environment, followed by transport outdoors, and their fate in an urban area. Toronto is used as a case study for illustration. Homes in Toronto have levels of OPEs from 1-3 orders of magnitude higher than BFRs. Concentrations of several OPEs (TBEP, TCPP, and TCEP) can reach low $\mu g \cdot L^{-1}$ levels in Toronto rivers. One pathway to surface waters is through clothes laundering, which efficiently releases these highly soluble compounds. Results from the Multimedia Urban Model (MUM) suggest that washout of measured outdoor air concentrations (low µg/m3 levels) is sufficient to supply rivers with sufficient OPEs to achieve measured concentrations. Estimated total OPE emissions to Toronto air reach tonnes per year, which are orders of magnitude higher than BFRs. These results are discussed in terms of implications for aquatic biota.

Environmental health impacts of road dust emissions: The case of platinum metals

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Road dust is a major sink for a variety of toxic metals and metalloids (e.g., lead, cadmium, chromium, arsenic and antimony) and a potentially important source of pollutants to the atmosphere. In addition to geogenic sources and human activities such as construction, traffic exhaust and non-exhaust emissions generated from the wear and tear of asphalt and automotive components such as tires and brake linings are primary sources of metal-enriched particulate matter on roadways. While most particles accumulating on roads fall in the size range expected to accumulate in the roadside environment, road dust has been found to contain a significant fraction of fine particles that are capable of being resuspended and transported longer distances. Total elemental concentrations of road dust in geochemical particle size fractions (e.g., <63 microns) have been characterized in a several studies. However, very little data are available on the metal and metalloid

concentrations in road dust fractions capable of being resuspended and their capacity to impact human and ecological receptors via atmospheric transport pathways. In particular, there are limited data on the emerging metals of concern contained in mobilizable road dust fractions such as platinum group elements (PGE), which are heavily used as automotive exhaust catalysts. This presentation focuses on concentrations of PGE in road dust collected in the City of Toronto. Data on PGE were generated as part of a larger study to investigate the bioaccessibility of priority metals in road dust, in fulfillment of identified information needs under Canada's Chemical Management Plan and Clean Air Regulatory Agenda. Road dust samples were collected from a range of street types (residential, arterial and expressway) in Toronto in 2015 and 2016 in cooperation with Transportation Services, City of Toronto. Total PGE concentrations were measured in both coarse and fine dust samples using NiS fire-assay-INAA. Particle size distribution analysis of road dust sweeps indicated that the majority of particles range between 6.5 and 14.6 microns, thereby belonging to a size range capable of resuspension to the atmosphere. Pd was observed to occur at the highest relative concentrations in fine road dust, with a City median of 132 $\mu g \cdot kg^{-1}$, followed by Pt (median: 50 $\mu g \cdot kg^{-1}$) and Rh (median: 19 $\mu g \cdot kg^{-1}$) and Ir (median: 0.35 $\mu g \cdot kg^{-1}$), while Os and Ru were below limits of detection and/or limits of quantification. Fine/coarse enrichment ratios for PGE increased from lowest to highest in the following order: Pd(2.5)< Rh(2.6)<Pt(2.8)< Ir(7). The observed enrichment of PGE in the finer fractions of road dust is of concern, given the greater relevance of the mobilizable fraction to environmental health. Potential implications for ecological receptors will be discussed in this context.

Developing ambient air quality criteria for sulphur dioxide: Case study

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The Ontario Ministry of the Environment and Climate Change (MOECC) local air quality regulation (O. Reg. 419/05: Air Pollution - Local Air Quality) works within the province's air management framework to protect local communities from the effects of air pollution by regulating air contaminants released into communities by various sources, including industrial and commercial facilities. The Ministry derives Ambient Air Quality Criteria (AAQCs) which are then used to develop air standards and guidelines under O. Reg. 419/05. The AAQCs are developed following an evaluation of the scientific and jurisdictional information, and are based on sensitive critical effects. While the majority of AAQCs have been established to protect human health, some AAQCs are derived to protect against other environmental impacts including impact on vegetation, corrosion and visibility. This presentation will provide an overview of the science that the Ministry uses to develop AAQCs, with focus on sulphur dioxide (SO_2) which is currently being reviewed. Exposure to SO_2 is associated with number of adverse effects on human health and the environment. Inhalation of SO_2 can contribute to respiratory morbidity. Asthmatics and those with underlying respiratory disease are particularly susceptible to the direct health effects of SO_2 exposure. SO_2 also contributes to the formation of fine particulate matter (PM2.5). PM2.5 is associated with an increased risk of premature mortality and

aggravation of respiratory and cardiovascular disease, resulting in increased hospital emergency department visits, doctor visits and restricted activity days. SO_2 can also have direct and indirect effects on the environment. Plants can directly uptake SO_2 which can interfere with photosynthesis and energy metabolism, leading to direct injury and reduced plant growth and yield. SO_2 can affect ecosystems, both terrestrial and aquatic, indirectly through the deposition and retention in soils of sulphur-containing acidifying compounds such as sulphuric acid and sulphate particles. The deposition of acidifying compounds can lead to increased acidification of ecosystems, causing a decrease in growth and increased susceptibility to disease and injury of sensitive species including lichens. Using SO_2 as a case example, discussion will aim to elaborate on the approaches the ministry uses in weighing all the scientific information in determining the most appropriate sensitive adverse effect as means of establishing regulatory limits for contaminants released into the air, hence protecting human health and health of the environment.

Mercury in Ontario fish under changing environment

Satyendra Bhavsar[1],

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Mercury can be naturally elevated in the environment; however, anthropogenic activities can increase environmental mercury levels even at remote locations. Mercury is considered a global pollutant due to its widespread presence and bioaccumulative and toxic nature. Mercury can damage gonads and alter production of sex hormones in freshwater fish. Mercury is also harmful to humans, causing damage to the neurological, immune, genetic, enzyme, cardiovascular, respiratory, and gastrointestinal systems. Environmental levels of mercury are impacted by a multitude of complex factors including local geochemistry and biology, local/regional climate, and local/regional/global emissions. This talk will present recent findings on the impacts of these factors, which are ever-changing, on mercury in fish from Ontario waterbodies including the Great Lakes.

Poster Presentations

Tracking all the variables: Application of a soil fingerprinting framework into soil ecotoxicity assessments

Natalie Feisthauer[1], Catherine Fox[1], David Kroetsch[1], Gabriele Broll[2], Charles Tarnocai[1], Elizabeth Kenney[1], Monika Joschko[3]

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Soil health, or soil quality, is fundamental to soil biodiversity, function and the provision of services such as food production, and is derived from characteristics of soil biological, chemical and physical components. Each of these components influences the response of soil organisms to contamination and should be considered in the interpretation of ecotoxicity assessments. However it is difficult to describe the constituents of soil physical, chemical and biological components in a manner that is succinct, repeatable and has the potential to be quantified. A unique "soil fingerprinting" framework was developed to record and monitor the impacts on soil quality from environmental stressors and land use management. The framework provides an enhanced soil A horizon descriptor methodology to systematically track and record change in soil chemical, physical and biological characteristics. The enhanced horizon designation generated is analogous to a genetic code or "soil fingerprint" for an individual soil sample at the time the sample was collected. The framework monitors A horizon characteristics that not only represent dynamic soil properties (e.g., soil structure, organic carbon, etc.) but also soil and land information (e.g., soil texture, land use), providing data to allow comparative interpretations of soil quality changes and identify trends due to human activities (e.g., soil management practices, remediation, etc.) among different soils, or the same soils under different conditions or at different times. The design of the framework is modular, meaning any level of information can be removed and new levels can be added. For example, levels for soil microbial respiration, root biomass, microarthropod toxicity etc. can be added. The framework then generates fingerprints that are unique identifiers of the nature and context of the key dynamic soil properties related to soil quality as one integrated descriptive label together with ecotoxicity information. For instance, by including soil structure, the framework can integrate physical characteristics that significantly influence soil biological endpoints but that are not often included in ecotoxicity assessments. A database of fingerprints, generated either over time or among sites, provides enhanced opportunities to quantitatively analyze multiple lines of evidence in contaminated site assessments. Examples of the application of the soil fingerprinting framework in soil under different land uses and land management practices will be provided.

Effects of bismuth on germination and growth of ryegrass exposed on filter paper and spiked soils

Pierre Yves Robidoux[1], Zohra Omouri[2], Michel Fournier[2]

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Phytotoxicity of Bi citrate and Bi nitrate were evaluated on perennial ryegrass (*Lollium perenne*) using filter paper and soil tests. Endpoints include seeds germination, root and shoot growth. The results showed that Bi nitrate seemed to be more toxic than Bi citrate, and root growth more sensitive than seeds germination and shoot growth. Filter paper tests indicated that Bi nitrate significantly decreased root elongation at tested concentration ≥ 30.4 $mg \cdot L^{-1}$ and seedling germination at 485 $mg \cdot L^{-1}$. Whereas Bi citrate decreased significantly root elongation and seedling germination at concentration ≥ 99.52 $mg \cdot L^{-1}$ and at 398.08 $mg \cdot L^{-1}$, respectively. Data from artificial soil spiked with Bi nitrate indicated a significant reduction on root wet mass at 485 $mg \cdot kg^{-1}$ soil and no significant effect was observed on shoot wet mass and germination at concentration ≤ 485 $mg \cdot kg^{-1}$ soil. In natural sandy soil, Bi nitrate significantly reduced root wet mass and root elongation at concentration ≥ 4.8 and 48.5 $mg \cdot kg^{-1}$ soil, respectively, while no effects were observed on seed germination and shoot mass. Sandy soil spiked with Bi citrate showed no significant decrease in root growth and germination. The Bi availability varied with the physicochemical soil characteristics, and the toxicity of Bi nitrate/citrate on perennial ryegrass varied with the matrix in the following order: filter paper > natural sandy soil growth > artificial soil.

Characterization of complex non-aqueous phase liquids by nuclear magnetic resonance spectroscopy

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Non-aqueous phase liquids (NAPLs) formed from the pooling of immiscible solvents in the subsurface pose a unique challenge for characterization, risk assessment, and remediation. The lack of understanding as to exactly which compounds are present at a site impairs the ability to develop both accurate risk assessments and efficient and effective remedial strategies. Until removed or sequestered, the constituents of NAPL will continue to leach into groundwater, posing an ongoing risk to groundwater contamination. At many current and former chemical facilities, the composition of NAPL can be complex, and, for several reasons, is often incompletely characterized. Analysis of NAPL constituents by conventional chromatographic methods is impaired by the high-levels of solvents present, which require dilutions of several orders of magnitude before analysis in order to ensure the safety and integrity of the instrumentation. These dilutions reduce the ability to study the components present in NAPL mixtures at lower concentrations, including pesticides and other hydrophobic compounds that partition into the hydrophobic NAPL substrate. In addition, conventional analytical methods based on the targeted analysis of known or presumed compounds often fail to identify the structures of compounds that are unknown or for which standards are not available, including degradation products. This poster

discusses the use of Nuclear Magnetic Resonance (NMR) Spectroscopy as a non-destructive and unbiased analytical tool to assist in the characterization of complex NAPL samples with minimal sample manipulation. NMR has many beneficial attributes that are of use in the characterization of these concentrated, complex mixtures of chemical contaminants, including: the non-selective fingerprinting of all classes of organic compounds present, the ability to quantify constituents without individual standards and calibrations, and the ability to provide structural information on unknown constituents. Advanced multidimensional NMR methods are discussed, including diffusion ordered spectroscopy, that are able to assist in the deconvolution of the overlapping signals in the spectra of complex NAPL mixtures such that the NMR spectra for individual constituents can be isolated.

Assessing upper profile-homogenization as a method for remediation of recalcitrant contaminant metals in soils

Beverley Hale[1], Ryan Thorn[1], Luba Vasiluk[1], Michael Dutton[2], Maria Bellantino-Perco[2]

[1]University of Guelph, [2]Vale Canada Limited

Surface deposition of trace metal contaminated particulates onto soil often results in the non-proportional distribution of contaminants throughout the profile. Surrounding the decommissioned Ni refinery in Port Colborne, Ontario, Ni concentrations in the upper 10 cm of the profile reached upwards of 4500 mg·kg-1, while deeper soils remain at near background concentrations. Laboratory and field-based studies were undertaken to test the viability of reducing the trace metal concentrations in the rhizosphere by homogenizing the upper 30 cm of the soil profile. The laboratory study studied the growth of soybean in pots under controlled conditions in field-collected soils from Port Colborne and clean soil from Elora region. Three profile depth-based treatments from each soil type were used (0-15 cm, homogenized 0-30 cm, and 15-30 cm). Homogenization of the 0-30 cm depth reduced the concentrations of Ni and Cu ten-fold relative to the 0-15 cm depth, for Port Colborne soils. Soybean growth was significantly improved in the homogenized Port Colborne soils relative to the 0-15 cm treatment; however, growth was significantly reduced relative to controls. Therefore, this method of remediation will likely reduce the toxicity of Ni that would occur on soils with similar concentrations of total Ni, but will not eliminate it. A field trial is underway to evaluate soil homogenization capabilities of heavy machinery and assess how the associated reduction in trace metals in the rhizosphere affects toxicity in crops, using normal agronomic practices. Following homogenization, Ni was reduced from 3700 to 1532 mg·kg-1 and Cu was reduced from 353 to 159 mg·kg-1 in the top 10 cm of the profile. Crop trials have yet to begin, but the initial assessments of trace metal reductions at the profile surface demonstrate the potential of this method as a large-scale method of remediation in the Port Colborne region.

Validation of a new ecotoxicity test method for assessing the effect of contaminants in soil using orbatid mites

Rick Scroggins[1], Juliska Princz[1], Patrick Boyd[1], Heather Lemieux[1]

[1] *Environment and Climate Change Canada*

Oribatid mites are currently under-represented in soil ecotoxicity testing, but are an important taxonomic group found in abundance within surface soils. Oribatids contribute to a healthy ecosystem by assisting with nutrient cycling through litter breakdown and to soil formation processes. Method development and validation efforts directed by Environment and Climate Change Canada (ECCC) have led to a standardized ecotoxicity test method using the species *Oppia nitens* (C.L. Koch, 1839). The new ECCC test method involves a 28-day soil exposure to capture effects on adult lethality and reproduction (measured as the number of live juveniles produced). Validation of the new test method is currently under way through inter-laboratory testing involving nine participating laboratories from Canada and Europe. There are three rounds of round robin testing which include a performance test in two field soils and an artificial soil (Phase 1), a reference toxicant test using boric acid in a field soil (Phase 2), and a full survival and reproduction test involving a known toxicant and standard natural soil such as Lufa 2.2 (Phase 3). The results from completed phases will be presented, along with an analysis of test variability, and performance across different soil types. The new test method is the first standardized effort for oribatid mites and will complement an existing suite of soil invertebrate test methods (e.g., earthworms and collembolan), with applicability to both forest and agricultural soils.

Remediation of nickel toxicity by liming: Field validation

Luba Vasiluk[1], Judith Vandeven[1], Amanda Pellegrino[1], Michael Dutton[2], Maria Bellantino-Perco[2], Beverley Hale[1]

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Toxicity of cationic metals is dependent on soil chemistry, especially pH. Changes in pH not only affect bioavailability of metals but also soil nutrients and may cause adverse effects to plants. Liming of field soils contaminated with nickel (Ni) in Port Colborne, Ontario, was studied as a remediation technique. Treatments of 88 tonnes per hectare of calcitic or dolomitic lime as well as positive control with no lime (mechanical equipment only) and a negative control with no treatment were applied to a field in Port Colborne early in 2015. The soil was sampled before and after lime application, and soybeans planted in 2015 (Y1) and 2016 (Y2). Soybean was harvested manually in October of Y1 and yield calculated. Soil pH, pseudo-total Ni, plant-available Ni (using $CaCl_2$) were measured on samples collected before and after liming. It was found that both types of lime increased soil pH, though calcitic lime resulted in a greater increase than dolomitic. A decrease in pseudo-total Ni was seen following liming, though this could be explained by the dilution effect of adding 88 t/ha of lime to the top 5 cm of soil. Plant-available Ni was reduced by both types of lime; however, calcitic had a greater effect than dolomitic. It was found that there is a very predictable relationship between soil pH and plant-available Ni, which does

not depend on the way the pH was increased. Soybean yield for Y1 was significantly lower than that observed for Ontario due to late seed planting on the experimental plots. The greatest yield was seen on the negative control; due to large error for that treatment the negative control yield was statistically greater than only the calcitic treatment and not the dolomitic. Higher yield was seen in the dolomitic treatment than calcitic, which may be due to calcitic pH being too high for optimal soybean production. In Y2, agricultural yield of soybean had improved in comparison to typical Ontario yield, but was still lower. It was found that type of lime, positive control (mechanical effect only) and pH before liming no longer showed a significant effect on soybean yield, although spatial variation had been shown to play a key role in soybean growth in both years.

Toxicogenomic characterization of the effects of 17α-ethinylestradiol and fluoxetine in fathead minnows: Identifying signature toxicity pathways to predict adverse outcomes

Alper James Alcaraz[1], Derek Green[1], Kerstin Bluhm[1], Taylor Lane[1], Markus Brinkmann[1], Nicole Baldwin[1], Jamal Taghavimehr[1], Anita Massé[1], Doug Crump[2], Nil Basu[3], Natacha Hogan[1], Markus Hecker[1]

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

Pharmaceuticals and personal care products (PPCP) are becoming an increasing concern to water managers due to their toxicity to non-target organisms, pseudo-persistence and continuous discharge into surface water systems. Among these chemicals are the endocrine disrupting compound 17α-ethinylestradiol (EE2) and the selective serotonin reuptake inhibitor fluoxetine (FLX). Little is known about the (sub-)chronic effects of these compounds to aquatic organisms that are chronically exposed to low concentrations. In particular, information is limited on the molecular pathways that drive cascades of events that ultimately result in phenotypic adverse outcomes. Recent advances in next generation omics technologies provide a platform for the unbiased characterization of toxicity pathways in target organisms, as they allow entire biological systems to be probed without a priori knowledge of the mechanism by which a chemical causes toxicity. Therefore, the main goal of our research is to identify and validate key molecular toxicity pathways that are predictive of EE2- and FLX-induced apical responses in the model fish *Pimephales promelas*. We will utilize sequence-by-synthesis-based whole transcriptome (RNASeq) and high-resolution mass-spectroscopy-based shotgun proteomics to characterize the molecular toxicity pathways and associate these with downstream biological responses of ecological and regulatory relevance in fathead minnow embryos exposed to graded concentrations of EE2 and FLX. We anticipate that this strategy will allow us to identify a set of signature toxicity pathways, including rare transcripts, which are predictive of EE2- and FLX-induced toxicity. This approach would facilitate the identification of key pathways and core genes involved in toxic responses, and therefore lead to the development of an early life-stage gene expression assay that captures critical toxicity pathways for the prediction of apical outcomes of regulatory relevance beyond the two chemicals listed here. As this assay would take place prior to swim up, it would not be

considered as a live animal test, hence would address the need for alternative approaches in chemical screening. This study is part of the EcoToxChip project (@ecotoxchip).

Novel approaches in toxicokinetic modeling to meet the challenges of chemical risk assessment in the 21st century

Markus Brinkmann[1], Markus Hecker[1]

[1]University of Saskatchewan

Ever-increasing numbers of chemicals are released into the environment that are potentially toxic to humans and wildlife. Current regulations for the assessment of the toxicological risks of these chemicals rely on extensive live-animal testing, using a few standard model species. In addition to the huge costs and ethical objections regarding animal testing, these approaches are often not adequately protective of organisms of concern, such as native species or humans. Recent advances in omics technologies and systems biology, as well as in the field of *in vitro* high-throughput testing, provide promising tools to address these challenges. Application of these methodologies in context with chemical risk assessment, however, requires the translation of their results into outcomes of regulatory relevance. To bridge these gaps, this study explores the use of novel approaches in toxicokinetic modeling to extrapolate effects obtained with *in vitro* assays to whole organisms, as well as among species, life stages and levels of biological organization. Successful implementation of these approaches in chemical risk assessment will represent a great step towards more sustainable, efficient, and unbiased assessments of the safety of the large number of chemicals in use today and in the future, and may potentially save billions of dollars and millions of animals.

Characterizing early changes in molecular toxicity pathways to predict adverse outcomes of ethinyl estradiol and chlorpyrifos in amphibians

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Amphibians are of vital importance to aquatic ecosystems and are generally regarded as a sensitive bioindicator of ecosystem health. Exposure to contaminants can have adverse outcomes on amphibian health, such as altered rate of metamorphosis, reproductive effects, immune suppression, and behavioural effects. Alterations in molecular processes often precede these apical effects, giving early indications of subsequent physiological changes and their modes of action. Toxicogenomics therefore shows great promise as an early screening tool to prioritize chemicals with potential risk for adverse effects, without the need for long-term, animal-intensive exposures. The main goal of this study is to identify and validate key molecular toxicity pathways that are predictive of contaminant-

induced apical responses in the model amphibian *Xenopus laevis*. Specifically, this study focused on chlorpyrifos (CPY) and ethinyl estradiol (EE2), two anthropogenic contaminants of concern with different modes of action and characterized apical effects in amphibians. Post-hatch individuals were exposed for 96 hours to CPY (0.5, 2, 8 $\mu g \cdot L^{-1}$) and EE2 (0.04, 0.2, 1 $\mu g \cdot L^{-1}$) and sampled for whole transcriptome (RNASeq) and mass-spectroscopy-based shotgun proteomics to characterize their molecular toxicity pathways. A subset of tadpoles was then transferred to a flow-through diluter system for exposure to metamorphosis (~ 40 days) and assessed for developmental stage, morphometrics, organ histopathology and genetic sex. We anticipate that this work will identify critical toxicity pathways and associated specific key genes to be used in an early life-stage gene expression assay to predict apical outcomes of ecological and regulatory relevance in amphibians, providing an alternative approach in chemical screening. This study is part of the EcoToxChip project (@ecotoxchip).

Use of reference toxicant challenges for studying multiple stressor impacts

Lindsay Beyger[1], Jordan Anderson[1], John Guchardi[1], Douglas Holdway[1]

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Over the years a large focus has been placed on the effects of individual pharmaceutical compounds on non-target organisms. While this is highly important from a mechanistic point of view, it is also important to consider the real environment in which fish would be exposed. In surface waters, wild fish are exposed to numerous compounds over multiple generations with many different stressors and modifying factors. Adult American flagfish (*Jordanella floridae*) were exposed to ibuprofen, naproxen, and 17 α-ethinylestradiol alone and in mixtures during a short-term reproduction study. Offspring were collected and used in a copper reference toxicant challenge. The challenge was completed to assess if their sensitivity would alter based on prior life history. Both control and exposed offspring exhibited a slight change in sensitivity during the challenges. The methodology of testing subsequent exposures on offspring as a secondary stressor has not been well studied. This research aims to contribute to a better understanding of the impact of multiple factors, including single and mixture pharmaceutical exposures and subsequent toxicant challenges, which fish may encounter in the environment.

Identification of toxicity pathways that can be used to predict adverse outcomes of chlorpyrifos in fathead minnows

Kerstin Bluhm[1], Alper James Alcaraz[1], Derek Green[1], Taylor Lane[1], Markus Brinkmann[1], Jamal Taghavimehr[1], Anita Massé[1], Nicole Baldwin[1], Doug Crump[2], Niladri Basu[3], Natacha Hogan[1], Markus Hecker[1]

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Chlorpyrifos is an organophosphate insecticide that acts as a neurotoxicant through inhibition of the enzyme cholinesterase. The mode of action of organophosphates in target and non-target organisms, including mammals, is similar. The aim of the project is to

develop an early life-stage gene expression assay (EcoToxChip) that captures critical toxicity pathways of chlorpyrifos for the prediction of apical outcomes of regulatory relevance. As this assay is intended to use early life-stages that are not feeding independently, it would not be considered as a live animal test, and therefore, would address the need for alternative approaches in chemical screening. As part of the project, critical toxicity pathways and associated core genes will be identified following exposure of fathead minnows (*Pimephales promelas*) at early life-stages to three sub-lethal concentrations of chlorpyrifos. Specifically, sequence-by-synthesis-based whole transcriptome (RNASeq) and high-resolution mass-spectrometry-based shotgun proteomics will be used to characterize key molecular toxicity pathways. Pathways will then be correlated with downstream biological responses of ecological and regulatory relevance, and critical genes linked to apical outcomes will be identified for inclusion on EcoToxChips. Chlorpyrifos concentrations were selected based on a preliminary test as well as concentrations in published data. These tests revealed a threshold level of mortality between 1 and 10 $\mu g \cdot L^{-1}$ chlorpyrifos. To ensure the determination of solely sub-lethal effects in at least two of the tested concentrations, 0.5, 1.5 and 4.5 $\mu g \cdot L^{-1}$ chlorpyrifos solutions were investigated in the fathead minnow early life-stage assay with larvae samplings after 7 and 32 days of exposure. None of these concentrations affected survival or growth, resulting in a sub-chronic NOAEC and LOAEC of 4.5 and 10 $\mu g \cdot L^{-1}$ chlorpyrifos, respectively, in fathead minnows. Samples are currently being further analyzed for molecular and physiological endpoints to gain insight into critical toxicity pathways. This study is part of the EcoToxChip project (@ecotoxchip).

Time to sampling after capture impacts site-specific ovarian steroid production in rainbow darter (*Etheostoma caeruleum*)

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Studies on male fish in the Grand River (Southern Ontario, Canada) have shown a variety of reproductive issues such as increased intersex condition (ova-testes) and vitellogenin production, and decreased gonadsomatic index and testosterone production. Many of these effects have potentially been linked to proximity to the effluent outflows of wastewater treatment plants (WWTP). WWTP effluents are complex mixtures that contain numerous chemicals with the potential to disrupt the endocrine systems of exposed fish. This study investigated the sex steroid production levels of female rainbow darter (RBD, Etheostoma caeruleum) at two locations in the Grand River: a location upstream (reference) of the Waterloo WWTP effluent outflow and a site that is downstream of the outflow (exposed). Secondly, this study examined the impact of the collection method on the steroid production. Given that field collections of small-bodied fish in rivers often employ electrofishing methods and that stress is known to affect steroid levels, fish collected following electrofishing from both locations were sacrificed and ovaries removed at varying times over two sampling periods, one year apart. Ovarian tissue was cultured *in vitro* for 24h with or without the addition of human chorionic gonadotropin (hCG), an analog to endogenous luteinizing hormone that stimulates steroid production. Reference

fish sacrificed 1h post-collection had stimulated ovarian estradiol production levels of 54.4 ± 4.8 pg·mg^{-1} of tissue in 2015 and 46.1 ± 5.5 pg·mg^{-1} in 2016, which were significantly higher than exposed fish sampled after 1 hour in both years (34.3 ± 2.2 pg·mg^{-1} and 23.4 ± 3.5 pg·mg^{-1}, respectively). In 2016, at 3 hours post-collection, stimulated estradiol production in reference fish was no longer significantly higher than the exposed fish (38.6 ± 2.6 pg·mg^{-1} vs. 24.3 ± 3.2 pg·mg^{-1}). In 2015, at 7 hours post-collection, basal estradiol production levels for reference fish ovarian tissue (5.0 ± 0.44 pg·mg^{-1}) were significantly higher than those of exposed fish (3.1 ± 0.40 pg·mg^{-1}). Over the 24 h, stimulated estradiol levels decreased significantly in reference fish ovaries (1h 54.4 ±4.8, 7h 40.7 ± 4.0, and 24h 25.6 ± 2.9 pg·mg^{-1}). At 24 h, there were no significant differences in basal or stimulated estradiol production levels between the reference and exposed fish ovaries in both years. These results suggest that standardized collection and sampling methods are important to interpreting reproductive steroid levels of fish collected through electrofishing and in the identification of site-specific differences. The impact of time held post-shock may be a stress-related response with enough magnitude to impact the ability to detect reproductive endocrine impacts of point/non-point sources on small-bodied fish and is being further investigated.

Assessment of the effect of water quality on copper toxicity in two strains of *Hyalella azteca*

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At the Chalk River Laboratories (CRL) site, sediment toxicity testing is typically conducted using a standard 5-salt culture water (SAM-5S) that is prepared in the laboratory. However, this water may have a different composition than the water found in local water bodies. A study was undertaken to assess the copper tolerance of two strains of *Hyalella azteca* in SAM-5S (high ionic strength), Ottawa River water (low ionic strength) and diluted SAM-5S (similar ionic strength to Ottawa River water). Acute (96-hour) copper toxicity tests were conducted with 9 to 16 day-old *H. azteca*. One of the strains tested was obtained from the Canadian Centre for Inland Waters (CCIW). This CCIW strain can tolerate waters with low ionic strength but cannot reproduce in Ottawa River water. The other strain tested was from Twin Lake (low ionic strength) located on the CRL property. For a given water type, the two strains of *H. azteca* yielded comparable responses to copper. The highest copper tolerance was found in Ottawa River water, closely followed by SAM-5S, whereas the lowest copper tolerance was found in diluted SAM-5S. This study also demonstrates no difference in sensitivity to copper between the two *H. azteca* strains.

The effects of anti-sea lice therapeutants on sensitive life stages of non-target species in combination with different stressors

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The environmental consequences of aquaculture chemotherapeutant use as a treatment for sea lice was assessed by generating data on the acute and sublethal toxicity to sensitive marine organisms (crustaceans; Family Pandalidae) under environmentally realistic conditions and multiple stressor scenarios (chemical: single and multiple pulse exposure scenarios; physical: variable oxygen, temperature; biological: complete life stage testing). Exposures were short-term and based on environmental data reported near aquaculture operations of each of the formulations Salmosan®, Paramove® 50, and SLICE®, either individually or in a mixture. For SLICE®, exposures were done using spiked fish feed or in water; exposures with Salmosan® and Paramove® 50 were via water. Acute toxicity of Salmosan® was greater than Paramove® 50 in both juveniles and adults of several species (coonstripe shrimp [*Pandalus hypsinotus*], pink shrimp [*Pandalus jordani*], dock shrimp [*Pandalus danae*], spot prawn [*Pandalus platyceros*], ghost shrimp [*Neotrypaea californiensis*], and sand shrimp [unidentified species]). No mortality was seen after consumption of feed medicated with SLICE®. Species were equally sensitive to chemotherapeutants. Data for three developmental stages (larval stages I, III, and V) of the spot prawn indicate that these early life stages are far more sensitive to all chemotherapeutants compared to adults. Prawns actively avoided all chemotherapeutants. Sublethal exposures resulted in increases in oxygen consumption, and in some cases olfaction, and feeding behaviours. Data indicate that mixtures are more toxic than when exposed to each chemical alone, and that environmental temperature increases toxicity by as much as 3-fold. Hypoxia also exacerbated acute toxicity; however, no effects of environmental salinity were seen on toxicity thresholds. The data obtained from this project is required to ensure the proper and safe use, and appropriate regulation of these aquaculture chemicals in Canada.

The environmental effects of diluted bitumen on Pacific estuarine and marine organisms in the Straights of Georgia/Juan de Fuca area of British Columbia

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The environmental consequences of diluted bitumen (dilbit) exposure to estuarine and strictly marine species was assessed. WSF of dilbit (WSFd) was generated by using an oiled substrate method in which dilution water flows through a polyvinyl chloride column filled with ceramic beads pre-soaked in dilbit (Cold Lake Summer Blend) or by the CROSERF method for the preparation of a water-accommodated fraction (WAF) and a chemically enhanced water-accommodated fraction (CEWAF). The susceptibility of different groups of marine organisms in acute toxicity tests with WAF/CEWAF varied from being relatively non toxic at 100% WAF to lethal at less than 5% dilutions. CEWAF was always more toxic to all species compared to WAF alone; however, the magnitude of CEWAFs' higher toxicity

was not the same in all species. Susceptibility trends for WAF were kelp (*Macrocystis pyrifera*) > echinoderm (*Strongylocentrotus purpuratus*) > mysid (*Mysidopsis bahia*) > Topsmelt (*Atherinops affinis*) = spot prawn (*Pandalus platyceros*) = pink salmon (*Oncorhynchus gorbuscha*) = rock greenling (*Hexagrammos lagocephalus*) = tidepool sculpin (*Oligocottus maculosus*). LC_{50} values for the latter five organisms could not be determined for WAF. Several performance abilities showed significant alterations in crustaceans and teleosts upon exposure. Swimming performance in fish was affected that could reduce many aspects of survival, migration, and predator avoidance. Physiological effects were also seen in several parameters in crustaceans and fish including growth and feeding. Teleosts and crustaceans responded variably to the presence of low concentrations of dilbit; some organisms were either not able to detect or to respond to dilbit, suggesting a greater risk of exposure (particularly if attracted). These studies also show that the olfactory ability of crustaceans is affected by exposure to dilbit. This information highlights the need for realistic toxicity testing methods that will generate toxicity data that will ensure that the risk assessments to organisms are accurate.

The environmental fate and non-target effects of sea lice pesticides used in Canadian salmon aquaculture

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The environmental consequences of aquaculture chemotherapeutant use as a treatment for sea lice were assessed by generating data on their environmental partitioning and persistence, and acute and sublethal toxicity to a variety of Pacific marine organisms. Deltamethrin (DM), emamectin benzoate (EB), and cypermethrin (CP) all demonstrated partitioning into sediments, while azamethiphos (AZ) was almost undetectable in the sediment, and hydrogen peroxide (HP) was detected in water only. The estimated water $t_{½}$ of HP was <9 hours. AZ was the next least persistent ($t_{½}$ ~12 days), followed by DM ($t_{½}$ water ~18 days; sediment ~45 days), CP ($t_{½}$ water ~ 20 days; sediment ~ 557 days), and EB ($t_{½}$ sediment ~230 days). For all toxicity experiments, exposures were short-term (<96 hours) and were based on environmental data reported from aquaculture facilities. Generally, susceptibility to each chemotherapeutant in acute toxicity tests with a variety of marine organisms (giant kelp [*Macrocystis pyrifera*], topsmelt [*Atherinops affinis*], mysids [*Mysidopsis bahia*], blue mussels [*Mytilus* spp.], Pacific purple sea urchins [*Strongylocentrotus purpuratus*], and spot prawn [*Pandalus platyceros*]) was species-specific and no general trends were evident. Spot prawns were actively attracted to, and actively avoided, EB, HP, and AZ at various concentrations. Naïve pink salmon (*Oncorhynchus gorbuscha*) fry avoided EB, HP, CP, and AZ. All chemotherapeutants reduced swimming performance, and sublethal exposures to CP and DM resulted in the loss of attraction to food. This research provides targeted information on the environmental fate and acute/sublethal toxicity required to ensure the proper and safe use of chemotherapeutants through science-based regulations for these important aquaculture chemicals.

The effects of the aquatic herbicide Reward® on fathead minnow

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Diquat dibromide is a herbicide widely used in North America for industrial and recreational control of terrestrial and aquatic weeds. The objectives of this study were to assess the lethal and sub-lethal effects of this herbicide in a commercial formulation, Reward® (240 $g \cdot L^{-1}$ diquat dibromide). Therefore, this research provides novel toxicity data on this active ingredient combined with any additives or adjuvants in the commercial formulation of Reward®, and on application scenarios prescribed by the manufacturer. Specifically, a 14-day period between applications of Reward® in a water body undergoing treatment is required, yet the effects of these 'pulse' exposure scenarios on aquatic wildlife such as fish are unknown. Several experiments were conducted to address this knowledge gap. The first was an early life-stage fathead minnow (*Pimephales promelas*) exposure that was conducted according to an Environment and Climate Change Canada Biological Test Method entailing a 7-day continuous, chronic exposure (0.105-12.6 $mg \cdot L^{-1}$). A larval LC_{50} of 2.04 $mg \cdot L^{-1}$ was derived from this experiment and a significant decrease in average biomass was observed at 1.17 $mg \cdot L^{-1}$ Reward®. The second fathead minnow larval exposure was a modified version of the 7-day chronic test whereby larvae were exposed for 24 hours and then reared in clean water for 14 days followed by a second 24 hour exposure to Reward®. The LC_{50} derived from this experiment was 4.19 $mg \cdot L^{-1}$ and an EC_{50} for weight of 6.71 $mg \cdot L^{-1}$ was also obtained. A third experiment on adult fathead minnows revealed an LD50 of 6.71 $mg \cdot L,^{-1}$ and while there was no change in gonadosomatic index of adults, the F1 generation reared in clean water displayed a significant non-monotonic dose-response in hatch success. These findings suggest that concentrations causing adverse effects on morphometrics occur above the maximum concentration predicted by the manufacturer upon application to water bodies (i.e., >0.37 $mg \cdot L^{-1}$). However, there is some evidence that effects on development of offspring occur at or below this level, and further investigations at the molecular level are currently underway to examine the sub-lethal adverse effects of this commercial formulation of diquat dibromide.

Effects of ocean acidification combined with multiple stressors on early life stages of the Pacific purple sea urchin, *Strongylocentrotus purpuratus*

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Decreases in ocean pH through ocean acidification have been shown to have direct negative impacts on the early life stages of the Pacific purple sea urchin, *Strongylocentrotus purpuratus*. Research has suggested that multiple stressors could exacerbate, cancel, or even alleviate the impacts of ocean acidification on echinoderms. This study assessed the combined effects of changes in pCO_2 concentrations (390, 800, 1500 $mg \cdot L^{-1}$), salinities (28, 31, 34 parts per thousand) and temperatures (12, 15, 18°C) on fertilization and larval development in *S. purpuratus*. Increased pCO_2 was the predominant stressor, with additive and antagonistic effects from temperature changes, and no effect from salinity changes.

Stressor combinations significantly decreased the rate of normal larval development by 28-68%, whereas fertilization and larval survival were unaffected. Decreases in larval development were observed in combined stressor treatments with pH decreases as low as 0.1-0.3 from ambient. With the projected pH decrease of 0.3-0.4 by the year 2100, the detrimental effects of multiple stressors such as the combination of CO_2 and ocean warming may occur sooner than realized. The strong impact on normal larval development likely indicates that later development stages could be detrimentally affected and could thereby influence the population dynamics of *S. purpuratus* along the Pacific Coast. This may cause detrimental cascading effects on various other species in Pacific Coastal ecosystems in the near future.

DFO's National Contaminants Advisory Group: Highlights on research investigating the biological effects of contaminants in aquatic environments

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As a science-based federal government department, Fisheries and Oceans Canada (DFO) requires scientific evidence to facilitate the sound management of Canada's fisheries and to advance sustainable aquatic ecosystems while fostering economic prosperity across maritime sectors and fisheries. The National Contaminants Advisory Group (NCAG) provides scientific information and advice to DFO on priority issues related to the biological effects of contaminants on aquatic species. The main functions of the group are to facilitate research projects through external researchers, to synthesize results and to develop science advice in support of DFO decision-making. Current priority research themes are: (1) oil and gas, (2) pesticides, (3) aquaculture therapeutants, and (4) contaminants and issues of emerging concern. The NCAG has funded a variety of multi-year research projects at Canadian universities and not-for-profit research institutions. A summary of NCAG supported research projects and their highlights is presented.

Assessing the lethal and sublethal toxicity of two thiocarbamates to aquatic invertebrates

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Canada's Chemicals Management Plan (CMP) was implemented by the federal government in 2006 to regulate chemicals detrimental to human health and the environment. Few toxicological data are associated with the numerous industrial chemicals listed in the CMP. Thiocarbamates are high-production-volume chemicals, and are imported into Canada primarily for uses related to rubber and motor vehicle parts manufacturing, although they are also used as pesticides. Despite their widespread use, there is little information on the ecotoxicity of thiocarbamates, and the research that is

available focuses primarily on acute lethality. Therefore, our objective was to conduct chronic, spiked-sediment exposures to assess the toxicity of two thiocarbamate compounds, tetramethyl thiuram disulfide (TMTD or Thiram, CAS RN 137-26-8) and dipentamethylene thiuram tetrasulfide (DPTT, CAS RN 120-54-7), to three species of aquatic invertebrates: *Hyalella azteca* (amphipod), *Hexagenia* spp. (mayfly), and *Daphnia magna* (cladoceran). Three-week range-finding tests with *Hyalella* and *Hexagenia* were conducted initially, and as DPTT demonstrated little toxicity, chronic DPTT exposures were only conducted with *Daphnia*. Chronic sediment exposures were six weeks (*Hyalella* and *Hexagenia*; Thiram only) or three weeks (*Daphnia*; Thiram and DPTT), and effects on survival (all species), growth (all species), and reproduction (*Hyalella* and *Daphnia* only) were assessed. Results are currently based on nominal concentrations ($mg \cdot kg^{-1}$ dry weight sediment), as chemical analysis of water and sediment is ongoing. Survival in Thiram exposures was significantly reduced at 100 $mg \cdot kg^{-1}$, with LC_{50}s of 86, 110, and 48 $mg \cdot kg^{-1}$ for *Hyalella*, *Hexagenia*, and *Daphnia*, respectively. Thiram also caused a significant decrease in growth of *Hexagenia* (EC_{50}=69 $mg \cdot kg^{-1}$). Growth and reproduction of *Hyalella* and *Daphnia* were not affected by Thiram (up to 300 and 1000 $mg \cdot kg^{-1}$, respectively). Chronic DPTT exposures with *Daphnia* caused a significant decrease in survival (LC_{50}=1000 $mg \cdot kg^{-1}$), but there were no significant effects on growth or reproduction (up to 1000 $mg \cdot kg^{-1}$). Thiram was similarly toxic to the three invertebrate species tested, with effects occurring between 48-110 $mg \cdot kg^{-1}$, and was 10-fold more toxic than DPTT. The results of this study will be compared to thiocarbamates measured in Canadian environmental samples, and will support environmental risk assessments to determine if thiocarbamates could impact aquatic organisms.

Assessing the lethal and sublethal toxicity of perfluorooctanoic acid (PFOA) to *Hyalella azteca* and *Pimephales promelas*

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[1]*Environment and Climate Change Canada*

Perfluoroalkyl substances (PFASs) are used in a variety of industrial and commercial products, including surfactants, polymers, lubricants, adhesives, paints, household cleaners, pesticides, and fire-fighting foams. Significant environmental concerns are associated with PFASs due to their persistence, potential for bioaccumulation, toxicity, and capacity for long-range transport. PFASs, including perfluorooctanoic acid (PFOA), are widely present in the Canadian environment, particularly at some contaminated sites due to historic firefighting training operations. Despite a large body of research on environmental exposure, the toxicity of PFOA is an emerging field of study, and insufficient aquatic toxicity data exist to develop water quality guidelines. Thus, our objective was to conduct chronic, aqueous exposures with PFOA to assess the lethal and sublethal toxicity to *Hyalella azteca* (amphipod) and *Pimephales promelas* (fathead minnow). Amphipod exposures were 6 weeks (1-100 $mg \cdot L^{-1}$ nominal) and examined survival, growth, and reproduction. Fathead minnow exposures were 21 days (0.01 to 100 $mg \cdot L^{-1}$ nominal), and endpoints included hatching success, deformities at hatch, larval survival, and growth.

Measured PFOA concentrations in exposures were 80-90% nominal; therefore, toxicity data were expressed as nominal. Amphipod survival was significantly reduced at 100 $mg \cdot L^{-1}$, with a 6-week LC_{50} of 53 $mg \cdot L^{-1}$. Growth and reproduction of amphipods were more sensitive endpoints than survival, with 6-week EC_{50}s of 2.3-2.4 $mg \cdot L^{-1}$. Fathead minnows were less sensitive than *Hyalella*, with only a 10% decrease in larval survival at 100 $mg \cdot L^{-1}$. There were some indications of increased deformities in larval fish at 100 $mg \cdot L^{-1}$, but these were not statistically significant. Hatching success and growth of larval fish were not affected by PFOA exposure up to 100 $mg \cdot L^{-1}$. Maximum concentrations of PFOA in the surface waters of the Great Lakes are generally < 50 $ng \cdot L^{-1}$, and as the toxicity of PFOA to amphipods and fathead minnows occurred at concentrations > 1 $mg \cdot L^{-1}$, it is likely that most environmental concentrations are far below those that cause toxicity to these species. However, localized areas could be highly contaminated due to historical activities or recent spills (where concentrations as high as 11 $\mu g \cdot L^{-1}$ have been found). Our data will provide valuable information with which to assess the risk of PFOA at contaminated sites and to set a target for site remediation.

The effects of hydroxypropyl-β-cyclodextrin (HPβCD) on the toxicity of intraperitoneally injected steroids in rainbow trout (*Oncorhynchus mykiss*)

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Pharmaceuticals and personal care products (PPCPs) have been studied for their toxicity to non-target organisms in the aquatic environment. The majority of this research has involved understanding the toxicity of individual compounds. While this information is essential for understanding individual toxicity of a particular toxicant, it does not take into consideration the effects of chemicals in a mixture. This is a concern since PPCPs most commonly enter surface waters through wastewater treatment plant (WWTP) effluent as a complex mixture. This study examined how the odour suppressant and excipient hydroxypropyl-β-cyclodextrin (HPβCD) alters the toxicity of select synthetic steroids commonly used as human oral contraceptives. HPCβD is amphiphilic and toroidal in shape, with the ability to include non-polar guest compounds within its central cavity. HPCβD is used as both an excipient in the pharmaceutical industry and an odour suppressant in Febreeze®. The steroids of interest include: 17α-ethinylestradiol (EE2), levonorgestrel (LNG), and etonogestrel (ENG), all of which are commonly found in human oral contraceptives. To identify the individual toxicity of each compound, juvenile female rainbow trout (*Oncorhynchus mykiss*) were intraperitoneally injected with either 0.001 $nmol \cdot kg^{-1}$, 0.0032 $nmol \cdot kg^{-1}$, 0.010 $nmol \cdot kg^{-1}$, 0.032 $nmol \cdot kg^{-1}$, 0.100 $nmol \cdot kg^{-1}$ or 0.320 $nmol \cdot kg^{-1}$ of EE2, LNG, ENG, or HPCβD n=10 fish per treatment. Blood, liver, gonads, and brains were sampled 7 days post injection and analyzed for changes in vitellogenin (VTG), endogenous steroid concentration, and alterations in gene expression. Next, juvenile female rainbow trout were injected with a mixture of HPCβD and either EE2, LNG, or ENG, and sampled 7 days after injection. Results are pending. Preliminary results from this study indicate that in the presence of HPCβD, the 96-hour acute toxicity of EE2 to larval American flagfish (*Jordanella floridae*) was significantly reduced in a 1:1 molar ratio (EE2:HPβCD)

($p \leq 0.05$). Thus an interaction between HPCβD and EE2 appears to be able to be detected though a biological assay.

Bisphenol A as a disrupter of lipid metabolism in zebrafish embryos

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Bisphenol A (BPA) is a widely used plasticizer that raises major concerns due to its activity as an endocrine disruptor. As a known xenoestrogen and potential obesogen, its use has been regulated or restricted by different regulatory bodies around the world. However, there are still large uncertainties about its effects at embryonic stages, both at the short and at the long term. In order to identify molecular footprints of the exposure of to BPA in early zebrafish (*Danio rerio*) development, we exposed zebrafish embryos during 2-5 dpf (days post fertilization) to different BPA concentrations (control, 0.1, 1 and 4 $mg \cdot L^{-1}$ of BPA) to analyze effects at transcriptomic, metabolic and morphological levels. Transcriptome changes were analyzed by RNA-seq (5 dpf, 3 replicate per condition, 10 embryos per replicate). Statistical analysis using DESeq2 (R package) revealed 1517 differentially expressed genes (DEGs, adjusted p-value <0.05), with 810 up-regulated and 707 down-regulated genes. Gene ontology (GO) analysis showed that these genes were related to response to estrogen stimulus, oxidation-reduction process, metabolic processes, and lipid transport and metabolism. The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis showed that retinol metabolism and PPAR signaling pathways were also significantly affected by the treatment. Due to the significant number of DEGs related to lipid metabolism and to elucidate possible alterations in lipid composition, we analyzed total lipid profiles of exposed and control embryos (4, 6 and 8 $mg \cdot L^{-1}$ of BPA, 5 embryos per replicate) at 4, 5 and 6 dpf. Lipids were analyzed by high-performance thin layer chromatography (HPTLC), which provides an integrated system to measure the different lipid classes (each lipid inside the same family) and, at the same time, to separate the different families. Moreover, the relatively high throughput of the technique facilitates dose-response studies under very different parameters. The results show that BPA-treated animals maintain a significant proportion of triglycerides (TAGs) until at least 6 dpf, the stage at which the yolk sac (main TAG reservoir in ZF embryos) is completely reabsorbed in control conditions. We also observed changes (both decreases and increases) in the majority of other lipid classes during the 4-6 dpf. These results agree with the observed deregulation of lipid genes upon BPA exposure and demonstrate the physiological connection between gene deregulation and metabolic profiles. Morphological analyses also agreed with the lipid and transcriptomic data. BPA-treated embryos showed a significant tendency to retain yolk sac remnants after 5 dpf, and an increase in yolk sac size at early stages, which is consistent with the higher TAGs content measured in the same BPA conditions. We propose that this effect reflects either a yolk sac malabsorption syndrome or an impairment of the general lipid metabolism, which in turn would lower yolk sac consumption. We also suggest that these effects may be linked to the suspected interaction

of BPA with the PPAR (peroxisome proliferator-activated receptor) signaling pathway, although they may involve other lipid-metabolism related receptors such as PXR (pregnane X receptor) and CAR (constitutive androstane receptor). As a final conclusion, we propose that the actual legislation, mainly focused to prevent the estrogenic effects of BPA, may actually underestimate the negative effects of BPA for environmental and human health.

In vitro comparison of sensitivities among four Canadian fish species to emerging contaminants of concern

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Large numbers of chemicals are discharged into aquatic ecosystems as a result of human activities. While some of these compounds have been widely studied and adverse effects on fishes have been identified, there are an increasing number of emerging contaminants (ECs), including pharmaceuticals and personal care products (PPCPs) or brominated flame retardants (BFRs), for which little or no toxicity data regarding aquatic organisms is available. Many of these ECs, such as 17α-ethinylestradiol (EE2), a potent estrogen agonist used in oral contraceptives, fluoxetine, a common antidepressant, and hexabromocyclododecane (HBCD), a widely used flame retardant, may pose significant risks to aquatic ecosystems due to their prevalence in the environment. Specifically, large quantities of these chemicals have been identified in municipal wastewater effluents (MWWEs). Widespread use of ECs has raised concerns regarding their possible risks to the environment, particularly to native species of cultural, recreational and commercial importance to Canadians, including lake trout (*Salvelinus namaycush*), northern pike (*Esox lucius*) and white sturgeon (*Acipenser transmontanus*). While data for some of these compounds are available on model laboratory species, such as rainbow trout (*Oncorhynchus mykiss*), little is known of the effects of these ECs to species in northern ecosystems, and as such, there are approaches needed that allow us to assess potential effects to such species. However, there is increasing concern with regard to live animal testing, particularly with regard to endangered or long-lived species. Therefore, alternative testing methods, such as *in vitro* tissue explant assays, are needed. The aim of the current study is to validate the predictivity of our *in vitro* results by comparing to a parallel *in vivo* study with the same species. Following an *in vitro* tissue explant assay in which lake trout, northern pike, rainbow trout and white sturgeon livers were exposed to serial concentrations of EE2, fluoxetine or HBCD, transcript abundance of select genes was measured in these species and a species-specific response was characterized. Rainbow trout had the greatest response among species when exposed to EE2, followed by white sturgeon, northern pike, then lake trout. With exposure to fluoxetine, rainbow trout and white sturgeon had equal responses while lake trout and northern pike had lesser responses. With exposure to HBCD, rainbow trout was again the most responsive species, followed by northern pike, lake trout, and white sturgeon.

Exploring 17α-ethinylestradiol exposure tolerance in mummichog (*Fundulus heteroclitus*) across the ovarian maturation cycle

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The endocrine disrupting compound 17α-ethinylestradiol (EE2) is linked to organism-level (hormone physiology/development) and population-level (egg production) effects in fish. Compared to model freshwater teleosts, estuarine mummichog (*Fundulus heteroclitus*) egg production is less sensitive to EE2 exposure; this may be due to differences in ovarian physiology, including 17β-estradiol (E2) regulation. In most teleost fish, there is a shift from estrogens (such as E2) to progestogens (such as maturation inducing steroid; MIS) as follicles progress to early maturation. However, in mummichog there is no shift detected, as levels of E2 are consistently higher in all stages of follicular development. Plasma and ovarian follicles from maturing mummichog were collected and grouped into five stages of maturation; follicular steroid production and/or gene expression in hormone signalling and steroidogenic pathways were assessed by stage. Plasma and follicular production of E2 increased as the ovarian maturation cycle progressed, and dropped after maturation; whereas MIS was equally expressed during development and early maturation stages, and increased in late maturation and ovulation stages. Differences between mummichog and other teleosts in maturational gene expression include: expression of P450 c17 (converts pregnenolone to 17α-hydroxypregnenolone and dehydroepiandrosterone) and follicle stimulating hormone receptor (FSHr; key gonadotropin responsible for ovarian development) drop earlier in the maturation cycle; P450 aromatase (converts testosterone to E2) is evenly expressed through all stages prior to dropping at late maturation, and luteinizing hormone receptor (LHr; key gonadotropin responsible for ovarian maturation) peaks earlier in maturation than expected. To determine EE2 effects, follicles grouped per stage were exposed to 50, 100 and 250 nM of EE2 *in vitro*; after 24 hours, P450 aromatase and LHr expression show no exposure differences regardless of ovarian stage. The dissimilarity in mummichog from other model teleosts in E2 regulation plus EE2 insensitivity in maturing follicles may be partially responsible for the lack of sensitivity of egg production in EE2-exposed mummichog.

Zebrafish (*Danio rerio*) high throughput testing to monitor estrogenic compounds in environmental samples

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For the past 15 years, researchers have demonstrated and documented the adverse effects of estrogen and other endocrine disrupting compounds (EDC) on humans and wildlife. In a direct nuclear method, estrogen interacts with specific target sequences of DNA known as estrogen response elements (ERE) or units. ERE are palindromic and non-palindromic sequences through which estrogen regulation is mediated. The zebrafish

(*Danio rerio*) has been developed as a valuable screening tool for detection of estrogen and EDC in water samples. Zebrafish embryos are small and transparent, and organs develop rapidly which facilitates the *in vivo* screening of estrogenic compounds. Zebrafish transgenic lines can be generated effortlessly, which makes it an exceptional choice for high-throughput screening. Our lab has expertise in generating various transgenic zebrafish lines and we have a state-of-the-art zebrafish high-throughput screening facility to screen drugs and water samples. The lowest available detection level of an estrogenic compound by a transgenic zebrafish is approximately 2.5 $ng \cdot L^{-1}$. The only Canada-wide water quality criteria for an estrogenic compound for protection of aquatic life is lower (e.g., the British Columbia Ministry of the Environment criteria for the synthetic estrogen EE2 is 0.5 $ng \cdot L^{-1}$). In order to increase the sensitivity of the transgenic fish to detect estrogenic compounds in a water sample, we have designed and synthesized a construct containing six tandem copies of ERE sequence that will drive the expression of Green Fluorescent protein (GFP). We examined the 5 flanking sequence of Estrogen Receptor alpha, Sox9a, cyp19a1b and various other genes to identify the sequence that matched the zebrafish ERE consensus sequence [GGTCA NNN TGACC]. We identified various potential zebrafish EREs that roughly matched the consensus sequence. In order to generate a sensitive reporter line for detecting estrogen in water samples, we synthesized a construct containing 6 tandem repeats of ERE sequence and cloned upstream of EGFP vector. This vector also contains Cmlc2 promoter, driving the expression of blue fluorescent protein in the heart for selection of transgenic fish and Tol2 transposon to enable transgenesis. We have injected the donor construct with Tol2 transposases and generated the transgenic line expressing EGFP under 6X ERE in the presence of estrogen, and we are currently validating the transgenic line. This transgenic zebrafish line will be employed as a tool for high-throughput zebrafish-based screening assays to monitor estrogenic activities in complex mixtures such as municipal wastewater effluents and surface waters from Ontario. This zebrafish-based *in vivo* reporter gene assay will add to the suite of novel tools being investigated in Ontario.

Molecular evidence of the impacts of model androgens on the gonadal steroidogenic pathway in male mummichog (*Fundulus heteroclitus*)

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The hypothalamus-pituitary-gonadal axis in fish is responsible for synthesis of sex hormones testosterone (T), 17β-estradiol (E2) and 11keto-testosterone (11KT) and is a target for endocrine disrupting chemicals. Androgenic endocrine disruption is typically under-studied compared to estrogenic endocrine disruption, even with a variety of anthropogenic sources, including sewage effluent and animal feedlots, introducing androgens into aquatic environments. Current research has identified decreased plasma sex steroid hormones in mummichog (*Fundulus heteroclitus*), an estuarine killifish, after exposure to androgenic compounds, in conjunction with decreased egg size and reduction of plasma vitellogenin. The molecular cause(s) are currently unknown. To elucidate the impacts of androgens on the gonadal steroidogenic pathway in mummichog, testis tissue

was exposed *in vitro* to control (ethanol only), 10-6, 10-9 and 10-12 M of the non-aromatizable androgen 5α-dihydrotestosterone (DHT) and the aromatizable androgen 17α-methyltestosterone (MT) for 6, 12, 18 and 24 hours. A suite of genes encompassing the entire pathway from cholesterol mobilization into the mitochondria to the final steroidogenic pathway products of T, E2 and 11KT were analyzed to determine androgen interaction within the steroidogenic pathway. Genes were normalized to elongation factor 1-α. Temporal impacts of gene expression occurred at 18 and 24 hour time-points, with upregulation of many genes from both MT and DHT treatments. The upregulation found in the current study does not explain the depression of plasma sex steroids, as upregulation should result in higher production of steroidogenic end products. 3βHSD, responsible for the conversion of pregnenolone to progesterone, was the only gene to respond after 6 hours of exposure, indicating it may be a biomarker for androgenic activity. Downregulation of androgen receptor was found in MT treatments at 24 hours but not in DHT treatments, indicating potential estrogenic effects of MT. Comparison of aromatizable and non-aromatizable model androgen responses will increase knowledge of potential differences in androgen impact due to androgen classification. Variable impacts across time periods show the transience of gene expression alteration and indicate that gene expression may not closely correlate to noted reductions in plasma sex steroids. Further work utilizing precursors of the steroidogenic pathway, such as pregnenolone, or specific enzymatic inhibitors, such a trilostane, will further elucidate the impacts of androgens within the gonadal steroidogenic pathway. These results will strengthen the understanding of androgenic perturbation at the molecular level and may be linked to higher levels of biological organization in future studies.

Obesogenic action of Bisphenol A on human adipoblastes

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Bisphenol A (BPA) is a chemical widely used in the production of polycarbonate plastics, epoxy resins, thermal paper, paints, water-pipes, electronic equipment, toys, packaging, printing inks, flame retardants, electronic devices, coatings for metal cans, dental sealants and laboratory equipment, among others. Some of these applications lead to the exposure of humans to BPA through food, dermal contact and drink. BPA binds to various receptors such as estrogen and androgen receptors, aryl hydrocarbon receptor and peroxisome proliferator-activated receptor, all of them associated with the endocrine system. BPA is prone to disrupt function of sex hormones, leptin, insulin and thyroxin. More recently, it has been suggested that BPA increases risk of obesity, diabetes and heart disease in humans, and it is related to genotoxic activity and epigenetic modifications. The objective of this work was to determine the obesogenic action of BPA on human adipoblasts. For this, an experimental study was carried out in which a sample was taken of a female voluntarily subjected to a liposuction of peripheral adipose tissue. The ADSCs (Adipocyte Derived Stem Cells) were obtained and then suspended in the basal medium and grown in 10 cm cell culture dishes. To evaluate the proliferative activity, the viable cells in each dish were counted on the trypan blue staining exclusion method at the end of

the culture. Seven days after the sowing of cells in “passage 0”, cell differentiation was initiated. To determine adipogenic differentiation, confluent cultures were incubated for four weeks in the control medium with 10% FBS supplemented with 0.5 mM isobutyl methylxanthine, 1 mM dexamethasone, 10 mM insulin, and 200 mM indomethacin. Exposure was performed using DMSO as a control and concentrations of 0.1 µM, 0.3 µM, 1 µM, 3 µM, 10 µM, 30 µM, 100 µM, 300 µM, 1000 µM BPA. Mortality of 100% of cultured cells was determined in all replicates (n=3) for concentration of 1000 µM BPA. For the measurement of adipocyte differentiation, staining with Oil Red O was performed as follows: 0.35 g of Oil Red O were dissolved in 100 ml of isopropanol. The filtered solution was added to the cells. The adherent cells were rinsed with PBS and fixed in 4% formaldehyde. The staining was then performed with 0.1% Oil Red O and images were taken from the dishes. To quantify, the pigment was extracted with isopropanol and the absorbance was determined with a spectrophotometer at wavelengths of 492 nm. The results were calculated in relation to the control. Triglycerides were extracted from the cells in 200 µL of chloroform/isopropanol/nonylphenol 40 (7:11:0.1) in a microhomogenizer. The extracted cells were centrifuged for 10 minutes at 15,000 G and the organic liquid was transferred to a new test tube and dried at 50 °C and placed under vacuum for 30 minutes. The dried lipids were dissolved in newton-x-100 in 200 µl of lipid-specific assay buffer. The percentage proliferation of adipoblast on exposure to 1% DMSO was similar in all three replicates and was lower than when exposed to minimal amounts of BPA. As the concentration of BPA was increased, a proportional increase in the percentage of adipoblast proliferation was observed until, at a concentration of 1000 µM, adipoblasts did not survive, clearly demonstrating the toxic effect of BPA on human adipoblasts. Adipoblasts exposed to DMSO 1% accumulated an equivalent amount of lipids in the 3 replicates and the value was lower than the values obtained with any concentration of BPA. As the concentration of BPA increased, a proportional increase in the amount of accumulated lipids up to a concentration of 300 µM, where maximum values were obtained, was observed. We demonstrated the obesogenic action of BPA. With the increase of BPA concentration, the lipid accumulation of adipoblastes and the quantity of triglycerides increased until BPA 300 µM.

In vitro assessment of pH-dependent uptake and toxicity of ionizable organic chemicals in fish

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Uptake and effects of ionizable organic chemicals in fish can significantly differ as a function of ambient pH. These differences are driven by the rate of passive diffusion of the uncharged species across the gill epithelium, which is considerably greater than that of charged species. Consequently, the flux of chemicals will peak at different pH values depending on their acid dissociation constants. Here, we propose a rapid *in vitro* screening method to assess the pH-dependent uptake of ionizable organic chemicals, specifically weak acids, at the fish gill. To this end, the permanent rainbow trout gill cell line RTgill-W1

was grown in transwell tissue culture inserts for two to three weeks, and allowed to establish tight monolayers as characterized by stable transepithelial electrical resistance (TEER). After acclimatization to the reduced complexity exposure medium L15/ex at pH 6.0, 7.4, or 8.5, the permeation of chemicals from apical to basal transwell chambers was determined during a 24-hour exposure period by means of liquid chromatography with high resolution mass spectrometry (LC-HRMS). The neutral red retention assay was conducted prior to exposures to exclude interference of cytotoxicity with the measurements. The assay was then used to investigate individual model chemicals (chlorophenols, carboxylic acids) and a technical mixture (nonylphenol) that had been previously shown to cause pH-dependent toxicity. To explore the applicability domain of the assay, we chose to investigate the pH-dependent permeation of chemicals present in acidic, neutral, and basic fractions, as well as reconstituted total extracts of oil sands process-affected water (OSPW). The acute toxicity of OSPWs has been shown to be mostly associated with the acidic fraction, specifically naphthenic acids. We observed a substantial pH-dependency of the cytotoxicity and permeability of weak acids, as well as the acidic fraction and reconstituted total extract of OSPW. We conclude that our *in vitro* assay can be used to screen for pH-dependent uptake and toxicity of ionizable organic chemicals in fish. It is intended to validate the test for application to prediction of pH-dependent uptake and effects *in vivo*. Thus, it may be a highly valuable tool for *in vitro*-*in vivo* extrapolation and prioritization of chemicals in non-target chemical screenings.

Establishing an *in vitro* model system for mechanistic amphibian immunotoxicity research

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Recent large-scale disease-driven population declines have highlighted the need to understand mechanisms of immunotoxicity in amphibians. *In vitro* assays represent an ideal method for studying the molecular basis of contaminant-induced immunotoxicity. Using the *Xenopus* A6 cell line, derived from kidney epithelial cells, we are developing an *in vitro* approach to screen chemicals for their potential to impair the innate immune response in amphibians. Lipopolysaccharide (LPS), a major component of bacterial outer membranes, was used as an immune challenge. *Xenopus* A6 cells exposed to 1 and 10 $\mu g \cdot mL^{-1}$ LPS for 3- and 6-hour induced expression of immune-related genes (tumor necrosis factor α (TNFα), interleukin -1 β (IL-1β), colony stimulating factor-1 (CSF-1)), indicating the capacity to mount an inflammatory response. Exposure to benzo[*a*]pyrene (BaP) (50 – 3200 $ng \cdot mL^{-1}$), a model immunotoxicant, resulted in a dose-dependent increase in the expression of cytochrome P450 1A1 (CYP1A1) but no change in aryl hydrocarbon receptor (AhR) after a 3-hour exposure. Results from these assays will be used to design contaminant/pathogen co-exposures to determine if exposure to BaP impacts the ability of the cells to respond to an LPS challenge. This *in vitro* model system will allow us to examine the mechanism of immunotoxicity in amphibians for a variety of environmental contaminants of concern.

Optimization of clearance rate as an effect endpoint in toxicity testing with freshwater mussels

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Freshwater mussels play an integral role in aquatic ecosystems by assisting in nutrient cycling, energy transfer, habitat structure, and water quality. Populations of freshwater mussels can significantly impact water quality by filtering particulate material from the water column which can lower nutrient levels. When investigating the potential risks that waterborne contaminants pose to freshwater mussels it is important to assess ecologically relevant sub-lethal endpoints since these organisms will most likely be exposed to low concentrations of contaminants in aquatic systems. This study examined how to optimize our current method of quantifying the filtering capacity (clearance rate: CR), of mussels after exposure to contaminants in toxicity tests. CR was defined as the amount of algal cells that an individual mussel can remove from the overlying water by filtration and was determined using spectrophotometric absorbance and direct microscopic examination. Optimization of the method included consideration of the following factors: concentration of algae mixture at test initiation, duration of clearance rate assay, and statistical power. Experimental vessels were set up with juvenile (ten, approximately 6 months old) and adult (one, approximately 2.5 years old) *Lampsilis siliquoidea*. The optimized adult clearance rate assay was required to run for 48 hours with 2.70×10^7 cells·mL^{-1} of algae added at test initiation and a minimum of six replicates per treatment to detect a 10% effect. The optimized juvenile clearance rate assay was required to run for 48 hours with 1.77×10^7 cells·mL^{-1} of algae added at test initiation and a minimum of four replicates per treatment to detect a 25% effect. After a reference toxicant exposure to sodium chloride, LC_{50}s from the optimized CR assay were compared to established mussel toxicity endpoints: survival and burial (ability of mussels to bury in clean sand). CR by direct microscopic examination was less sensitive in adults and slightly more sensitive in juveniles compared to the less labour-intensive endpoints of survival and burial. No significant differences were detected between the LC_{50} values determined from the three endpoints.

Application of the positive control approach in standardized biological test methods

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Standardized biological test methods published by Environment and Climate Change Canada (ECCC) require the performance of routine reference toxicant tests to assess the sensitivity of the test organism population over time. Over the past two decades, many standardized toxicity test methods have shifted from short-term lethality endpoints to longer-term sub-lethal endpoints such as reproduction, growth and development. In contrast, the endpoints and duration of reference toxicant tests has often remained unchanged, relying on short-term LC_{50} values. To address this incongruence and increase

the relevance of reference tests, ECCC has decided to introduce the option of conducting reference toxicant testing using a single concentration reference toxicant — in essence, a positive control. The option for using a positive control would be implemented by adding a treatment with each definitive test, and would be run the same as a negative control (i.e., same replication, organism requirement, test vessels, etc.) except exposed to a single concentration of a known toxicant. The endpoint for a positive control would be consistent with the endpoints derived from definitive tests (e.g., weight, growth, etc.), but would involve a target effect level (e.g., 40%), pre-defined within a suitable effect range (e.g., >30 to <50% effect) by the laboratory. The application of a positive control will be described with two examples. First, we will outline the steps to be followed for incorporating a positive control in a new ECCC soil test method, the 28 day reproductive inhibition test with the oribatid mite, *Oppia nitens*. Second, we will review how data from a recent inter-laboratory study with leopard frogs (*Lithobates pipiens*) can support the selection and utility of a positive control.

Working towards a physiologically based oiling model (PBOM) for predicting thermoregulatory responses in seabirds

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The risk of adverse effects on birds in marine and freshwater environments after an oil spill (namely, loss of feather insulation leading to decreased thermoregulatory capacity and subsequent hypothermia and mortality) can be expressed as the product of the probability of encountering a spill and the volume/thickness of that spill. This paradigm is the basis of the seminal resource for assessing the effects of spilled oil on wildlife: the *Comprehensive Environmental Response, Compensation, and Liability Act* (CERCLA) Type A Natural Resource Damage Assessment Model for Coastal and Marine Environments (NRDAM/CME). In that report, a deterministic threshold volume of 350 ml is considered a lethal dose, which translates to a threshold thickness of 10 µm for spills with diameters larger than 230 m. Some stakeholders in the risk assessment process consider oiling to be more like a non-threshold event, with even the smallest amounts of oil related to large enough changes in metabolism to bring about mortality. What is absent from the risk assessment practice is a dynamic model for predicting thermoregulatory responses in seabirds after an oiling event. Here we present (1) data, obtained through a literature search, about key variables related to avian metabolic responses to oiling (e.g., ambient temperature of both air and water, body condition, feather structure, insulative capacity, oil type); (2) what is known about the avian oiling dose-metabolic relationship under different conditions; (3) an illustrative conceptual map of the biotic and abiotic interactions related to this relationship; and (4) results of preliminary modelling (based on points 1-3) from a collaborative effort to establish a Physiologically Based Oiling Model (PBOM) for quantifying thermoregulatory responses in seabirds after oiling. From this exercise, we provide our interpretations on how to make scientifically justified risk characterizations for birds interacting with spilled oil.

An Investigation of Cause: How multiple lines of evidence determined the probable cause of toxicity for two mining effluents

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Biological monitoring pursuant to the *Metal Mining Effluent Regulations* (MMER) showed effects on a benthic invertebrate community in a creek receiving discharges from a combination of municipal, urban, and industrial sources, including treated mine effluent from two facilities. The biological effects observed included an absence of members of the sub-phylum Crustacea. This was consistent with sub-lethal toxicity tests showing reproductive impairment in *Ceriodaphnia dubia* exposed to the treated mining effluents. To understand the potential for the mining effluent constituents to contribute to the observed effects, a two-part Investigation of Cause (IOC) study was carried out using *C. dubia* as a surrogate test species. In Part 1, regulated metals were screened based on toxicity and corresponding chemistry data. Based on this assessment, copper (Cu) and nickel (Ni) were put forward as metals of potential concern for effluent discharged from the waste water treatment plant (effluent #1) and Ni was put forward for effluent discharged from the waste water treatment system (effluent #2), while As, Pb, and Zn were eliminated. In Part 2 of the IOC study, toxicity tests with *C. dubia* (combined with chemical analyses) were conducted on final effluent samples collected over a 1-year period (9 samples for effluent #1 and 11 samples for effluent #2). The results of this testing showed consistent reproductive impairment (i.e., calculated IC_{25}s ranging from 15.2% to 74.9% (v/v) and from 1.1 to >100% (v/v) for effluent #1 and #2, respectively) and comparatively few occurrences of acute lethality. Application of the toxic unit (TU) model to the data subsequently showed that all of the total dissolved Ni TUs were higher than the TU IC_{25}s for effluent #1, thus indicating that levels of Ni were high enough to have caused toxicity. When assessed from a bioavailability perspective (i.e., TUs calculated based on the summed activities of bioavailable Ni species), calculated TUs and TU IC_{25}s diverged even less, with the exception of 2 samples where the bioavailable Ni TUs were much higher than the TU IC_{25}s. Copper was ruled out in Phase 2 of the IOC as dissolved concentrations were well below toxicity threshold values (thus rendering Cu TU values negligible relative to those calculated for Ni). For all effluent #2 samples, total dissolved Ni TU values similarly indicated that levels of Ni were high enough to cause toxicity and TUs calculated as a function of bioavailable Ni again brought the TUs and TU IC_{25}s closer together. However, for 5 of the samples, the bioavailable Ni TUs remained much higher than the TU IC_{25}. To address the uncertainty prompted by the few samples where TU values were well above the IC_{25}s, correlation analysis was performed to understand water quality impacts on Ni toxicity. This analysis showed that IC_{25}s increased as a function of increasing concentrations of dissolved organic carbon (DOC) for both effluents—a correlation which explained the cases where the Ni TUs and measured IC_{25}s diverged, as the higher DOC in these samples would have offered a protective effect against Ni toxicity. The collective results obtained support a conclusion that Ni was the principal toxicant responsible for the observed impairment to *C. dubia* reproduction for both effluent #1 and #2. This study highlights the importance of lab testing as an investigative tool for understanding field effects and building science-based burden of evidence linkages.

The influence of community composition on selenium bioconcentration in field-collected freshwater periphyton

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In freshwater ecosystems, bioconcentration and biotransformation of inorganic selenium occurs primarily in phytoplankton and periphyton, and associated biofilms. This process is the most important step towards the introduction of organic selenium compounds into local food chains. There is a large body of evidence to suggest that selenium enrichment functions can vary by up to five orders of magnitude for different species of planktonic algae and that biotransformation processes can differ among taxa. Similarly, previous work has shown that many bacterial genera are also capable of biotransforming and accumulating selenium using different strategies. Currently, the influence of freshwater periphyton community composition on the accumulation and subsequent food-chain transfer of selenium compounds is poorly understood. The aim of this research is to collect different periphyton community assemblages in order to address the primary question asked of this project: Does community composition matter when estimating selenium accumulation in periphyton-dominated biofilms? Field-sampled periphyton from five different lakes was exposed to aqueous selenite or selenate at concentrations of 5 or 25 μg $Se \cdot L^{-1}$ for a period of 8 days. Selenium enrichment functions varied by up to 20-fold among the different field-collected periphyton communities and displayed saturation kinetics at environmentally relevant selenium oxyanion concentrations. The results of this work will aid in more reliable modelling of selenium transport through Northern freshwater food-webs. This will enable regulators and industry to more accurately predict the effects of selenium transfer to higher order consumers, such as fish and waterfowl.

Characterization of nickel-biotic ligand interactions in seawater

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Potential impacts of metals in saltwater (SW) environments are significant. Globally, the largest anthropogenic releases of metals are from fossil fuel, predominantly coal and oil, combustion, mining and smelting. The binding of metal by ligands is assumed to reduce the bioavailability of metal to the biotic ligand (organism), thereby reducing toxicity in the system, and this relationship can be predicted using the biotic ligand model (BLM). However, the effects of nickel (Ni) in saltwater ecosystems has not received as much research attention as in freshwater (FW) ecosystems. Thus, there is a pressing need to obtain experimental data and to test models of Ni binding in order to predict, mitigate, and manage potential Ni pollution and toxic impacts. My study predicts that model ligands will reduce short-term toxicity (EC_{50}) to purple sea urchins (*S. purpuratus*) and that this toxicity will vary depending on the binding affinity (log K_f) of the ligand. It is hypothesized that the EC_{50} of metal free ion (Ni^{2+}) will be similar between model ligands if toxicity is explained by $[Ni^{2+}]$. It is also hypothesized that the binding affinity of the biotic ligand can be determined

using known binding affinity constants. Defined solutions of artificial seawater (ASW) containing different model ligands (i.e., ethylenediaminetetraacetic acid (EDTA), glutamic acid (GA), tryptophan (TRY), histidine (HD), and citric acid (CA)) were used for Ni toxicity and speciation tests. Total dissolved Ni concentration [NiD] of the treatments has been measured using the Graphite Furnace Atomic Absorption Spectrometer (GFAAS) and [Ni^{2+}] has been predicted using Visual Minteq 3.1. The dose-response curves were expressed both as percentage abnormal development of the sea urchin embryo by [NiD] and [Ni^{2+}] and corresponding EC_{50} values were determined. In the same defined solutions, Ion Exchange Technique (IET) coupled with GFAAS was performed to determine [Ni^{2+}] and was compared against Visual Minteq results in order to evaluate the feasibility and applicability of the IET method. The methods developed with model compounds can be applied to natural samples in order to determine the binding affinity and protectivity of dissolved organic matter (DOM) and Ni EC_{50} values. The results showed that: (1) protection of the biotic ligand occurred when model ligands were added; (2) protection increased with increased Ni binding affinity; (3) consistent with the BLM, Ni toxicity can be explained by [Ni^{2+}]; and (4) IET-GFAA is a suitable technique for determining [Ni^{2+}] in sea water. The results of this research contribute to the development of biotic ligand-based prediction models for estimating the impacts of Ni in marine water. Funding was provided by Natural Sciences and Engineering Research Council of Canada (NSERC), VALE and NiPERA (Nickel Producers Environmental Research Association).

Toxic effects of nickel salts on the nematode *Caenorhabditis elegans*

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Nickel (Ni) is a transition metal widely distributed in the environment, with several industrial applications. However, its toxicity mechanisms have been poorly studied. To assess the toxicity of nickel salts, the wild type *Caenorhabditis elegans* strain Bristol N2 was used in bioassays through different toxicity endpoints such as lethality, locomotion, growth and fertility. GFP transgene integrated with the mtl-1, sod-4 and gpx-6 genes were used to assess changes in gene expression. *Escherichia coli* OP50 was used as food in K agar prepared with KCl, NaCl, agar, peptone, cholesterol, $CaCl_2$ and $MgSO_4$. The larval age synchronized nematodes L4 and L1 were exposed to concentrations of 0.1, 1.0, 10, 100 and 1000 µM of three nickel salts: $NiCl_2.6H_2O$, $NiSO_4.6H_2O$ and Ni (NO_3) $2.6H_2O$, respectively; and also, to mixtures of these. Medium K (52 mM NaCl and 32 mM KCl in ultrafiltered water) was used as the control and as dilution water for the preparation of the solutions. Four replicates were performed for the treatment and each experiment was done in triplicate. For the evaluation of survival, larval stage nematodes L4 were exposed for 24 hours to solutions of the nickel salts. Approximately 10 ± 1 worms were used for treatment. The number of live and dead worms was then counted by visual inspection using a dissecting microscope. In order to evaluate changes in locomotion, the frequency of body curves was measured, for which the nematodes previously exposed for 24 hours were transferred to a K agar plate and recorded for the number of body curvatures in 20 seconds. Each curve of the body was counted as a change in the direction of the posterior

bulb of the pharynx along the Y axis, assuming that the nematode moved along the X axis. Approximately 30 nematodes were examined per treatment. Developmental assessment was performed by measuring body length in L1-age larvae after 48 hours of exposure to nickel salt solutions. *E. coli* OP50 was inoculated as a food source. Body length was measured by analyzing a photograph recorded by a dissecting microscope and using ImageJ software. Approximately 30 nematodes were examined per treatment. To evaluate reproduction, the size of the rearing was measured. L4 larval nematodes were previously exposed for 24 hours to solutions of nickel salt. Each nematode was individually fixed on K agar plates seeded with *E. coli* OP50 and the number of offspring was counted in all steps after 72 hours. Approximately 10 nematodes were examined per treatment. Changes in gene expression were assessed using transgenic GFP reporter strains containing mtl-1, sod-4 and gpx-6 genes. Equal aliquots of nematodes at all larval stages were plated onto non-fluorescent, 96-well U-bottom black microplates with the nickel salt solutions. The plates were incubated at 15 °C and fluorescence was recorded after 24 hours using a Perkin-Elmer Victor 1420 multi-level plate reader at 485/525 nm as excitation/emission wavelengths, respectively. The K medium was used as control. Normality and homogeneity of variance were verified using the Kolmogorov-Smirnov and Bartlett tests, respectively. Significant differences were determined between the means with ANOVA. The Dunnett test was applied to compare each sample with the control. The results obtained show that the survival was dose dependent, with nickel chloride being the most toxic salt. At 1000 µM, all salts caused the mortality of all exposed animals. Regarding the effects on locomotion, the basic motions were inhibited up to 66, 54 and 39% for 100 µM of nickel chloride, nitrate and sulfate, respectively. In turn, body length decreased by 15.5, 19.4 and 20.1% to 100 µM of nickel chloride, nitrate and sulfate, respectively. On the other hand, brood size was dose dependent, with 86, 84 and 80% inhibition of exposure to 100 µM of nickel chloride, sulfate and nitrate, respectively, related to the control. Changes in gene expression showed that mtl-2 was overexpressed 2.9-fold than the control for 100 µM of nickel sulfate. The stress response genes sod-4 and gpx-6 were overexpressed 3-fold and 3.7-fold control of nickel chloride and nickel nitrate, respectively. In conclusion, these results verify that the toxicity of nickel depends on the type of salt and the concentration of the exposure, which affects the behavior of locomotion, reproduction, growth and increases the expression of stress-response related genes.

Assessing fish health and food-web structure in small sub-Arctic lakes in Nunavut in relation to mining activity

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Mining activity in Canada's subarctic region has increased significantly in recent years. Potential impacts that resource extraction might have on the ecological integrity of aquatic systems in this sensitive region, however, are poorly understood. In this study, we monitored the health of several fish species as part of a baseline study for an open pit gold mine located near Rankin Inlet, Nunavut. The mine is in advanced exploration and construction phase with operations commencing in 2019. Lake whitefish (*Coregonus*

clupeaformis), Arctic grayling (*Thymallus arcticus*), lake trout (*Salvelinus namaycush*), and 3- and 9-spine sticklebacks (*Gasterosteus aculeatus* and *Pungitius pungitius*) were sampled from six lakes between 2014 and 2016 within the current zone of exploratory activities (road building and camp construction, infrastructure development, exploration, drilling, blasting and stripping the natural cover off the land) and/or eventual impact zone once full mining operations commence (where additional impacts such as lake dewatering, blasting, water retention dykes, ore processing, and effluent discharge into tailing impoundment areas are expected). Fish measurements included total weight and length, liver and gonad weight, sex, age (otolith and weight-length frequency), gut contents, and muscle tissue for stable isotope analysis. Gut contents indicate clear resource partitioning among fish species in these small lakes with lake trout predominantly eating small fish (including sticklebacks), grayling relying on amphipods, snails and some caddisflies (Trichoptera) and whitefish exhibiting a more generalist invertebrate diet. In sticklebacks, we also noted a high incidence (~30%) of parasitism for both the 3- and 9-spine sticklebacks. Our goal is to compare pre-and-post incidences of parasitism as a basis for evaluating stress in these ecosystems. Characterizing the fish communities in these lakes will help to develop a strong baseline against which potential post-mining operation impacts can be compared.

Benthic community structure in subarctic lakes in relation to mining disturbance

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A gold mine operated by Agnico Eagle near Rankin Inlet, Nunavut, is currently in exploration phase and is scheduled to commence full operation in 2019. Operations do and will include open pit mines, water retention dykes, lake dewatering, blasting, ore processing, and tailings ponds occupying approximately 2400 hectares. The landscape within and surrounding the mining area contains many small (<20 ha) lakes which could be impacted by increased dust deposition, sediment loading due to run-off and/or leaching from waste rock piles, and increased water/sediment metal concentrations. To assess the potential risks of the mining operation to these lakes, we initiated a monitoring study in 2014 to assess water chemistry, food web structure (stable isotopes analysis), community composition of benthic invertebrates, phytoplankton, zooplankton, and the relative health and condition of fish populations. In this poster, we present our findings on benthic community composition in relation to physicochemical lake characteristics using standard composition metrics and multivariate analysis (PCA, CCA). In all lakes, the benthic community is dominated (50-80%) by larval Chironomidae. Amphipods (*Gammarus lacustris*), snails (*Physella, Stagnicola*), clams (*Pisidium*), Trichoptera (*Limnephilus, Grensia*), and Plecoptera (*Nemoura, Isoperla*) comprise the remaining community. Deep lakes (Z_{max}>3m) tended to be less productive (based on chlorophyll a), less diverse and have higher proportions of Chironomidae; shallow lakes tended to be more productive, have higher proportions of *G. lacustris* and lower proportions of Chironomidae. Lake groupings based on PCA for water chemistry were largely driven by differences in hardness and dissolved organic matter.

Impact of two rare earth elements on the microbial health in a sandy loam soil

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The surface soil ecosystem is largely dependent on the health of its soil microbial community. At Environment and Climate Change Canada, soil microbial health is being assessed by amending a soil with a priority substance and looking for effects using a suite of microbial tests. These include organic matter (OM) decomposition, community-level physiological profiling (CLPP), and heterotrophic plate count (HPC). Currently, we are studying the impact of two rare earth elements, samarium (Sm) and praseodymium (Pr), on the soil microbial community. In individual experiments, our test substances have been added to sandy loam soil as SmCl3 or PrCl3 at different test concentrations. The soluble salt forms were used due to the high solubility of these compounds. Five concentrations were tested for both substances: 853, 1493, 2612, 4571, and 8000 $mg \cdot kg^{-1}$ dry weight soil. A calcium chloride control was also used in each experiment to check for the possible influence of the chloride ion on our test results. Since the purpose of this research is to measure whether there are differences in soil microbial health between reference soil and the same soil spiked with $SmCl_3$ or $PrCl_3$, it is important to quantify the influence of the chloride component. OM decomposition, CLPP, and HPC results displayed greatly decreased microbial activity, diversity, and biomass, respectively, at the highest tested concentration of both substances compared to the reference soil. However, we suspect that chloride ions are acting as a confounding variable and may be contributing in part to the increase in toxicity to soil microorganisms at higher test concentrations of $SmCl_3$ and PrCl3. This suspected chloride effect will be addressed in planned additional experiments.

Comparison of metal toxicity and metabolic rate between northern and temperate fish species

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Canadian Water Quality guidelines (WQG) are developed from acute and chronic toxicity data, usually done using standard toxicity testing methods. Within the data sets used for deriving WQG, northern species are rarely represented and this represents a gap in our understanding as well as a concern. There is potential that metabolic rate (MR) could be used as an indicator in determining metal toxicity, due to its role in delaying toxicity. The objective of my research will be to determine if metabolic rates can be used as an indicator in determining toxic stress. MR, measured as the rate of oxygen consumption (MO_2), will be measured for rainbow trout (*Oncorhynchus mykiss*) and fathead minnows (*Pimephales promelas*). Environmentally relevant concentrations of copper, including the LC_{50} and LC_{20} will be used to observe differences in MR during metal exposure between species. Lastly, similar tests will be done using northern caught slimy sculpin (*Cottus cognatus*) to determine if similar changes are seen. Preliminary results from one trial with added Cu (100 $\mu g \cdot L^{-1}$), control MO_2 ranged from 9.75 to 11.75 µmol/g/hour (5 cycles, 11.05 ± 0.99 µmol/g/hour, mean ± s.d.). When Cu was introduced, the MO_2 increased by 45% (3

cycles, approximately 1 hour, 15.99 ± 0.88 μmol/g/hour, mean ± s.d.). Subsequently, MO_2 decreased to 12.13 ± 0.36 μmol (mean ± s.d.). This study will be helpful in determining if water quality guidelines are relevant in the protection of northern species.

Polymers that "substantially degrade, decompose or depolymerize": Draft interpretation affecting the notification of new polymers under Canadian *New Substances Notification Regulations*

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The *New Substances Notification Regulations* (NSNR) of the *Canadian Environmental Protection Act, 1999* (CEPA) requires the notification of substances new to Canada with prescribed information. These substances are assessed to determine whether they are potentially "toxic," in order for appropriate control measures to be implemented to mitigate risk to human health and/or the environment. Some substances meet criteria that allow them to be notified as "reduced regulatory requirements" (RRR) polymers. One criterion relates to polymer stability. Polymers that "substantially" degrade, decompose or "depolymerize" do not meet the description of a RRR polymer. An interpretation is that "substantial" be judged against both the degree of degradation, and the influence of the transformation products in risk assessment. Canada's draft policy regarding that interpretation and its implementation will be explored.

Comparing predicted and observed ecotoxicity of polycyclic aromatic hydrocarbon mixtures in sediments from the Athabasca system

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The target lipid model (TLM) is a relatively novel approach applicable to predicting the combined toxicity of polycyclic aromatic hydrocarbon (PAH) mixtures to aquatic organisms. It applies the critical body residue principle, which proposes that, once absorbed into an organism, the combined toxicity exerted by multiple individual PAH compounds will be additive, with a mode of action involving non-polar narcosis. Combining the TLM with equilibrium partitioning allows individual PAH concentrations in sediment to be converted to a toxic unit; the toxic units can be summed for all PAHs in the sediment to estimate the overall toxic potential of the PAH mixture. This toxic units approach is potentially useful as a tool for screening the toxic potential of naturally-occurring and anthropogenic PAH mixtures in aquatic sediments of the Athabasca Oil Sands Region. While the method has shown promise based on validation with data sets in the United States, the wealth of co-occurring sediment PAH and ecotoxicity data (500+ synoptic chemistry and toxicity measurements) collected since 1997 through the Regional Aquatics Monitoring Program (RAMP) and Joint Oil Sands Monitoring (JOSM) program provides an opportunity for further validation of the method, specific to conditions present in the Athabasca system. In this poster, we compare chemistry-derived toxicity predictions to corresponding

laboratory-observed sediment toxicity for standard midge, oligochaete and amphipod test species. We test the prediction accuracy of two versions of the TLM (available in the literature and US EPA guidance) as well as differing equilibrium partitioning assumptions (partitioning to organic carbon vs. free-phase hydrocarbons). By examining the similarities and differences between predicted and observed toxicity it is possible to evaluate the utility of the toxic units method as a screening tool for PAH mixtures in sediments of the Athabasca system, and better understand the factors that may influence sediment toxicity in the field.

Ecological screening assessment of quaternary ammonium compounds under the Government of Canada's Chemicals Management Plan

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Under the Chemicals Management Plan (CMP), the Government of Canada is assessing and managing, where appropriate, the ecological and human health risks of substances. Building on previous work under the CMP (over 2700 substances addressed from 2006 to 2015), 1550 substances have been identified as priorities for assessment over the subsequent five years (2016-2020). Examples of the 1550 substances include a group of quaternary ammonium compounds (QACs). This poster provides a description of the QAC group, an overview of the available data, and preliminary assessment approaches for these compounds under CMP. Activities related to characterizing these substances, which are representative of a wide range of QACs, may be used to inform a broader evaluation of QACs in the future.

An assessment of the response of freshwater organisms to thiamethoxam under laboratory and field conditions

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Thiamethoxam is a neonicotinoid insecticide used extensively in agriculture across Canada and around the world. It has been detected in surface waters (typically in the ng/L range) next to areas of use, raising questions about possible non-target effects. To better characterize the risk thiamethoxam might pose to freshwater organisms, a series of laboratory and mesocosm studies have been conducted. We report the results of laboratory toxicity testing (acute and chronic) conducted under good laboratory practices (GLP) for over 30 freshwater species (insects, molluscs, crustaceans, algae, macrophytes, and fish). As expected for this compound, fish and primary producers were insensitive, with acute median effect concentrations (LC_{50}/EC_{50}) observed to be ≥ 80 mg·L^{-1} in all cases. Tested molluscs, worms, and rotifers were similarly insensitive (EC_{50} ≥ 100 mg·L^{-1}). In general,

insects were the most sensitive group, with most acute EC_{50} values <1 mg·L^{-1}. In addition, an outdoor mesocosm study was conducted examining the response of zooplankton, insects, and phytoplankton up to 93 days following a single treatment with thiamethoxam. The No Observed Ecologically Adverse Effect (NOEAE) was 100 µg·L^{-1} and the NOEC community was 30 µg·L^{-1}. Finally, in order to address the uncertainty of effects associated with possible chronic exposures, a 35-day outdoor mesocosm study with a formulated product was conducted with the mayfly *Cloeon dipterum*. This species was particularly sensitive to thiamethoxam under chronic exposure conditions in laboratory studies. Significant reductions in both larval abundance and adult emergence were observed at 10.0 and 3.0 µg·L^{-1} within 6 to 14 days of exposure, respectively, with transient effects at 1.0 µg·L^{-1}. Exposure to 0.1 and 0.3 µg·L^{-1} had no statistically significant effect on larval mayfly abundance or adult emergence at any point in the study. These findings support a 35-day NOEC of 0.3 µg·L^{-1} for *C. dipterum* under chronic exposure conditions. Overall, these data will reduce uncertainty in ecological risk assessments of thiamethoxam.

The impact of the aquatic herbicide diquat on phytoplankton in the presence of macrophytes and a simplified food-web

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Information on the effects of chronic, ecologically relevant concentrations of current-use aquatic herbicides on non-target biota is lacking. We determined the effects of the aquatic herbicide diquat on natural phytoplankton communities in outdoor mesocosms (300 L) that included macrophytes as well as amphipods and tadpoles. The experimental design consisted of a control and five treatments (five replicates each) with applications ranging from 6.4 to 100% of the commercial diquat formulation Reward® label rate, which corresponded to concentrations of 74 to 1153 µg·L^{-1}. Biota were exposed to a single application of diquat for six weeks. Macrophytes were negatively affected across all concentrations within a couple of days and this was followed by a rapid rise in phytoplankton biomass that was proportional to diquat treatment. Both pigment and microscopic analyses showed similar trends in biomass. Phytoplankton communities were dominated by chlorophytes across all treatments. Species diversity was negatively affected by diquat, leading to dominance of *Chlorella* or *Scenedesmus* spp., depending on the treatment.

Analysis of pesticide residues in cannabis regulated by Oregon state using LC-MS/MS

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Cannabis, being consumed for both medicinal and recreation purposes in the legalized states of the United States, requires testing for pesticides, since chronic exposure to pesticides can lead to considerable health risks. The State of Oregon has issued regulatory limits for 60 pesticides residues in cannabis flower and edibles. We studied the 60

pesticides spiked in cannabis flower extracts using LC-MS/MS. Pesticides were simultaneously analyzed in both positive and negative mode using electrospray ionization. We were able to detect all the pesticides using this methodology, well below the action limits specified by Oregon. Ground cannabis flower (1 gm) was hydrated with water (5 mL), spiked with pesticide and surrogate standards, followed by the addition of acetonitrile (5 mL). To this mixture, QuEChERS salts (3 g $MgSO_4$ and 0.75 g sodium acetate) were added, vortexed for 0.5 hours and centrifuged at 7000 rpm (5 minutes). The supernatant was analyzed directly or further cleaned up using dispersive SPE (dSPE containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18). Negative controls with no pesticide spikes were taken through the extraction process. The samples were then diluted and injected on column for chromatographic separation followed by detection in multiple monitoring mode (MRM) using the PerkinElmer QSight 210TM mass spectrometer. The pesticides were analyzed using a triple quadrupole mass spectrometer fitted with a hot coaxial flow electrospray ionization source combined with a heated laminar flow interface. The overall sensitivity for most of the pesticides, including those that are normally analyzed by gas chromatography (GC), was between 1-100 $\mu g \cdot kg^{-1}$, well within the regulatory limits set by the State of Oregon.

Roundup WeatherMax® and amphibian development: An environmentally relevant field study on the effects of a glyphosate herbicide formulation on gonadal differentiation of *Lithobates sylvaticus*

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Several laboratory, mesocosm and field studies have evaluated the effects of glyphosate-based herbicides on frog development. However, little is known about the possible effects of glyphosate-based herbicides on frog gonadal differentiation in animals undergoing natural development in the wild. We used whole wetland manipulations to determine if exposure to an agriculturally relevant application of Roundup WeatherMax®, a herbicide formulation containing the potassium salt of glyphosate and an undisclosed surfactant, influences gonadal development of wood frog tadpoles (*Lithobates sylvaticus*) under natural conditions. Experimental wetlands were divided in half with an impermeable curtain so that each wetland contained a treatment and a control side. Tadpoles were placed inside *in situ* enclosures in each wetland half and exposed to two pulses of Roundup WeatherMax® at the predicted maximum environmental concentration (PMEC, 2.89 mg acid equivalent (a.e.)$\cdot L^{-1}$). Results showed that under an environmental realistic scenario, exposure to Roundup WeatherMax® does not influence gonadal differentiation in *L. sylvaticus* tadpoles. We conclude that detected differences on sex ratios and gonadal differentiation at the molecular level, studied by measuring mRNA levels of cyp19a1a, foxl2, cyp17 and star, are more likely due to intrinsic differences between the wetlands and the wetland sides than to exposure to Roundup WeatherMax®.

Organophosphates and American lobster larvae: From gene expression to physical effects

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Aquaculture, agriculture, and the American lobster (*Homarus americanus*) fisheries are three economically important industries in the Atlantic regions of Canada and the United States and have great potential to interact within the marine ecosystem. A common practice in aquaculture and agriculture industries is the use of pesticides. These pesticides have the potential to interact with non-target organisms, such as the American lobster, through runoff, spray drift and dispersal. In recent decades, lobster landings have declined in the Northumberland Strait, a body of water with surrounding agricultural industries, and recruitment of larvae has decreased in areas of the Bay of Fundy containing nearshore salmon farms. Larval stages of *H. americanus* are vulnerable to both hydrophilic and hydrophobic pesticides, since early larval stages reside in the pelagic zone before settling to the benthic zone. Both of the organophosphates Salmosan® (an aquaculture pesticide with the active ingredient azamethiphos) and chlorpyrifos (an agricultural pesticide) target arthropodic pest insects and have been shown to affect the survival of some larval decapod crustaceans. Azamethiphos has been shown to impact the growth and survival of *H. americanus* yet no data exist on the impacts of chlorpyrifos to this species. Using 3- and 48-hour acute exposures of Salmosan® and chlorpyrifos, respectively, median lethal concentrations were established for stage IV American lobster. During sublethal exposures, the growth parameters intermoult period, moult inhibition and specific growth rate were found to be significantly affected when compared to the control treatment. RNA sequencing was carried out using Illumina Hiseq technology and subsequent confirmation of the expression of genes of interest was performed via RT-qPCR. In the current study, it was determined that both organophosphates affected expression of genes encoding for stress responses and xenobiotic metabolism. Chlorpyrifos exposures additionally affected expression of hypoxia response and axonal growth-related genes while azamethiphos exposure corresponded to changes in expression of genes related to oxidative stress and immunity.

The presence of pesticides in river sediments is related to toxicity on *Caenorhabditis elegans*

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Pesticides are a group of compounds utilized for crop protection in agriculture, domestic application, and disease control, among others. However, their continuous application and release to aquatic environments has led to their presence in sediments, becoming a matter of concern due to their capacity to bioaccumulate and persist. Some pesticides have been considered toxic at different levels: endocrine disrupters, carcinogens, and neurotoxicants, among others. In Colombia, a country with vast agricultural activity, the use of pesticides is generalized in many processes, including illegal crops eradication.

The Magdalena River, the most important waterway in Colombia and the main source of drinking water and fish, has sink runoff from agriculture, and the presence of pesticides in its sediments is unknown. In this study, sediment samples were collected in 20 sites of the Magdalena River and analyzed for 40 pesticides among organophosphorus, chlorinated, uracil, triazines, chloroacetamides, and thiocarbamates. Methanolic leachate of each sample was obtained by centrifugation-filtration and used to expose *Caenorhabditis elegans* in L4 larval stage for 24 hours to evaluate survival, locomotion, growth, fertility, egg-production, reproduction, and changes in the expression of stress response related genes. The most abundant pesticides in the Magdalena River sediments were atrazine (0.76 ± 0.99 $ng \cdot g^{-1}$), bromacil (0.74 ± 0.52 $ng \cdot g^{-1}$), 4,4´DDE (0.66 ± 1.38 $ng \cdot g^{-1}$), and chlorpyrifos (0.45 ± 0.64 $ng \cdot g^{-1}$). The highest concentrations of these pesticides were found in samples taken in towns located in agricultural areas where oil palm growth is very intensive and the use of pesticides is poorly controlled. All samples presented DDT/(DDE+DDD)<1, indicating historical contributions of DDT. The most of the samples showed DDD/DDE ratio < 1, suggesting aerobic conditions. Effects on survival, growth, and locomotion were observed in the samples related to industrial, gold mining and petrochemical activities. Changes in gene expression were evident for cyp-34A9, especially in the sampling site located near an oil refinery, and at the seaport in Barranquilla City. Nematode fertility, egg-production and brood size were inhibited up to 22, 33, and 37%, respectively. There was strong and negative correlation between fertility, egg production, and brood size and the concentration of some pesticides such as dichlorvos, mevinphos, metachlor, trans-chlordane, cis-chlordane, DDE, endosulphan sulfate, famphur, and chlorpyrifos. In addition, dichlorvos correlated with the expression of gst-1 and cyp-34A9; molinate was associated with the expression of hsp-16.2. The concentration of etotrophs was related to survival. The β,γBHC content was associated with locomotion. The presence of bromacil corresponded to survival and with expression of sod-1, cyp-34A9, and gpx-6. Fenthion and parathion were correlated to survival and locomotion. Chlorpyrifos correlated to the expression of hsp-16.2, gst-1, and cyp-34A9. Coumpafos had strong associations with hsp-16.2, gst-1 and gpx-6. In conclusion, the presence of pesticides such as chlordane, DDT-related compounds, and chlorpyrifos, among others in Magdalena River sediments is associated with survival, locomotion, reproductive toxicity, and the expression of stress response related genes on the biological model *C. elegans*.

Assessing the trophic transfer of waterborne selenium to an amphipod (*Hyalella azteca*) through a diet of field-collected microorganisms

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The mobilization and contamination of selenium (Se) into environmental systems is of significant global concern. A variety of natural and anthropogenic sources and site-specific differences in biogeochemistry are the source for different forms and concentrations of Se in aquatic environments (eg. selenate [SeO_4^{-2}] and selenite [SeO_3^{-2}]). These inorganic forms of Se are efficiently assimilated, biotransformed, and bioaccumulated by aquatic

microorganisms into organoselenium compounds, which are transferred to higher trophic levels via dietary pathways. The present study aims to quantify the trophic transfer factors of selenomethionine to a primary consumer through the biotransformation of inorganic oxyanion Se forms (selenate and selenite) by field-collected communities of microorganisms. Biofilm samplers were placed in the epilimnetic zone of uncontaminated lakes in northern Saskatchewan and allowed to accumulate natural communities of periphytic microorganisms representative of these lake habitats. DNA samples were collected from each biofilm sample for microscopic and metagenomic characterization of the biofilm communities. Samples were exposed in the laboratory to aqueous concentrations of selenite and selenate respectively, at concentrations of 0, 5 and 25 μg $Se \cdot L^{-1}$, and uptake and biotransformation by the biofilm to selenomethionine was quantified. The amphipod *Hyalella azteca*, a primary consumer characteristic of Canadian freshwater ecosystems, grazed on the Se-spiked biofilm communities with the intent to determine trophic transfer efficiencies as a function of microorganism community structure. This research will serve to assist in improving environmental risk assessment strategies for the release of Se into aquatic environments.

CYP3C gene regulation by the aryl hydrocarbon and estrogen receptors in zebrafish

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Cytochrome P450s (CYPs) are a large family of enzymes that are critical for xenobiotic and endogenous compound metabolism. The CYP3 family is extensively studied in mammals for their incredible ability to metabolize many, structurally unrelated, exogenous compounds. In mammals, CYP3 genes appears in one subfamily (A) and these are responsible for the metabolism of over 50% of pharmaceuticals. Interestingly, CYP3s in fish include four subfamilies (A, B, C, D) with function that is poorly studied but commonly thought to be similar to mammalian CYP3A. In fact, mammalian and fish CYP3As may be functionally different and appear to be regulated by different pathways. Differences in expression and regulation likely exist between the fish CYP3 subfamilies as well. Duplication of piscine CYP3 subfamilies has been hypothesized to produce genes with new functions regulated by unique receptor pathways. This study focuses on the novel CYP3C genes in zebrafish (*Danio rerio*), an important model species. Zebrafish have four CYP3C genes, CYP3C1, CYP3C2, CYP3C3 and CYP3C4, and these genes are expressed in detoxification organs including intestine and liver. In this study, zebrafish were exposed to 17β-estradiol (0.001-10μM; E2) and β-naphathoflavone (0.001-1μM; βNF), prototypical ligands of the estrogen receptor (ER) and the aryl hydrocarbon receptor (AhR), respectively, to determine if these receptor pathways are involved in CYP3C gene regulation. Exposure to βNF resulted in the dose-dependant up-regulation of all CYP3Cs genes in the intestine but not in the liver. CYP3C gene expression was induced in the ovary. CYP3C induction was not observed in female intestine, ovary and testis after E2 exposure but all genes were induced in the male intestine. Finally, CYP3Cs were induced in the female liver but was down-regulated in the male liver with E2. Overall, CYP3Cs were not highly inducible by βNF or E2 and most genes were induced between 2-4 fold over control.

Yet, this data suggests that the AhR and ER may play a role in the regulation of CYP3Cs that is gender and tissue specific. The goal of this study is to provide insight on an important family of detoxification enzymes and potentially contribute to further characterizing the capacity of the commonly used model organism, the zebrafish, for metabolizing xenobiotic compounds.

Effects of water-borne benzo[*a*]pyrene on early-life stages of the fathead minnow (*Pimephales promelas*)

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Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitously distributed environmental pollutants that mainly originate from petrogenic and pyrogenic sources such as combustion of fossil fuels and other organic material. Various PAHs, including benzo[a]pyrene (BaP), have been demonstrated to cause a wide range of effects in exposed wildlife, including alterations of immune responses, impaired development and reproduction, as well as mutagenesis and carcinogenesis. Most studies to date, however, have used comparably high exposure concentrations, dietary routes of exposure or intraperitoneal injection to administer BaP, and knowledge of low-dose effects at concentrations around water solubility (approx. 4 $\mu g \cdot L^{-1}$) is generally limited. This route of exposure, however, must be considered highly relevant in light of the distribution of PAHs even into remote aquatic systems. To bridge this knowledge gap, early-life stages of the fathead minnow (*Pimephales promelas*) will be exposed to waterborne BaP as a model compound to characterize toxicity pathways that drive the sensitivity of early life-stage fish to PAHs. Molecular responses at the whole transcriptome, proteome and metabolome level will be investigated at the swim-up stage, and quantitatively correlated with effects on apical (growth, survival, development), histopathological, and biochemical endpoints 28 days post-hatch. The data generated within this experiment will help to better understand the relevance of aqueous exposure to BaP specifically, and PAHs in general, and provide important insights into the relevance of molecular responses in early-life stages as early-warning biomarkers for apical outcomes in juvenile and/or adult fish. This study is part of the EcoToxChip project (@ecotoxchip).

Adverse outcome pathways for selenomethionine exposure in four commercially and culturally relevant Canadian fish species

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Selenium (Se) is a nonmetal atomic element and essential trace nutrient for all domains of life. However, excess consumption of or exposure to various chemical species of Se can have detrimental effects on organisms that may ultimately lead to death of the individual or extirpation of populations. The most environmentally relevant form of Se is the organic form, selenomethionine (SeM). Selenomethionine is generated by primary producers and subsequently bioaccumulates in aquatic food webs, ultimately causing severe teratogenic effects in oviparous animals (e.g., fish, birds) as SeM is incorporated into and maternally transferred by the egg yolk protein, vitellogenin. As anthropogenic activities can cause Se influx to the aquatic environment, it is critically important to determine the toxic mechanisms of and species-specific sensitivities to SeM in order to protect vulnerable populations. This research will use microinjected SeM as an analogue for maternally transferred SeM in four commercially and culturally relevant Canadian fish species: fathead minnows (*Pimephales promelas*), white sucker (*Catostomus commersonii*), rainbow trout (*Oncorhynchus mykiss*), and endangered white sturgeon (*Acipenser transmontanus*). Biochemical and apical endpoint analyses will be used to determine the relative developmental sensitivities of these species to SeM and to construct adverse outcome pathways (AOPs) for SeM exposure in each species under study. These AOPs will identify the molecular initiating event (MIE) of SeM toxicity as it pertains to downstream deleterious effects on apical endpoints (survival, length, weight, condition, reproductive output, somatic indices, etc.). Omic (genomic, proteomic, transcriptomic, and metabolomic) analyses, biomolecular assays (glutathione:glutathione disulfide ratio, and thiobarbituric acid reactive substances, superoxide dismutase activity, and catalase activity assays), energetic (triglyceride and glycogen) concentrations of muscle and liver, and histological analyses will be used to help identify the MIE of SeM toxicity in these species. The data acquired and AOPs constructed in this research will subsequently aid in the of regulation of Se in the environment, and aid in the risk assessment of SeM exposure to these fish as well as novel species sharing similar biomolecular characteristics, all while minimizing loss of animal life.

Salmon aquaculture-derived nutrients and metals in biota from rocky habitats in the Bay of Fundy

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Salmon aquaculture has been present in New Brunswick for over 40 years and is one of the largest industries in the province. However, there have been concerns about the industry's environmental impacts, because of the release of wastes from the cages. Past

studies of such impacts have focused on soft sediment and little is known about impacts on other habitat types. For this reason, we aim to determine if aquaculture-derived nutrients and metals are being incorporated into the nearby benthic community in rocky habitats. Zinc is a micronutrient in salmon feed and is available to nearby organisms through excess feed and feces in the sediments. Meanwhile, copper from antifouling paint leaches into the water, binds to organic material and is also deposited in the sediments. The new organic food-source and metal contaminants can be consumed and impact the biology of marine benthic species. We hypothesize that internal metal content as well as the use of aquaculture-derived nutrients will change between species located near and away from pen sites and be feeding-type specific. To test this, cages filled with cobble, known as bio-collectors, were placed at paired sites near (<200 m) and away (800-1000 m) from each of 8 aquaculture sites (n=7 per site) in the Bay of Fundy from July to October 2016. Bio-collectors were colonized by a suite of invertebrates and fishes. Three species were analysed for metals and stable isotopes to assess the use of aquaculture wastes: a filter feeder ascidian, *Ciona intestinalis*, a scavenging fish, *Pholis gunnellus*, and a predatory fish, *Myoxocephalus scorpius*. Using dried muscle tissue or whole body samples, we measured concentrations of copper and zinc as well as C, N and S isotopes to evaluate differences between individuals from near and away sites within a species, identify differences between species, and compare isotope signatures of each species to that of the salmon feed. While our isotope results are pending, the metal data showed no significant difference between near and away locations for each species. However, there was a difference in the concentration of metals between species. *P. gunnellus* had copper levels between 1.35 and 1.93 $\mu g \cdot g^{-1}$, while *M. scorpius* levels ranged between 2.1 and 4.5 $\mu g \cdot g^{-1}$ and *C. intestinalis* had higher copper values of 5.0 and 17.7 $\mu g \cdot g^{-1}$. Moreover, *P. gunnellus, M. scorpius* and *C. intestinalis* had zinc levels between 0.25 and 0.6, 0.4 to 0.6, and 4.53 and 15.9 $\mu g \cdot g^{-1}$, respectively. Overall, there is little evidence from this study to support that aquaculture-derived metals are being incorporated by benthic species in rocky habitats near pen sites.

A new methodology to evaluate toxicity and chemistry of natural oil sands: Evaluation of dissolved and particulate fractions of bitumen ore

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In the tributaries and mainstem of the Athabasca River in the oil sands region, inputs of bitumen-derived constituents from natural oil sands as well as potential contaminants associated with mining activities will affect water quality, thus raising concerns of cumulative impacts on aquatic organisms. Erosion of bitumen ore by ice and water leads to the partitioning of constituents into air, water and sediment as a function of the physical and chemical properties of the constituents and the surrounding environment. The partitioning of bitumen-derived constituents influences the exposure to and effects of constituents on aquatic organisms. There is a need to better understand the environmental impacts associated with bitumen-derived constituents as a function of erosional processes in order to address the issue of potential cumulative impacts. The objective of this study was to isolate the dissolved and particulate fractions of bitumen ore mixed with water and

evaluate the toxicity of the individual and combined fractions on the survival of *Hyalella azteca*. Two bitumen ore samples were evaluated: 1) bitumen ore collected from a McMurray Formation exposure in the MacKay River valley, and 2) bitumen ore collected from an open pit mine and stored at the InnoTech Sample Bank. Bitumen ore was mechanical mixed for 24 hours in water to simulate erosion, and then filtered to separate the dissolved and particulate fractions. Toxicity tests were conducted using a 96-hour *H. azteca* survival test at bitumen concentrations of 0-2.5 dry weight $g \cdot L^{-1}$ for four different treatments: bitumen ore (unmixed), bitumen ore (mixed), dissolved fraction and particulate fraction. The results for both bitumen ore samples indicate significantly higher toxicity for all treatments that contain particulates relative to the dissolved fraction. Water chemistry analyses of metals and polycyclic aromatic compounds (PAC) were used to estimate threshold concentrations. Threshold concentrations were compared with available data on environmental concentrations of metals and PACs in suspended sediments collected from the Athabasca River. The findings of this study are important to establishing a comprehensive environmental monitoring program and developing an effective watershed management strategy in the oil sand region.

Impacts of gold mining on plants and arbuscular mycorrhizal fungi in northern Canada

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Gold mining continues to be an important part of economic development in Northern Canada. Since minerals and metals are non-renewable, mining is ongoing and it is important that mining development is carried out in a responsible and sustainable manner. A large portion of the gold that is found in the Northern territories is contained within arsenopyrite rock, therefore arsenic byproduct is of special concern. Currently, not much research has been done on the impacts of arsenic on plants and mycorrhizae in Northern ecosystems. Previous research in temperate regions has shown that the symbiosis formed between plants and arbuscular mycorrhiza can accelerate the remediation process in gold mines by supporting plant growth in poor soil conditions. The mine studied in this project is Tundra Mine, an inactive gold mine 240 km northeast of Yellowknife. Soil was collected from un-vegetated areas previously used as tailings ponds, which have since been treated to remove contaminants. Other sites were chosen at varying distances from the original tailings area to include a range of contamination levels and disturbances. Soil was analyzed for arsenic contamination as well as to find mycorrhizal inoculum potential by quantifying spore density at each site. Several sites near the original tailings contained biologically relevant concentrations of arsenic in the soil up to 3.2 $mg \cdot g^{-1}$. Mycorrhizal spores were found at all sites, ranging from 800 spores/100 mL to 15 spores/100 mL of soil. Mycorrhizal plant species were also collected from each site in June and August 2016 for assessment of mycorrhizal colonization. Arbuscular mycorrhizal colonization varied from site to site, but was found regardless of soil arsenic contamination. Tissue arsenic concentrations were determined for plant species that were common to all sites. It was

found that samples exceeded the Canadian Food Inspection Agency in livestock feed of forage by several orders of magnitude.

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